Islet Preservation During Isolation: A New Concept in Cell Transplantation

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THE widely used hyperosmotic Ficoll or dextran gradients do not completely separate islet from exocrine tissue using standard isolation solutions. Moreover, part of the islets are lost due to osmotic damage or an increased islet-density. Theoretically isoosmotic Percoll gradients should improve islet recovery. Nevertheless previous attempts were not successful. We previously demonstrated that almost pure canine islets are obtained by using the University of Wisconsin (UW) cold storage solution as the isolation medium before hyperosmotic dextran gradients. Thus, prevention of cell swelling plus dehydration would explain the superior purity. We tested whether prevention of cell swelling using UW as the Percoll gradient solution successfully replaced dehydrating hyperosmotic gradients.

MATERIALS AND METHODS

Methods are illustrated in Fig 1. Briefly (Fig 1) we used collagenase in Hanks' balanced salt solution (HBSS) for islet isolation from 35 canine left pancreatic segments. After intraductal infusion of collagenase and digestion at 38°C the solution was decanted. Next, either RPMI or UW in two other groups was added on ice. Ducts and vessels were discarded. The tissue was dispersed by stirring and syringing through a 14-gauge needle. After sieving, the digest was spun. Islets were purified after using either RPMI (group I; n = 8) or UW (group II; n = 9) by dextran gradients in HBSS (Fig 2), or (group III; n = 18) after using UW by Percoll gradients in UW (Fig 3). To assess islet yield and purity, samples were taken from the pancreas, digest, and purified suspension. The islet content of the pancreas was determined by point counting on hematoxylin and eosin–stained sections. During isolation, islet yield was determined from the mean diameter of islets and expressed as the islet volume per gram of processed tissue, and purity was estimated from amylase recovery and expressed (assuming 98% acinar volume in the digest) as the islet volume fraction.

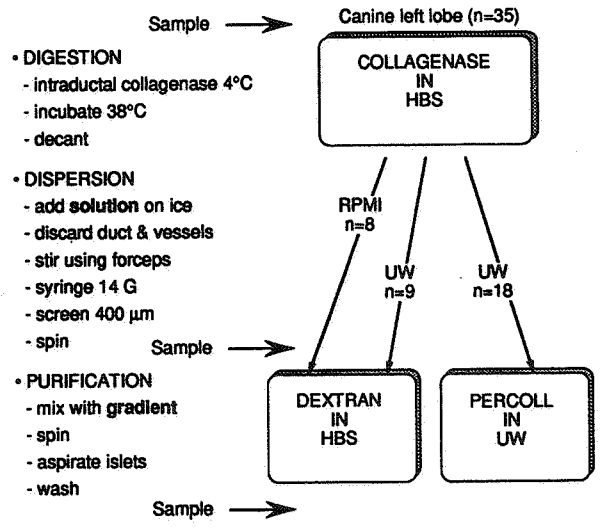

Fig 1. Method of canine islet isolation using either the physiological RPMI and Hanks' (HBS) solutions or UW solution.

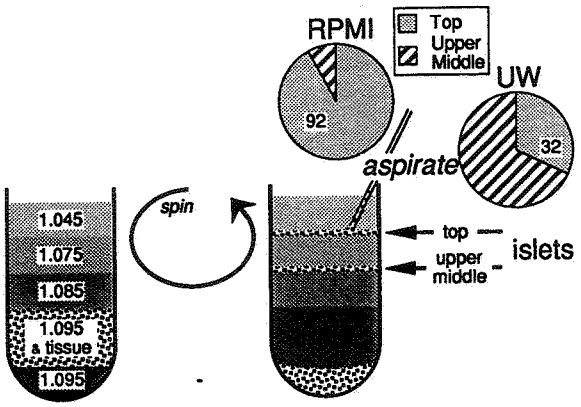

Fig 2. With RPMI as the isolation solution most islets are found at the top layer of dextran gradients, whereas with UW most islets are found at the denser upper-middle layer.

RESULTS

Before purification, islet yield did not differ using either UW or RPMI. Islet yield in the digest was reduced to 50% of the pancreatic content ($P < .001$). However, both islet

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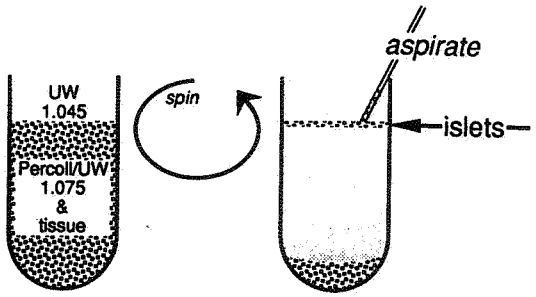

Fig 3. The density of islets is lower in isoosmotic percoll–UW gradients compared with dextran gradients; acinar tissue remains in the pellet.

recovery and purity improved markedly with density gradient purification after using UW during dispersion and sieving. Using the UW solution before purification consistently resulted in an islet volume fraction >90% with dextran gradients and 100% with Percoll–UW gradients, compared with a mean 33 ± 5% purity using RPMI before dextran gradients ($P < .001$). Mean islet recovery also increased from 40% with RPMI (group I) to 60% with UW (group II) after dextran purification ($P < .05$) and 70% after Percoll purification ($P < .01$). Preliminary results indicate >90% purity may also be attained with human islet isolation using the UW solution.

DISCUSSION

Replacing RPMI with UW before dextran gradient purification increased yield and dramatically improved islet purity. Also replacing dextran gradients with Percoll–UW gradients further improved both islet yield and purity. How do we explain these results? First consider the dextran gradients (Fig 2). Tissue is mixed with the bottom layer and, after spinning, islets are aspirated from the top and upper-middle interface. After using RPMI, 92% of the purified islets were found at the top still contaminated with acinar tissue, whereas after UW was used, most islets were found at the denser upper-middle and all acinar tissue was in the pellet. These results demonstrate that UW differs from RPMI in protecting cell density, by preventing the uptake of water by islet and acinar cells. Because these hyperosmotic dextran gradients also affect density by dehydrating the tissue, the effect of UW is further explained by our results with Percoll gradients. Percoll is an aqueous solution of plastic-coated silica particles and, like the components of UW, is an impermeant; that is, it does not cross the cell membrane. Using Percoll, islets are recovered at a density <0.075 (Fig 3) because Percoll hardly affects the physiologic osmolarity of UW. Still, acinar tissue is completely absent. We recently obtained similar results (100% purity and ~80% recovery) with Ficoll–sodium diatrizoate, another gradient solution of impermeants. Thus, the impermeant nature of UW is both necessary and sufficient for complete islet purification from acinar tissue.