Small Bowel Preservation
Comparison of Perfusion and Nonperfusion Systems
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Twenty-four canine small bowels were used to compare the different perfusion and nonperfusion systems previously studied in kidney preservation. Four systems were evaluated: (a) two pulsatile perfusion systems (Mox-100 and Mox-300), (b) a nonperfusion technique (Collins method), and (c) a combined method (Collins method for 6 hours followed by pulsatile perfusion for 18 hours). Final evaluation was determined by restoration of function in long-term (>20 days) small bowel transplant survivors. All the dogs receiving an allotransplant of bowels continuously perfused were long-term survivors. Causes of death were related to cachexia, obstruction, or rejection. The animals receiving nonperfused bowels developed hemorrhagic necrosis and died 2.8 ± 1.2 days after transplantation.

At the present time there is no small bowel preservation technique suitable for clinical application and the literature offers conflicting data on the experimental systems already used. The development of systems for extracorporeal preservation of the small bowel will permit the utilization of this organ for future cadaver transplantation. Therefore, we have made several experimental attempts to obtain a reliable comparison of the different perfusion and nonperfusion systems previously studied in kidney preservation.

Material and Methods
Experimental Model.—Twenty-four canine small bowels preserved for 24 hours were divided in four groups of six small bowels each. Two commercially available pulsatile perfusion systems (group 1, Mox-100 and group 2, Mox-300)12 were compared with each other and with a nonperfusion technique (group 3, Collins method).1 Group 4 utilized a combination of six hours hypothermia at 4°C by the Collins method followed by 18 hours of pulsatile perfusion. Final evaluation was based on restoration of function in long-term (>20 days) small bowel transplant survivors.

Operative Technique.—Adult mongrel dogs of either sex weighing between 15 and 24 kg were anesthetized with methohexital sodium for induction and halothane for maintenance. The entire small bowel from the third portion of the duodenum to the ileocecal valve was resected. The superior mesenteric artery was cannulated and the small intestine of groups 1 and 2 was flushed with a cold (4°C) Ringer lactate solution containing heparin sodium (10,000 units/liter) and procaine hydrochloride (1 gm/liter) until the venous effluent was clear. The small bowel of groups 3 and 4 was flushed with 300 ml of C4 Collins solution (4°C) containing MgSO4, 50% glucose, methylprednisolone, and chlorpromazine. The small bowel was then preserved either by pulsatile perfusion (groups 1 and 2), nonperfusion (group 3), or a combined system (group 4).

Following preservation the entire small intestine was transplanted orthotopically into an unrelated dog, according to standard techniques.1 Neither the donor nor the recipient dogs were allowed to have food; they were given fluids orally for five days prior to surgery. Kanamycin sulfate, 500 mg, and neomycin sulfate, 1.0 gm, were administered orally three times a day for four days. Following transplantation, azathioprine 2.5 mg/kg/day was given intravenously until death. Hypertonic glucose (10%) and albumin (25 mg/100 ml) solutions were given intravenously each day for one week according to the clinical state of hydration. Chloramphenicol, 500 mg, was given intravenously every eight hours and kanamycin, 500 mg, was given intramuscularly every 12 hours for three days and then the dosage was decreased to once a day for one week. Oral feeding was begun by the seventh postoperative day.

All animals were examined daily until death. Values for hemoglobin, hematocrit, white blood cell count, electrolytes, total proteins, albumin, and globulins were determined every other day for one week and once a week thereafter. The xylose absorption test was performed at weekly intervals in all dogs according to standard techniques.10

Postmortem examinations were performed on all dogs.

Perfusion and Preservation Techniques.—The perfusion system (pH, 7.4; temperature, 7°C, oxygen pressure, 200 mm Hg; pulse rate, 60 beats per minute; and perfusion pressure, 60 mm Hg) was primed with 750 ml of cryoprecipitated plasma. The plasma was frozen, thawed, and filtered.14 Prior to use, MgSO4, insulin, phenolsulfonphthalein (PSP), kanamycin, ampicillin trihydrate (Polycillin), methylprednisolone, and chlorpromazine were added. Flow rate, perfusion pressure, vascular resistance, fluid loss, weight gain, enzymes in the perfusate (lactic dehydrogenase, serum glutamic oxaloacetic transaminase, β-glucuronidase), lactic acid, and oxygen consumption during perfusion were noted initially and at 2, 6, 12, 18, and 24 hours. Xylose (10 gm in 100 ml of water) was infused into the cannulated proximal portion of the duodenum immediately after the start of perfusion and then at 12 and 24 hours. Perfuse xylose samples were taken at 30-minute intervals following administration. Intraluminal small bowel excretion samples for determination of total protein, globulin, and electrolyte levels were collected every six hours. Small bowels from group 3 were transferred to the perfusion system at 12-hour intervals to obtain all perfusion determinations.

