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MORPHOLOGICAL QUANTITATION BY PERFUSION STAINING OF THE ENDOCRINE MOUSE PANCREAS

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ABSTRACT

The endocrine mouse pancreas was visualized by perfusion staining the islets of Langerhans with the zinc-chelating agent dithizone. Morphological quantitation of the projected images showed a strong correlation between islet area, body weight, insulin secretion and pancreatic insulin content. The size distribution i.e. islet area and volume was best fitted to a power-generalized inverse Gaussian distribution.

INTRODUCTION

The detection of the islets of Langerhans, situated in an exocrine tissue and their significance in relation to diabetes has created a great need for a better understanding of the physiology and morphology of the endocrine pancreas. However, related to the characteristic anatomy (Bencosme et al, 1955) it has been difficult to establish structural and functional relationships in the gland. Perfusion staining has been used to visualize the islets (Bensley, 1911), but it was not until recently that a combined procedure for islet quantitation and islet function in mice was introduced (Bonnevie-Nielsen et al, 1983).

MATERIALS AND METHODS

The pancreas was perfused with 38^{o}C Krebs-Ringer-bicarbonate (KRB) with 40 mg/ml bovine serum albumin (Miles laboratories). A whole organ perfusion was obtained by perfusing the pancreas from the aorta. Stimulated insulin secretion was studied by adding 20 mM D-glucose to the perfusion media for different time periods. After perfusion, KRB supplemented with 1.24 mM dithizone (Merck, GFR), 71 µl/ml ethanol, 1 mg/ml procaine hydrochloride and 500 KIU/ml aprotinin (Novo,

Denmark) was infused via a sidearm syringe for 3 min. The pancreas was then perfused for 5 min with KRB to rinse the vessels from the stain. The organ block was secured on a round white teflon plate (diameter, 5 cm) with small pins. Glycerol in water (87 µl/ml) made the exocrine tissue transparent and a systematic dissection made all the islets visible. Photographs (Agfa ortho 26, Agfa-Gevaert) were taken at a final magnification of 14 diameters in a photomicroscope (Wild, Switzerland). Islet counts and a quantitative image analysis on the prints was performed with an electronic texture analysing system (T.A.S., Leitz, GDR). Experiments showed no effect on islet areas when placing a coverglass on the gland until the distance between the teflon plate and the coverglass was 50 µm (Bonnevie-Nielsen et al, 1983).

RESULTS

The staining procedure for the pancreatic islets utilizes the zinc chelating effect of dithizone, which stains the islets deeply red. Islet sized of 14 µm in diameter were easily detected. Fig. 1 shows a photomontage of one whole pancreas.

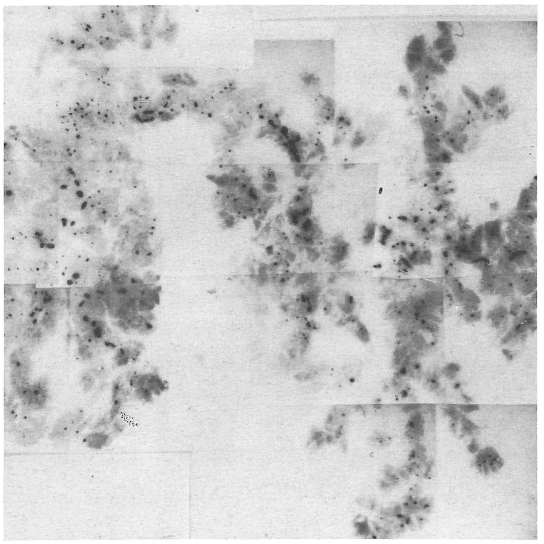

Fig. 1

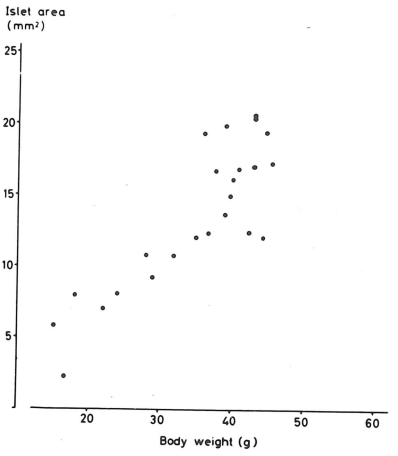

Fig. 2

In mice ranging from 4 weeks to 18 months of age the islet number showed no increase, whereas the islet area increased significantly from 2,2 to 23,15 mm^2. The ratio of islet area to body weight had a low variation and the two parameters correlated well with a p-value of 3×10^{-7} (Fig. 2). Total pancreatic insulin content was determined and the correlation

