RAPID ISOLATION OF PANCREATIC ISLETS FROM COLLAGENASE DIGESTED PANCREAS BY SEDIMENTATION THROUGH PERCOLL™ AT UNIT GRAVITY

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SUMMARY: A Percoll solution with a density of 1.045 g/ml was used to separate pancreatic islets and exocrine tissue from collagenase-digested human and ob/ob mouse pancreases by sedimentation at unit gravity. Most exocrine tissue from the mouse was found to range in density from 1.015 to 1.045 g/ml whereas the denser islets lay in a narrower range of 1.065-1.070 g/ml. Up to 400 islets were obtained from each mouse pancreas and 140 islets from 4 g of human pancreas; the isolated islets being essentially free from contamination with exocrine tissue. Glucose-stimulated insulin release was the same whether the mouse islets were isolated with or without Percoll. The simplicity of the method makes it suitable for large-scale islet isolation, a feature of potential importance for the treatment of diabetes by islet transplantation.

INTRODUCTION

The collagenase technique for isolation of pancreatic islets originally described by Moskalewski (1), was improved by Lacy and Kostianovsky (2), who also suggested the use of a sucrose gradient for the separation of islets from exocrine tissue during centrifugation. Lindall et al. (3) and Sharp et al. (4) substituted Ficoll for sucrose because it provided a better osmotic environment. Since some batches of Ficoll contained a toxic factor, prior dialysis was necessary to obtain reproducible islet function (4). In the present study Percoll, a new non-toxic density medium with optimal osmotic properties (5,6), has been used to separate pancreatic islets from collagenase digested pancreas. A major advantage of this medium is its low viscosity eliminating the need for centrifugation.

Abbreviations: HEPES,N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; TCM 199, tissue culture medium 199.

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MATERIALS AND METHODS

Chemicals. Collagenase (CLS IV, 140 U/mg) was obtained from Worthington Biochemical Corp., Freehold, N.J., USA. Sigma Chemical Co., St. Louis, USA, supplied HEPES, DNAase (DN-25) and bovine serum albumin (fraction V). TCM 199 (10X concentrated, lacking glucose, bicarbonate and phenol red)was from Statens Bakteriologiska Laboratorium, Stockholm, Sweden, and Percoll™ from Pharmacia Fine Chemicals, Uppsala, Sweden. All reagents were of analytical grade and distilled and deionized water was used.

Sources of pancreas; collagenase digestion. Adult male ob/ob mice were starved overnight and killed by decapitation. The pancreas was rapidly excised, transferred to ice-cold Hanks' medium and cut in small pieces with a pair of scissors. The pancreas pieces and 1.5 ml Hanks' medium were added to a vial containing 2.5 mg collagenase. After vigorous shaking for 18-20 min at 37°C the digest was diluted with 10 ml ice-cold Hanks' medium and thoroughly mixed. It was then kept on ice and allowed to sediment for 2 min. The supernatant was discarded and 25 µl DNAase solution (10 µg/µl) added. In one case pancreas was obtained from a 16 year old girl with brain death. The pancreas was excised immediately after circulatory arrest, and disrupted by injection of ice-cold Hanks' medium into the pancreatic duct. Four g of pancreas were cut into small pieces and added to 9 ml HEPES-buffered Hanks' medium (pH 7.4) containing 75 mg collagenase. After 25 min of vigorous shaking at 37°C the digest was diluted with 50 ml ice-cold Hanks' medium and allowed to sediment for 2 min. The supernatant was discarded and 50 µl DNAase solution added.

Separation of pancreatic islets. A stock solution of Percoll was prepared by mixing 9 volumes of Percoll with 1 volume of 10X concentrated Hanks' medium. For the separation of pancreatic islets 3.5 volumes of this stock solution were mixed with 6.5 volumes of Hanks' medium and poured into a 25-80 mm wide vessel. The pancreas digest was carefully placed on top of this medium with the aid of a pipette. The pancreatic islets were allowed to sediment. The pancreas digest was then removed by suction and the pancreatic islets collected from the bottom of the vessel.