Statistical Analysis.—Survival was analyzed with the use of the x2 method. Mean ± standard error of the mean was determined in all preservation and postoperative results. Technical and preservation failures were eliminated from the results.

Results
Changes During Perfusion and Preservation.—There were no significant differences in flow rate, perfusion pressure, and vascular resistance among the four groups of bowels. The 24-hour fluid loss for groups 1 and 2 was 1.4 ± 0.2 liter, whereas for group 3 it was 0.6 ± 0.1 liter. The weight gain was 10 ± 2.8% from the control in groups 1 and 2 and group 3 had 7.5% ± 2.5% increase. Group 4 showed values of fluid loss and weight gain similar to those of the first two groups of bowels.

The enzyme values were slightly increased in group 3, which was not significant (P > 0.3) in comparison with groups 1 and 2. Similar results were obtained for sodium, potassium, lactic acid, and osmolality. Moderately high values of xylose were observed at the end of the study in groups 3 (10.5 ± 2.5 mg/100 ml) and 4

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Xylose absorption pattern posttransplantation. Pentose is plotted against time of sample collections. Weekly determinations reflect gradual recovery of intestinal function, obtaining low normal values three weeks after grafting. Normal curve was observed at four weeks posttransplantation.

(7.5 ± 1.2 mg/100 ml), and significant differences were observed between groups 1 and 3 (P<.05). The blood gas levels were maintained within normal limits. The oxygen consumption at 24 hours for the small intestine continuously perfused was 1.3 ± 0.07 ml/min/100 gm; however, significant differences were observed between groups 1 and 3 (P<.05).

Survival.—All six dogs in group 3 died within two days (2.8 ± 1.2 days). Hemorrhagic necrosis was the usual cause of death in these poorly perfused small bowels. Transplantation after 24 hours of perfusion in group 1 and group 2 resulted in viable transplanted small intestines (> 20 days survival). In group 1, dog survival reached 30 ± 4.5 days and in group 2, 25 ± 6.2 days. The main causes of death were cachexia, intestinal obstruction, and rejection. Initial six-hour Collins preservation followed by perfusion for 18 hours (group 4) showed survival of 20.5 ± 6.2 days. Causes of death were the same observed for groups 1 and 2.

It was found that groups 1 and 2 showed a return to normal small bowel function (xylose) within three weeks after transplantation (Figure), whereas group 4 small bowel function did not return to normal values. There were no recorded determinations in group 3 where all dogs died before any examination was performed.

Clinical differences between groups were not striking. Anorexia and diarrhea were commonly present in most of the dogs. The weight decreased gradually to values at three

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**Table: Selected Literature on Experimental Small Bowel Preservation**