between insulin content and islet area had a p-value of 7,8 x 10^{-12}. Islet number and area were determined in mice that were made diabetic with 5 low doses of streptozotocin (SZ). The diabetic state developed gradually with increasing blood glucose, and destruction of the β-cells. The day after the last injection of SZ, the islet area was reduced to 31% and the islet number to 62% and a further reduction to 1% and 7% was reached on day 15 (Fig. 3 and 4).

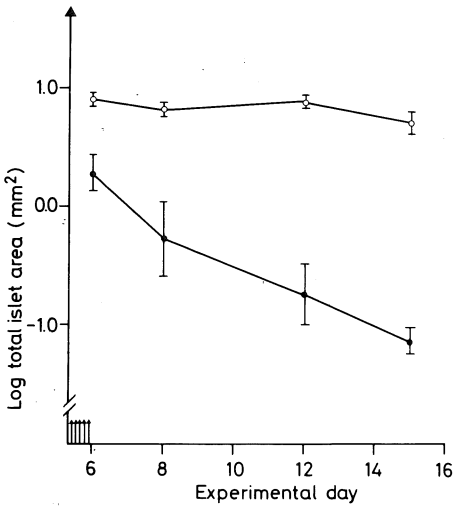

Fig. 3

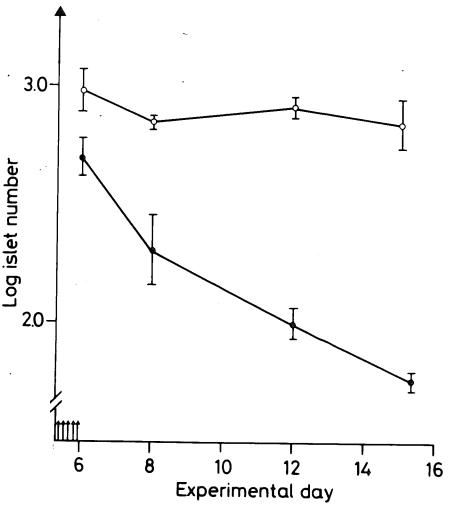

Fig. 4

The insulin secretory capacity was reduced to the same extent whereas the secretion pattern was qualitatively unaffected. A distributional study was performed on the areas obtained from normal pancreases because they did not follow any known family of distribution. The size distribution was characterized by a long tail to the right and an early peak mode with islet areas varying from 0,4 x 10^{-3} to 0,35 mm^2. The best fit was found with a pover-generalized inverse Gausian distribution.

DISCUSSION

The validity of the staining and morphometric method is dependent on a whole organ perfusion, and it is necessary that every single islet is clearly demarcated. This is easily obtained due to the high affinity of dithizone for the β-cell granules. There was no indication of an incrase in islet number with age but the total islet area increased by 350%. An increase in islet number was found by Hellmann (1959) and Bunnag (1966). However, since each islet was counted directly

the islet number in relation to age is concluded to be unchanged. The strong relationship between body weight and total islet area, total insulin content in pancreas and islet area might indicate a significant dependency among these parameters. It was also found that the insulin secretion capacity increased with age and body weight and furthermore was closely related to the pancreatic insulin content (Bonnevie-Nielsen et al, 1983). Isolated pancreatic islets from old rats had a decreased insulin secretion (Reaven et al, 1979), but these data were not related to body weight, DNA or insulin content. In diabetic mice an exponential loss was found in islet areas, islet number, pancreas insulin content and insulin release. However, the remaining islets showed a qualitatively normal secretion pattern, suggesting that the stimulus-secretion coupling was working normally (Bonnevie-Nielsen et al, 1981). As one dimension is lost by tissue sectioning for conventional microscopy, it is necessary to apply appropriate formulas for volume calculations (Wicksell, 1925). However, because the islet area does not change until the height of the third dimension is as low as 50 μm, the islet area is proportional to the islet volume. The observed relationship between islet area (islet volume) and pancreatic insulin content makes it possible to determine islet volume simply by measuring the insulin content radioimmunochemically. As the islet height is independent of the islet area it means that the power-generalized inverse Gaussian distribution will apply to islet volume as well as to areas.

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