Density determinations. Densities of media and tissue fractions were measured in an organic density gradient column (7). To determine the density of pancreatic islets and exocrine pancreas the collagenase-digested tissues were centrifuged for 30 min at 30 000xg in a mixture of 6 volumes of Percoll stock solution and 4 volumes Hanks' medium. Under these conditions Percoll forms a continous density gradient (6) and the tissue densities could be determined by measurements on the respective fractions in the organic density column.

Insulin secretion. Pancreatic islets were isolated either directly from the pancreas digest under a stereo-microscope and rinsed, or were separated by sedimentation through Percoll. Batches of 3 islets were preincubated for 60 min at 37°C, pH 7.4 in TCM 199 buffered with 25 mM HEPES and 4.2 mM $NaHCO_3$ and also containing 1 mg/ml albumin and 3 mM glucose. Preincubation was followed by 60 min of incubation at 37°C in 300 µl of the same medium containing either 3 or 18 mM glucose. The insulin released during incubation was measured radioimmunologically and expressed in relation to the dry weight of the islets.

RESULTS

The density of most pancreatic islets from the ob/ob mice were between the narrow limits 1.065-1.070 g/ml. Only few additional islets had densities

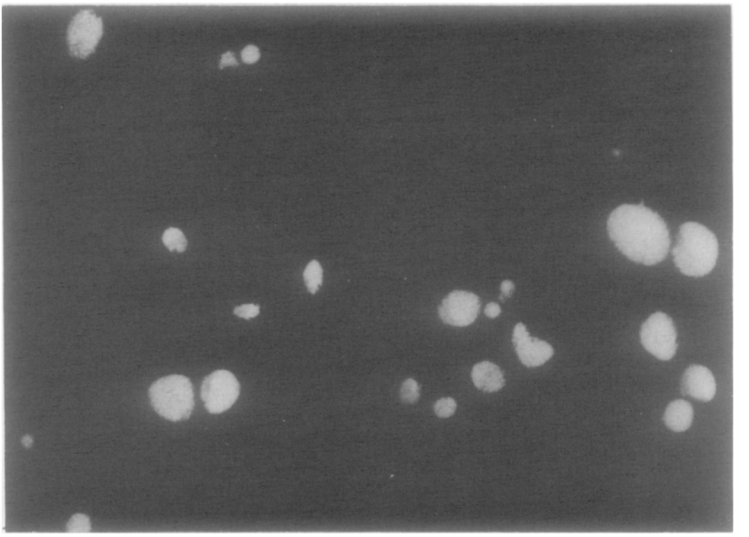

Fig. 1. Sediment obtained when collagenase-digested ob/ob mouse pancreas was layered on Percoll separating medium (density 1.045 g/ml). The picture shows isolated islets and minor amounts of tissue debris.

between 1.060-1.065 g/ml and 1.070-1.075 g/ml. The density of exocrine tissue from these mice was more variable. Most of this tissue was in the 1.015-1.045 g/ml range but minor amounts of dust-like fragment had densities up to 1.065 g/ml. The Percoll medium used for separation of islets from the pancreas digest had a density of 1.045 g/ml. Within 10 min essentially only very pure mouse islets sedimented through the medium and settled on the bottom (Fig. 1). Exocrine pancreas and islets with adherent exocrine tissue either did not enter the separating medium or sedimented very slowly. Islets from normal mice or human islets sedimented more slowly than the ob/ob mouse islets although clear separations were obtained. The addition of DNAase to the collagenase digest was a prerequisite for successful separation of islets by preventing aggregation of tissue fragments. Provided that collagenase digestion had been successful up to 400 islets were obtained from each ob/ob mouse pancreas and in one experiment 140 islets from 4 g of human pancreas. Fig. 2 shows a typical separation. In this Fig. the islets are shown floating on a high density medium. There were no differences in insulin secretion between ob/ob mouse islets isolated with or without Percoll. Both types of islets responded readily to glucose stimulation (Table 1).

