QUANTIFICATION OF CELLS IN ISLETS OF LANGERHANS USING DNA DETERMINATION

Jürgen Beckmann  
Sigurd Lenzén  
Susanne Holze  
Uwe Panten

The number of islets usually serves as reference parameter when secretory capacity or metabolism of isolated islets of Langerhans are studied. Since individual islets collected under visual control with the aid of a microscope vary considerably in size, marked quantitative variations occur between samples selected by different persons and on different days. Absolute data from different laboratories may be compared more directly, therefore, if based on a measure of islet mass rather than on the number of islets.

Freeze-drying and weighing samples composed of the small mammalian islets (down to less than 0.5 μg dry weight per islet) is tedious and demands much skill. It requires separation of the islets from any incubation medium without loss of tissue. This may often be impracticable, e.g. if islet cell coherence has been diminished by calcium depleted media or if islet metabolism has been stopped by strong inorganic acids prior to determination of metabolites like lactate or cyclic AMP.

Islet protein is dependent on the metabolic state of the tissue and, like dry weight, may be determined only after removal of albumin-containing medium.

The DNA content of the islets should be the ideal reference parameter since it is closely related to the number of cells. It has been determined by some investigators who used the technique of Kissane and Robins which is based on the reaction of dianinobenzoic acid (DABA) with the deoxyribose component of DNA. Compared to methods of DNA determination based on its fluorescence enhancement effect the DABA-technique has the advantage of being applicable even to

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damaged DNA and not to require homogenization of the tissue. On the other hand, DABA also reacts with some non-deoxyribose aldehydes which may derive from tissue lipids or incubation media. The original method was developed for the determination of DNA in nervous tissue which contains much larger amounts of lipids than the islets of Langerhans. It therefore includes three steps of lipid extraction from the tissue by ethanol.

In the present study we investigated whether pancreatic islets must also be extracted prior to DNA determination by DABA and whether some substances which are used in experiments on islet function and metabolism interfere with this determination. We developed a simple and accurate modification of the Kissane and Robins technique and used it to measure the DNA content of different mouse and rat islet preparations. Some of the results have been published in abstract form.

MATERIALS AND METHODS

Reagents and solutions - All reagents were of analytical grade and purchased from Merck (Darmstadt, Germany) unless stated otherwise. Collagenase type IV was from Worthington (Freehold/N.J., U.S.A.) and bovine serum albumin (fraction V) from Serva (Heidelberg, FRG).

3,5-Diaminobenzoic acid dihydrochloride ‘puriss.’ (DABA) was from Fluka (Buchs, Switzerland). On the day of use, 1.12 g were dissolved in 2 ml water, decolorized 2-4 times by mixing with charcoal (‘Norit A’, Serva; 10 mg/ml) and centrifugation, and kept cool in the dark.

Calf thymus DNA (type I) was from Sigma (St. Louis/Mo., U.S.A.). A stock solution in 1 M NH₄OH (2 mg DNA/ml) was stored in 0.1 ml aliquots at 4 °C up to 3 months. With 1 M NH₄OH final dilutions of 4.0, 2.0 and 1.0 μg DNA/20 μl were prepared daily.

Ethanol of analytical grade (Riedel, Hannover, FRG) was purified in order to reduce the acetaldehyde content: 1 l ethanol was mixed with 10 g semicarbazide hydrochloride and 30 g tris-(hydroxymethyl) aminomethane and distilled 3 times. After this, the fluorescence produced by allowing 20 μl ethanol to react with 50 μl DABA solution (see below) could not be lowered any further and amounted to about twice that of the blank (water + DABA).

Basal medium was a Krebs-Ringer bicarbonate buffer containing only 20 mM NaHCO₃ but 10 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid), 2 mg/ml bovine serum albumin, and NaOH to give a pH of 7.4 after equilibration with O₂ + CO₂ (95:5) at 37 °C.

DNA standards - Twenty μl of the DNA dilutions and of 1 M NH₄OH were pipetted in triplicate into the bottom of disposable 0.75-ml polypropylene tubes (type 30/8, Sarstedt, Nümbrecht, FRG) and dried at 60 °C. Standards of 0.4 μg DNA were obtained.

DNA standards in the presence of test solutions - Twenty μl of basal medium or other solutions were pipetted into dried standards of 0.4 μg DNA.

Freeze-dried pancreatic islets - Adult Wistar rats (Winkelmann, Borchen, FRG), adult lean albino NMRI mice (Gassner, Sulzfeldt, FRG), and adult non-inbred ob/ob mice (bred in our department) were used. The animals were not fed for 24 h.