A Rapid Method for the Separation of Rat Pancreatic Islets from Collagenase-Digested Pancreas using Percoll

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Abstract

The isolation of pancreatic islets from collagenase-digested Wistar rat pancreas was shown by the sedimentation method at a unit gravity using Percoll solution with a density of 1.041 g/ml. The density of digested exocrine tissues was in the range of 1.013-1.041 g/ml, while that of purely isolated islets was in the narrow range of 1.066-1.075 g/ml. More than a hundred islets were obtained freely from each rat pancreas without any gross contamination of digested exocrine tissues. A significant increase was observed in glucose-stimulated insulin release from islets isolated with Percoll in the same pattern as that without Percoll.

In addition to the well preserved morphology and function of pancreatic islets isolated by Percoll, the simplicity of the technique strongly commends the usefulness of this method.

Since the introduction of the collagenase technique for the isolation of pancreatic islets (Moskalewski, 1965), there has been a demand for an optimal medium to collect a large number of pancreatic islets from digested pancreatic tissue without perturbation of their own cellular environments. Separating agents such as sucrose and Ficoll employed so far have been shown to have disadvantageous physiochemical properties because of high viscosity, hyperosmolarity (Lindall *et al.*, 1969; Sharp *et al.*, 1973). Prior dialysis and lyophilization of Ficoll is required to observe pancreatic islet function (Sharp *et al.*, 1973).

Percoll (collica coated with PVP, phamacia Fine Chemicals) has recently been proven to be a suitable medium to isolate cells and/or cellular components (Pertoft *et al.*, 1979). The present report has shown a simple method for the isolation of

pancreatic islets using Percoll. The results of functional studies of pancreatic islets which were isolated directly without any separating agents and by density gradient using Percoll and Ficoll were compared.

Materials and Methods

Handling of pancreatic tissue (collagenase digestion)

The pancreatic tissue of male Wistar rat weighing 250-350 g was obtained according to the method of Lacy and Kostianovsky (1967) and minced into small pieces. After washing them three times with Hanks solution, 3 ml of Hanks solution containing 5 mg of collagenase (140 U/mg, Type I, Worthington Biochemical Co., Freedhold, N. J., USA) was added to them and they were all poured into a flask (volume: 15 ml), which was then sealed up with Parafilm (American Can Company, Dixie/Marathon, Greenwich, CT, 06830).

The flask was subjected to vigorous shaking by hand for 5 minutes and to subsequent shaking for 10 minutes (160 cycles/min) in a water bath at 37°C. The digest was added with 20 ml ice-cold Hanks solu-

tion and mixed completely in a glass centrifuge tube (3 cm in diameter, 10 cm in height). The supernatant was discarded carefully after centrifugation for 5 minutes at 3,000 rpm and 25 μ L DNAase (10 μ g/ μ L, Sigma Corp., ST. Louis, USA) was added to the sediment (final volume: ca. 1 ml).

Separation of pancreatic islets

The separation of pancreatic islets using Ficoll gradient was performed according to the method of Sharp (Sharp *et al.*, 1973). Pancreatic islets under microscope were picked up with a micropipette and put into Henseleit-bicarbonate buffer at 37°C, pH 7.4 containing 2 mg/ml of bovine albumin (Nakarai Chemicals, LTD) and 60 mg/dl glucose (control medium) and rinsed.

For the separation of pancreatic islets by Percoll, 3.5 volumes of stock solution of Percoll, which had been previously prepared by mixing 9 volumes of Percoll and 1 volume of 10X concentrated Hanks solution, were mixed with 6.5 volume of Hanks solution and poured into a glass centrifuge tube mentioned above. The digested pancreas was placed on the top of this solution. The pancreatic islets were allowed to sediment and collected from the bottom of this tube. The pancreatic islets collected were handled in the same manner as mentioned above.

Density determination

To determine the density of the medium and tissue fractions using Percoll solution, the centrifugation was performed using a 12 PA tube (Hitachi Koki Co., LTD) for 20 minutes at 20,000 $\times g$ in the mixture of 4 volumes of Percoll stock solution with 6 volumes of Hanks solution (total volume: 10 ml).

After centrifugation, 0.2 ml of this medium was obtained with a micropipette and its density estimated with the aid of an Atago Abbe Refractometer. Under these conditions, the Percoll solution formed a continuous density gradient. The tissue densities could be estimated by measuring the length from the surface of the solution to respective tissue fractions after centrifugation of digested pancreatic tissue on this medium.

Insulin secretion

Batches of 10 islets were preincubated for 30 minutes in 2 ml of control medium at 37°C, pH 7.4, which was continuously gassed with 95% O₂ and 5% CO₂.

After preincubation, the medium containing 10 islets was transferred to a Swinnex 13 millipore chamber (Bedford, Massachusetts) with a 5 millipore filter (Gelman) by pipette and simultaneously aspirated through a millipore chamber with a syringe. Islets were rinsed with control medium and they were settled firmly on the surface of the millipore filter. The millipore filter with islets was put into

2 ml of control or test medium containing 150 mg/dl or 300 mg/dl glucose. The test medium was same as the control medium except for the glucose concentration. The medium was collected in an ice-chilled sampling tube for insulin assay immediately after immersion of the millipore filter loaded with the islets (T-0). Then, 2 ml of control or test medium was added again and incubated for 30 minutes under the same conditions as those for preincubation. After this procedure, the medium was collected (T-30) and kept frozen until insulin assay. Insulin release during incubation in control or test medium was calculated by subtraction of T-0 from T-30. Insulin was assayed by one-antibody method (Asano *et al.*, 1975) using rat standard insulin, Statistical significance was calculated by Student *t*-test.