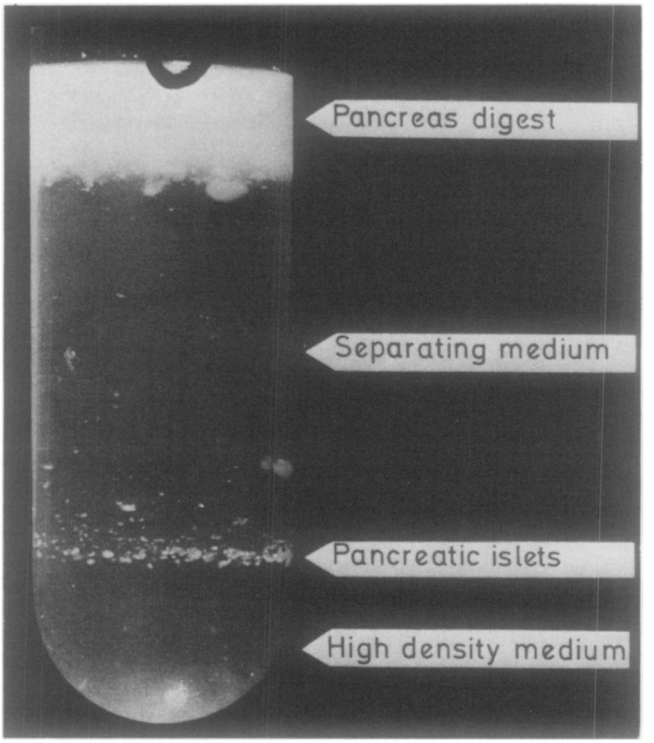

Fig. 2. Typical separation of pancreatic islets from collagenase-digested ob/ob mouse pancreas. The pancreas digest was placed on top of the isotonic Percoll separating medium (density 1.045 g/ml) and allowed 10 min of sedimentation at unit gravity. To aid visualization, a narrow test tube was used with a bottom layer of Percoll stock solution (density 1.118 g/ml).

DISCUSSION

Sedimentation of tissue constituents by centrifugation on sucrose or Ficoll gradients is complicated by high viscosity, undesired osmotic properties and in some cases also toxic effects (4). The use of Percoll as separating agent eliminates these problems. Iso-osmotic Percoll has a viscosity of 10 cP (5,6). This low viscosity eliminated the need for a centrifuge when separating pancreatic islets from exocrine tissue. Apart from the simplicity of separation by sedimentation at unit gravity, this procedure proved much more effective than separation by centrifugation through continous or discontinous gradients with regard to contamination of islets with exocrine tissue. The yield of pancreatic islets appeared to depend on the geometry of the separating vessel. A wide vessel allowing a thin layer of pancreas digest is therefore preferred. Methods for isolation of free cells often include the addition of DNAase to reduce clumping of the cells (8). In the present

Table 1. Glucose-stimulated insulin release (ng/μg dry islet)

Type of islet separation	Glucose concentration 1 mM	18 mM	Difference
With Percoll	1.76 ± 0.35	5.08 ± 0.71	3.32 ± 0.74^{XX}
Without Percoll	2.99 ± 0.84	6.78 ± 1.40	3.80 ± 1.40^{X}

Pancreatic islets were either isolated from collagenase-digested ob/ob mouse pancreases under a stero-microscope and rinsed or were separated by sedimentation through isotonic Percoll. After preincubation for 60 min in the presence of 3 mM glucose, batches of 3 islets were incubated for 60 min at 37°C in 300 μl medium supplemented with 3 or 18 mM glucose. The mean insulin secretions ± SEM from islets obtained from 7 animals are given.
$^{X}P < 0.05$, $^{XX}P < 0.005$

study DNAase was found to diminish the aggregation of tissue fragments in the collagenase digest, facilitating the separation. Most procedures for collagenase isolation of pancreatic islets involve elaborate rinsing of the pancreas digest to remove the enzyme. These problems are eliminated by the present method since the islets are effectively rinsed when sedimenting through the separating medium. In accordance with the non-toxic properties of Percoll observed during culture of different cell types (5) the present isolation procedure did not affect glucose-stimulated insulin secretion. Major obstacles in attempts to treat human diabetes mellitus by transplantation of pancreatic islets are the low yield and time-consuming procedures for isolation of islets from human pancreases. When the present technique was applied to collagenase-digested human pancreas, 140 pure islets were rapidly obtained from 4 g of tissue. This figure corresponds to approximately 3000 islets from the total pancreas, an amount that may well be attainable since such a simple method should be highly suitable for large-scale isolation of islets.

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