The efficacy of density gradients for islet purification: a comparison of seven density gradients

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Received March 6, 1990/Received after revision June 14, 1990/Accepted June 18, 1990

Abstract. Seven different density gradient-forming materials were compared as to their efficacy for rat islet purification. Continuous density gradients were used in order to determine the buoyant densities of the different pancreatic tissue components. Hand-picked islets served as a control. A significant separation of large numbers of islets from the exocrine tissue band was only seen in the albumin, dextran-40, and metrizamide gradients. Pure islet preparations could not be obtained with any of the gradients studied as none of the gradients completely separated lymph nodes, vessels, and ducts from the islets. Gradients containing sucrose resulted in low islet yields. The islet yields obtained with the other gradients were in the same range as those obtained by hand-picking. Metrizamide gave significantly higher yields than the widely used Ficoll. Judged both in terms of numbers of islets and their in vitro function, the best results were obtained with metrizamide and dextran-40.

Key words: Pancreatic islet purification – Density gradients in pancreatic islet purification

The purity of the islet graft is of the utmost importance for the success of islet transplantation. Exocrine tissue contaminating the islets can impair islet implantation [7], probably due to inflammation around the graft, and cause loss of transplanted endocrine tissue [6]. Suboptimal endocrine mass is thought to be a cause of early loss of graft function after transplantation due to exhaustion of the beta cells [1]. Furthermore, in humans, portal hypertension and disseminated intravascular coagulation have been reported after transplantation of nonpurified pancreatic tissue grafts [17]. Lymph nodes, exocrine tissue [5], and, in humans, also ductal endothelium [4] have been shown to contain MHC class II positive cells. As MHC class II positive cells play an essential role in the initiation of allograft rejection [12], these tissues should also be removed from the islet graft. Islet grafts can only be considered to consist of pure islets when they are depleted of exocrine tissue, lymph nodes, vessels, and ducts.

Purification of large numbers of islets from the collagenase-digested pancreatic tissue suspension cannot, for logistical reasons, be performed by hand-picking of the islets. Various other techniques have been applied [19], but generally purification by density gradient centrifugation is used.

Ficoll density gradients [15, 20] are often used for the separation of large numbers of islets from the collagenase-dissociated pancreatic tissue suspension, but they insufficiently separate lymph nodes, vessels, and ducts from the islets. Several other gradient-forming substances with different physiochemical properties, such as viscosity and osmolarity, have been described [2, 9, 11, 13, 18]. Their suitability for islet purification is usually determined by the number of functionally intact islets obtained, the amount of exocrine tissue contaminating the islet tissue, and islet function in vitro or in vivo after transplantation. However, the capacity of density gradients to separate lymph nodes, vessels, and ducts from the islets is not mentioned.

The present study compares the efficacy of seven different density gradient-forming materials for rat islet purification.

Materials and methods

Experimental design

In each experiment, eight male and fed Wistar rats with a mean body weight of 350 g (range 345–355 g) were used. The pancreases were dissociated using a standard collagenase digestion procedure. All of the digested pancreatic tissue was pooled and suspended in a total volume of 48 ml Krebs' Ringer HEPES (KRH), to which 0.25% bovine serum albumin (BSA, Sigma) was added. The tissue suspension was transferred to a 60-ml syringe. The syringe was continuously tipped to keep the suspension homogeneous. The suspension was divided into eight equal parts by weight so that each aliquot corresponded to the amount of pancreatic tissue obtained from one rat.
islet function due to the gradient-forming materials was assessed by testing in vitro four aliquots of ten islets obtained from each gradient.

Changes in numbers vary widely with each isolation procedure and since they do not contribute substantially to the total islet mass [23]. Changes in islet function were reversible or permanent, four aliquots of ten islets of each gradient were tested after the culture period and compared with cultured, hand-picked control islets.

The exocrine tissue band was examined to determine the number of islets larger than 100 µm that had not been separated from the exocrine tissue. These islets were not tested in vitro.

**Collagenase digestion**

The collagenase digestion method applied is a slight modification of a previously described procedure [21]. The abdomen of the rat was opened by a midline incision. The common bile duct was identified and the proximal segment was cannulated. A clip was placed on the entrance of the bile duct into the duodenum, and the pancreas was subsequently distended with 10 ml KRH containing 10% BSA [22].

After pancreatectomy, the pancreases were cut into small pieces with a pair of scissors and washed 4–5 times with KRH-10% BSA. Tissue from two pancreases in a total volume of 8 ml was transferred to a 25-ml Erlenmeyer flask. To each Erlenmeyer flask, 5 ml collagenase solution was added. The collagenase solution was prepared by dissolving 100 mg collagenase (Sigma type XI, 2200 U/mg) in 32 ml KRH-10% BSA. The flasks were stoppered and incubated in a horizontal position at 37 °C in a Dubnoff metabolic incubator and shaken at 200 cycles per minute. After 4 min the flasks were removed from the incubator and vigorously shaken manually for 1 min. The flasks were returned to the incubator and incubated for another 4 min. After a 2nd minute of vigorous manual shaking, the digestion was stopped by adding 10 ml KRH-10% BSA at room temperature to each flask. The contents of the flasks were poured into 100 ml conical vessels and the tissue was washed and sedimented twice with KRH-10% BSA. The volume of tissue sediment and KRH-10% BSA was brought up to 10 ml, transferred to the Erlenmeyer flasks, and 3 ml collagenase solution was added. The tissue was incubated a second time for 4–7 min in the water-bath, followed by 1 min of manual shaking. The digestion was stopped by adding KRH-0.25% BSA and the tissue was washed and sedimented four times.

**Density gradients**

The gradient materials studied were Ficoll 400 DL (Sigma), sucrose (Merck), Ficoll + 0.25 mM sucrose, Percol (Pharmacia), BSA (Sigma), dextran-40 (Sigma), and metrizamide (Nygaard). We always used 40 ml of gradient material in 50 ml graduated conical centrifuge tubes (Falcon 2098). The stock solutions of the gradients were made by dissolving the substances in KRH. The pH was adjusted to 7.4, if necessary. The continuous gradients were made with the aid of a multichannel peristaltic pump (Ismatic mp13 GJ10) by continuous dilution of the stock solution with KRH. The tissue aliquot, suspended one-in-one (v/v) in the lowest density of the gradient, was added to a 25-mi Erlenmeyer flask. To each Erlenmeyer flask, 5 ml collagenase solution was added. The collagenase solution was prepared by dissolving 100 mg collagenase (Sigma type XI, 2200 U/mg) in 32 ml KRH-10% BSA. The flasks were stoppered and incubated in a horizontal position at 37 °C in a Dubnoff metabolic incubator and shaken at 200 cycles per minute. After 4 min the flasks were removed from the incubator and vigorously shaken manually for 1 min. The flasks were returned to the incubator and incubated for another 4 min. After a 2nd minute of vigorous manual shaking, the digestion was stopped by adding 10 ml KRH-10% BSA at room temperature to each flask. The contents of the flasks were poured into 100 ml conical vessels and the tissue was washed and sedimented twice with KRH-10% BSA. The volume of tissue sediment and KRH-10% BSA was brought up to 10 ml, transferred to the Erlenmeyer flasks, and 3 ml collagenase solution was added. The tissue was incubated a second time for 4–7 min in the water-bath, followed by 1 min of manual shaking. The digestion was stopped by adding KRH-0.25% BSA and the tissue was washed and sedimented four times.

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