CONSISTENT and uniform isolation of pancreatic islets by collagenase digestion is a prime requirement for islet transplant therapy. Numerous factors contribute to the poor and inconsistent yields that characterize the current state of islet isolation. The existing methodologies permit isolation of 200 to 400 islets from a single mouse pancreas and 600 to 800 from a single rat pancreas. The extreme variability in activity and purity of collagenase preparations represents a major obstacle to successful high yield isolation of islets. The problem is further compounded by the variable degree of activation of native proteolytic enzymes within acinar cells that occurs during digestion and contributes to the variability of the results reported. Therefore, we tested the hypothesis that yield and consistency of islet isolation could be improved by modification of collagenase digestion mixture by addition of soyabean trypsin inhibitor (STI) and bovine serum albumen (BSA) and culturing the resultant cell suspension at 37°C for 48 hours in appropriate nutrient medium prior to islet separation to allow acinar cell disintegration and depletion.

MATERIALS AND METHODS

Four- to six-week-old Balb/c mice of either sex were obtained from the inbred colony maintained at the Animal House Facility NCCS, Pune, India.

Islet Isolation

Groups of two to four mice were killed by cervical dislocation, and pancreata were removed aseptically without ductal injection and distension. The pancreata were cut into small pieces and washed thrice with HBSS. The resulting pieces were subjected to collagenase digestion using a magnetic stirrer. The dissociation medium consisted of Dulbecco’s modified minimum essential medium (DMEM) supplemented with 1 mg/mL collagenase type V (Sigma), 2 mg/mL STI (Sigma), and 2% BSA fraction V (Sigma). The tissue digest was then transferred to 50 ml conical flask, and digestion was stopped by addition of chilled DMEM with 10% fetal calf serum. The mixture was then centrifuged at 1000 rpm for 10 min. Then it was vortexed, and the pellet was seeded in culture flasks (25 cm² Nunc, Denmark) containing RPMI-1640 (GIBCO), pH-7.2 supplemented with 10% volume/volume fetal calf serum (GIBCO). The flasks were incubated at 37°C for 48 hours in appropriate nutrient medium prior to islet separation to allow acinar cell disintegration and depletion.

Assessment of Islet Viability

Islet viability was assessed by Trypan Blue Dye Exclusion Test and specificity of islets was determined by dithiozone (DTZ) staining (Sigma, St. Louis, Mo). Dithiozone stock solution (39 mmol/L was prepared by dissolving 100 mg of DTZ in 10 mL DMSO, filtered, aliquoted, and stored at -15°C. Routine staining was carried out by adding 10 µL DTZ stock to islets suspended in 1 mL Krebs-Ringer Bicarbonate buffer (pH 7.4) with HEPES (10 mmol/L) and incubated at 37°C for 10 to 15 minutes. The stained islets counted under a inverted microscope (Olympus, Japan).

Insulin Release Assay

Triplicate groups of 10 islets each were placed in a single well of a 24-well plate (Nunc Denmark) each containing 1 mL Krebs-Ringer bicarbonate buffer (pH 7.4) supplemented with 10 mmol/L HEPES and 1 mg/mL BSA (hereafter referred as KRBH). The plates were incubated at 37°C in a CO2 incubator for 1 hr. The supernatant was collected and stored at -20°C and assayed for basal insulin level. The islets were then fed with KRBH supplemented with 16 mM glucose and incubated for a further period of 1 hour. The super-

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natant was collected and stored at −20°C. The insulin concentration of all the stored samples was determined by radio immuno assay (RIA) using the RIAK-1 kit from BRIT, Bombay, India.

Statistical Analysis
Results are expressed as mean ± standard error of the mean. The statistical significance of differences among groups was analyzed using Student’s t-test.

RESULTS AND DISCUSSION
The islet yield by normal collagenase digestion was 275.35 ± 58.98 islets per pancreas with a range of 120 to 358 (n=20) in group I and 1348.55 ± 128.22 islets per pancreas with a range of 913 to 1450 (n=27) in group II with a modified dissociation medium containing collagenase with STI and BSA (Fig 1). More or less equal number of islets were isolated from the pancreas of male and female mice (Fig 2), indicating utility of technique in both the sexes. Islets in both the groups responded well in vitro to insulin release with high glucose stimulation. (Table 1). The basal and glucose-stimulated insulin release in group I was 85 ± 6 μU/10 islets and 275 ± 42, respectively. In group II it was 93 ± 11 and 252 ± 22 μU/10 islets, respectively, and thus was comparable in the two groups. Viability as judged by trypan blue was above 90% in both the groups. Islets were also stained by DTZ, indicating their specificity and identity. Thus it is evident from the results obtained that addition of STI and BSA to collagenase containing dissociation medium significantly increased the islet yield from 358 to 1450. Improvement in the islet yield from rat pancreas has been reported due to protective action of BSA against warm ischemic injury.\(^6\) Similarly, addition of STI to dissociation medium probably helps in trypsin inactivation. Islet culture for 48 hours before islet separation permitted acinar cell disintegration and depletion resulting in less damage to islets and improving islet yield.\(^9\) These results clearly demonstrate that more than 1400 viable and functional islets can be consistently recovered from a single murine pancreas. However, the present technique has a limitation of 48-hour culture period prior to islet separation from acinar cells unlike the routinely used islet isolation procedures wherein islets are obtained on the same day of islet isolation. It would be a matter of choice to opt for a high yield within longer time or low yield within short time.

CONCLUSION
The method of islet isolation described here is simple and economical and yields far more islets per mouse pancreas.

### Table 1. Insulin Release From the Islets Isolated by Two Techniques

<table>
<thead>
<tr>
<th>Glucose Concentration</th>
<th>Routine Technique</th>
<th>Modified Technique</th>
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<tbody>
<tr>
<td>Basal (5.5 mmol/L)</td>
<td>85 ± 6</td>
<td>93 ± 11</td>
</tr>
<tr>
<td>Stimulated (16 mmol/L)</td>
<td>275 ± 42</td>
<td>252 ± 22</td>
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</tbody>
</table>

Data represent mean ± SD.
than those required for transplantation to reverse diabetes in mice irrespective of the sex of donor mice.

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