The volume and area of the capillaries in the endocrine and exocrine pancreas of the rat

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Summary. The capillary volumes in the endocrine and exocrine parenchyma of the pancreas were compared with a point-sampling technique. The islets were found to have a capillary volume of approximately 3.5%, while the value for the exocrine pancreas was significantly (P < 0.001) lower at 2%. When the capillary wall area was measured, however, both types of parenchyma had a similar value of approximately 20 mm²/mm³ tissue. The reason for the discrepancy between these parameters is probably the lack of lymphatic capillaries, with their relatively small lumen in the islets.

Introduction

The architecture and function of the vascular system of the pancreatic islets have been extensively studied with a variety of techniques ever since the vasculatures discovery (Kühne and Lea 1882). As well as conventional histological techniques, some of the methods used have included the analysis of the blood vessel morphology following the injection of various dyes (Wharton 1932; Thiel 1954) or plastic polymers (Fujita and Murakami 1973; Bonner-Weir and Orci 1982), direct observations of the circulation with intravital microscopy (Bunnag et al. 1963; Rooth et al. 1985) and measurements of islet blood flow with the microsphere technique (Lifson et al. 1980; Jansson and Hellerström 1983). These studies have provided a detailed morphological picture of the complex arrangement of the blood vessels in the endocrine pancreas, and have also given information on the islet blood flow under normal conditions.

However, there is still little quantitative information available on the dimensions of islet capillaries, i.e. their length, area and volume. Capillaries are difficult to identify and measure in glandular tissues unless they are specifically stained, and thereby contrasted against surrounding cells. In the present study we have used a classical histochemical staining for alkaline phosphatases (Gomori 1941), a group of enzymes which consistently appear in capillary endothelial cells of many species, including the rat. Although alkaline phosphatases are also present in some of the larger excretory ducts and in the pancreatic A-cells (Gomori 1941), the islet and exocrine capillaries can be easily identified (Githens 1983), and the technique has been used, in the present study, for a quantitative evaluation of the capillaries of the endocrine and exocrine pancreas.

Materials and methods

Histological techniques. Five adult, male Sprague-Dawley rats with free access to tap water and pelleted food (Type R3; Ewos, Anticimex, Södertälje, Sweden) were used. The animals were killed by cervical dislocation, and the pancreas removed through a midline incision. The gland was divided into two equal parts, which were transferred to metal grids covered by Tissue-Tek II O.C.T. Compound (Histo-Lab, Bethlehem Trading, New York, USA). The grids with the tissue were immediately submerged in 2-methylbutane (Kebo-Grave; Spånga, Sweden), cooled to just above its freezing point (−124 °C) and, after freezing, placed in a cryostat (Tissue-Tek II Cryostat; Histo-Lab, Bethlehem Trading, New York, USA) where the frozen tissue was cut into 10 µm thick sections, which were placed in groups of three on object glasses. To visualize the capillaries the pancreatic sections were postfixed in acetone at room temperature for 15 min, and stained for alkaline phosphatase according to the method described by Gomori (1941). Each section was treated for 40 min at room temperature in a solution containing 16.3 mM sodium β-glycerophosphate, 16.1 mM sodium diethylbarbiturate, 45.3 mM calcium chloride and 3.3 mM magnesium sulphide dissolved in distilled water and adjusted to pH 9.4 (all chemicals were purchased from E. Merck, Darmstadt, FRG). The object glasses were then rinsed in distilled water for 1 min, and transferred to a solution containing 68.7 mM cobalt nitrate (E. Merck, Darmstadt, FRG) in distilled water. After 5 min in this solution, the preparations were once again rinsed in distilled water for 1 min and then transferred, for 2 min, to the final solution containing 104 mM yellow ammonium sulphide (Carl Roth, Chemical Fabrik, Karlsruhe, FRG). The preparations were finally rinsed and then mounted with Eukitt.

Morphometrical techniques. To measure the volume percentage of the islet and exocrine capillaries according to the point sampling technique of Chalkley (1943), 20 randomly chosen sections from each animal were viewed in a light microscope at a magnification of 400X. A square ruled grid with 121 intersections was placed in the focal plane of the microscope eye-piece and projected onto the sections exceeding 100 µm in diameter, in the order in which they were encountered. The number of intercepts between intersections of the grid and capillary spaces were then counted in a total of 123 islet sections. The grid was also projected onto 123 randomly chosen areas in the exocrine parenchyma (25 areas in four of the animals and 23 in one) and the number of intercepts counted. At least 150 intercepts were counted in each animal. The areas in the exocrine parts of the gland were chosen with the aid of a table of random sampling numbers which were used as coordi-
Fig. 1. An islet stained for the presence of alkaline phosphatases. Note the darkly stained, elongated endothelial cells (solid arrows) and the larger, rounded and less intensely stained cells in the periphery of the islets (open arrow). Magnification 250 x

Fig. 2. The exocrine parenchyma of a pancreas stained for the presence of alkaline phosphatases. Darkly stained, elongated endothelial cells can be seen (arrows). Magnification 250 x

Fig. 3. An interlobular duct in the pancreas. A weak positive reaction for alkaline phosphatases can be seen in the stroma of the duct (arrows). Magnification 250 x