<table>
<thead>
<tr>
<th>Author, yr</th>
<th>System</th>
<th>Perfusate</th>
<th>% Organ</th>
<th>Time, hr</th>
<th>Results</th>
<th>Implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lillehei et al (1959)</td>
<td>Hypothermia (5C)</td>
<td>None</td>
<td>Entire</td>
<td>5</td>
<td>Successful</td>
<td>Autotransplantation</td>
</tr>
<tr>
<td>Lyons et al (1965)</td>
<td>Hypothermia (4C) &amp; hyperbaric oxygen (7.9 atm)</td>
<td>None</td>
<td>Partial ileum (20 cm)</td>
<td>48</td>
<td>Functional study</td>
<td>No</td>
</tr>
<tr>
<td>Eyal et al (1965)</td>
<td>Hypothermia (4C), hyperbaric oxygen (7.9 atm) &amp; chlorpromazine</td>
<td>None</td>
<td>Partial ileum (25 cm)</td>
<td>48</td>
<td>Good (4-day survival)</td>
<td>Autotransplanted in neck</td>
</tr>
<tr>
<td>Austen &amp; McLaughlin (1965)</td>
<td>Normothermia pulsatile &amp; nonpulsatile</td>
<td>Whole blood</td>
<td>Partial ileum (5 feet)</td>
<td>18</td>
<td>Pulsatile; viable for 18 hr; nonpulsatile 6 hr</td>
<td>No</td>
</tr>
<tr>
<td>Iijima &amp; Salerno (1965)</td>
<td>Normothermia perfusion</td>
<td>Heparinized Ringer lactate blood</td>
<td>Entire</td>
<td>5</td>
<td>Successful</td>
<td>Autotransplantation</td>
</tr>
<tr>
<td>Kavan et al (1967)</td>
<td>Normothermic nonpulsatile</td>
<td>Heparinized bovine blood</td>
<td>Entire</td>
<td>3</td>
<td>Functional study</td>
<td>No</td>
</tr>
<tr>
<td>Hohenleitner &amp; Senior (1969)</td>
<td>Normothermic nonpulsatile perfusion</td>
<td>Erythrocyte solution PVP &amp; low-molecular-weight dextran</td>
<td>Entire</td>
<td>5</td>
<td>Functional study</td>
<td>No</td>
</tr>
<tr>
<td>Alican et al (1971)</td>
<td>Hypothermic (9C) pulsatile perfusion</td>
<td>Homologous serum</td>
<td>Entire</td>
<td>24</td>
<td>Inadequate storage; only few hr survival</td>
<td>Allotransplantation</td>
</tr>
<tr>
<td>Ruiz et al (1971)</td>
<td>Hypothermia (4C) &amp; hyperbaric oxygen (4 atm)</td>
<td>Whole blood</td>
<td>Entire</td>
<td>48</td>
<td>Beyond 12 hr no acceptable viability measurements</td>
<td>No</td>
</tr>
</tbody>
</table>

* PVP signifies polyvinyl pirrolidone.
weeks of 25% ± 5.8% from the control weight. No regain in weight was observed. Anemia and hypoproteinemia were constantly present. There were no other significant laboratory value differences.

**Comment**

The entire small bowel has been perfused successfully with heparinized Ringer lactate blood at normothermia during five hours.1 With pulsatile perfusion and whole blood at 37 C the intestine was kept viable in vitro for 18 hours.2 When nonpulsatile flow was used, the small bowel survived only six hours.3 In 1969, Hohenleitner and Senior4 perfused the dogs' entire small bowel with either crystalloidal solution or high-molecular-weight dextran for five hours. They obtained indirect signs of viability. Two years later, Alican and associates5 stored two canine small bowel allografts for 24 hours by continuous hypothermic homologous serum perfusion at 9 C. After orthotopic transplantation the recipients died within a few hours from loss of fluid, electrolytes, and protein. This phenomenon was interpreted as inadequate small bowel storage. A selected review of experimental small bowel preservation studies is presented in the Table.

During perfusion, the flow, pressure, vascular resistance, weight gain, and fluid loss were acceptable measurements of viability. Perfusate oxygen consumption and enzymatic changes were good indicators of viability during preservation also. These perfusion characteristics were confirmed with long-term transplant survivors. The association of hypothermia, oxygenation, stable pH, cryoprecipitated plasma, and pulsatile perfusion were the main factors in the results obtained with this method. The perfused groups yield a more consistent outcome than storage in electrolyte solution alone, at least when 24-hour preservation is used.

The use of combined methods of preservation did not improve survival when compared to the perfused bowel groups. Significant differences were noted, however, between the Collins group and the combined technique. These results suggest that initial pulsatile perfusion may be the most satisfactory means of prolonged preservation available even for transportation. Similar results have been reported by Santiago and associates,6 when they analyzed the same techniques for renal preservation.

Although the xylose tolerance absorption test showed consistent results, its ability to predict small bowel viability was questioned. This doubt was based mainly on the allowance for this pentose to circulate freely during perfusion. High concentrations of xylose might be the consequence of damage to the small intestine cell membrane and impairment of the active mechanism of transport for this pentose.

Hemorrhagic necrosis was a consistent finding in the poorly perfused bowels, while in the well-preserved organs there was no evidence of edema, hemorrhage, or ischemic areas after allografting. Long-term allograft survival was achieved in the successfully preserved bowels. However, the rejection process appeared several weeks after transplantation.

The results obtained with the perfused bowels gives an encouraging basis for further work in small bowel allotransplantation. The use of immunosuppressive drugs during perfusion may result in longer periods of small bowel survival and would permit the possible use of such a system for future clinical application.

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**Nonproprietary Names and Trademarks of Drugs**


**References**