Results

Isolation of rat pancreatic islets using Percoll

The density of isolated rat pancreatic islets was within the narrow range of 1.066–1.075 g/ml. The density of digested exocrine tissues was more variable. Most of this tissue was in the range of 1.013–1.041 g/ml.

Very purely isolated pancreatic islets were sedimented through Percoll with a density of 1.041 g/ml and settled down to the bottom of the glass tube within 10 minutes. Typically isolated islets from collagenase-digested pancreas obtained with this method are shown in Fig. 1. (More than a hundred purely isolated islets were collected easily by this method. Fig. 2 shows a B-cell in the isolated pancreatic islets, seen under the electron microscope, which appeared normal, full of β -granules, mitochondria and nucleus in cytoplasm.

Insulin secretion (Table 1)

The mean value for insulin release per islet during 30 minutes incubation is shown in Table 1. The glucose-stimulated insulin release was somewhat lower in the islets isolated by Percoll as compared with the results by the other two methods. A significant increase in insulin release to different

Fig. 1. Photograph of islets isolated from collagenase-digested pancreas by sedimentation through Percoll solution ($\times 120$).

Fig. 2. Electronmicrograph of B-cell in the isolated islet observed in Fig. 1. ($\times 18,000$). Electronmicroscopically, B-cell appeared normal with the findings of well preserved Golgi apparatus (*), β -granules ($\downarrow$), mitochondria (m), nucleus (N) etc.

Table 1. Insulin Release (μ U/ML/ISLET)

Type of islet separation	Glucose concentration		
	60 MG/DL	150 MG/DL	300 MG/DL
with Percoll	18.2 $\pm$ 4.5 (N=6)	29.7 $\pm$ 5.8 (N=6)	37.0 $\pm$ 8.1 (N=6)
with Ficoll	23.9 $\pm$ 8.9 (N=6)	31.1 $\pm$ 7.2 (N=6)	85.2 $\pm$ 18.2 (N=6)
direct separation	19.1 $\pm$ 4.3 (N=6)	33.8 $\pm$ 3.5 (N=6)	46.3 $\pm$ 4.7 (N=6)

Mean $\pm$ S.D.

Mean insulin values released from 10 islets to the stimulation of different glucose concentrations during 30 minutes incubation.

glucose concentrations of 150 mg/dl and 300 mg/dl was observed in islets isolated by Percoll ($P<0.05$, $P<0.001$ vs glucose 60 mg/dl) similarly to that in islets directly separated without Percoll. In the case of Ficoll gradient separation, the insulin release in response to the stimulation by glucose was different from that observed as mentioned above with an abrupt increase in insulin release in the 300 mg/dl glucose concentration ($P<0.001$ vs glucose 60 mg/dl).

There was no significant difference between isolated by Percoll and those in direct separation with respect to the insulin release at any point in glucose stimulation. The mean value of insulin release in response to the stimulation by 300 mg/dl glucose concentration was significantly higher in islets isolated by Ficoll than any other two methods ($P<0.001$).

Discussion

During the process of gradient centrifugation using Ficoll or sucrose, pancreatic islets were exposed inevitably to a high viscous, hyperosmolar condition and/or some toxic effects (Lindall *et al.*, 1967; Sharp *et al.*, 1973). Iso-osmotic Percoll is not viscous and has no toxic effect on its own physiochemical properties. The isolation of pancreatic islets from pancreatic digest was attained by sedimentation through Percoll at unit gravity, which eliminated the need for the centrifugation of pancreatic digest and its elaborated rinsing to remove enzyme.

The addition of DNAase to the pancreatic digest before its application to Percoll was effective in preventing the aggregation of tissue components in the pancreatic digest (Shortman, 1972). And Percoll solution with a density of 1.041 g/ml, which has been proven to be the maximum density of digested pancreatic exocrine tissues, should make the separation of pancreatic islets from exocrine tissues complete. In a recent report, the mixture of 12.5% Ficoll and 33.4% Conray solution resulted to some degree in the elimination of high viscosity and toxic effects, but still remained in hyperosmolarity (Okeda *et al.*, 1979).

The glucose-stimulated insulin release in the present isolation method was proven to be the same pattern and no significant difference was observed, in the mean insulin value at any point in glucose stimulation, between these two methods. In case of Ficoll gradient separation, insulin release in response to the stimulation of different glucose concentrations did not show a significant staircase increase as compared with that in Percoll separation or in direct separation. The causes of these difference were unknown, but the exposure of pancreatic islets to hyperosmolar and viscous conditions would impose some unfavourable effects on cellular function in spite of its prior dialysis and lyophilization.

This method is most suitable for collecting a large number of pancreatic islets in a short time without exposing them to unfavourable conditions.

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