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**Topics and Trends**

# Organ Preservation Injury in Small Bowel Transplantation

HITOSHI FUJIWARA, MD
SESHADRI RAJU, MD
JAMES B. GROGAN, PhD
W.W. JOHNSON, MD

Department of Surgery
University of Mississippi Medical Center
Jackson, MS 39216-4505

**Abstract** *Histological features of preservation injury were studied in a dog model of total small bowel transplantation. It was remarkable that substantial microscopic injury was evident during cold ischemia, unlike in other organ systems. This was early in onset and was related to the duration of cold storage. There was further progression of injury during reperfusion, as expected. Nevertheless, the small bowel was noted to have substantial ability to recover from this storage- and reperfusion-related injury. Histological features of damage and recovery are described in detail.*

**Keywords:** Small bowel transplantation, organ preservation, hypothermia.

We have previously reported successful 12-h preservation of the entire small bowel by simple hypothermia in a canine model.[1] After 24 h of hypothermic storage, long-term survival of allotransplants was achieved with cyclosporine immunosuppression. However, there was considerable dysfunction of the small bowel graft in these animals, which appeared to be storage related. In an effort to clarify this problem, a histological study of the graft before, during, and after cold storage was undertaken. The results are reported here.

## Materials and Methods

### *Animals*

Adult mongrel dogs weighing 13–25 kg were used. The following experimental groups were utilized: group 1 ($n = 6$), autotransplants, 60–80 min intraoperative cold ischemia; group 2 ($n = 7$), allotransplants, 60–80 min intraoperative cold ischemia; group 3 ($n = 7$), allotransplants, stored for 12 h ($n = 4$) or 24 h ($n = 3$);

All correspondence should be addressed to Seshadri Raju, MD, Department of Surgery, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216-4505.

group 4 ($n = 4$), allotransplants, stored for 24 h and observed histologically for 48 h after transplantation.

### *Technique*

Total orthotopic small bowel transplantation was performed. The technique has been described in detail previously.[1-3] Briefly, the small bowel graft was cooled gradually in situ in the donor, followed by simultaneous intravascular infusion and intraluminal irrigation. The infusate consisted of lactated Ringer's solution with heparin (5000 U/L) and the luminal irrigant consisted of lactated Ringer's solution with 0.5 g kanamycin/500 mL. The small bowel graft was stored at 0–2°C. Revascularization of the graft was achieved by end-to-end anastomosis with the superior mesenteric vessels. Intestinal continuity was achieved by end-to-end anastomosis of the jejunum and ileum at the upper and lower end, respectively. A 15-cm length of Thiry–Vella loop of proximal jejunum was isolated with intact blood supply and ends were exteriorized for monitoring purposes.

Immunosuppression for allotransplants (groups 2 and 3) consisted of intravenous cyclosporine with a starting dose of 16 mg/kg · day, tapered to 8 mg/kg · day by the eighth postoperative day. Prednisone (5 or 10 mg/day) was added to the regimen in group 2, but not until later in group 3.

### *Biopsy Protocol (Table 1)*

Microscopy specimens of jejunum were taken from group 1 (4 of 6) and group 4 during graft harvest, storage, and transplant procedure. Biopsy of "normal" small bowel ($n = 4$) was taken immediately after laparotomy. In nonstored transplants ($n = 4$), biopsies were taken after cold flush, before reperfusion, and 5 min, 60 (or 90) min, 6 h, 24 h, and 48 h after reperfusion. In stored transplants ($n = 4$), biopsies were taken at 15 min, 60 min, 3 h, 6 h, 12 h, and 24 h during cold storage, and 5 min, 60 min, 6 h, 24 h, and 48 h after reperfusion. From the rest of the animals, biopsies were taken from a Thiry–Vella loop on a daily basis after transplantation. All specimens were oriented and fixed with 10% formalin and stained with hematoxylin and eosin (H–E) for light microscopy.

**Table 1**
Experimental Groups and Timing of Biopsies

| Group | Type of Graft | *n* | Timing of Biopsies | |
|---|---|---|---|---|
| | | | Control and Cold Ischemia | Posttransplant |
| 1 | Autograft 60–80 min cold ischemia | 6 | Control, 15 min, 60 min | 5 min, 60–90 min, 6 h Days 1–5 |
| 2 | Allograft 60–80 min cold ischemia | 7 | | Days 1–5 |
| 3 | Allograft stored 12–24 h | 7 | | Days 2, 5 |
| 4 | Allograft stored 24 h | 4 | Control, 15 min, 60 min, 3 h, 6 h, 12 h, 24 h | 5 min, 60 min, 6 h Days 1, 2 |

## Results

Microscopic findings of graft mucosa were as follows.

Normal intestinal epithelium was intact with tall villi of regular size.

*Immediate Transplantation with 60–80 min of cold ischemia (group 1).* Biopsies taken immediately after the cold infusion procedure revealed interstitial edema with detachment of epithelium at the tip of the villi. After 60 min of cold ischemia, biopsies revealed more advanced damage with epithelial detachment to the top of crypts. Even more marked microscopic changes were evident after reperfusion. At 5 min after reperfusion, the villi had become completely denuded of epithelium ($n = 2$) or nearly so ($n = 2$) with moderate contraction of villi. Considerable congestion and hemorrhagic extravasation were seen. After 60–90 min of reperfusion, epithelial recovery was already in evidence. Biopsies taken up to 6 h of reperfusion revealed only minimal congestion with nearly complete reepithelialization. The epithelium, however, appeared to be fragile and easily detachable by minor trauma during biopsy procedure. At 24 h, the villi appeared to have recovered their length, and epithelialization was complete. At 48 h, the villi appeared near normal with lacteals remaining somewhat dilated, and some edema was still evident in the mucosal layer.

*Storage and transplantation with 24-h cold ischemia (group 4).* At 15 min of cold storage, interstitial edema had begun to be evident in the lamina propria. Tiny fluid spaces were seen beneath the epithelium at the upper part of the villi. After 60 min of cold storage, epithelial detachment was seen extending down to the base of villi and some superficial crypts. At 3 h of cold storage, the villi tended to become shorter with contracted smooth muscle. Little further change was seen in the crypts. At 6 h of cold storage, the villi appeared significantly shorter and lacteals were occluded. The space between detached epithelium and the villi became wider, presumably due to increased fluid accumulation. The upper half of the crypts tended to show detachment of epithelium at this time (Fig 1). At 12 h of cold storage, some villi were almost completely denuded of epithelium and this process had begun to involve the depth of the crypts (two-thirds in depth) including scattered deepithelialization in the deepest part of the crypts (Fig 2). At 24 h of cold storage, the villi tended to become longer again, presumably owing to relief of smooth muscle contraction. Detachment of epithelium in deeper crypts progressed further.

After 24 h of cold storage, reperfusion produced marked microscopic changes. After 5 min of reperfusion, the villi were very short with strong contraction of smooth muscle and were completely denuded of epithelium. Congestion with hemorrhagic extravasation was a prominent feature (Fig 3). Disorganization of crypt structures, especially at the upper two-thirds, was evident. At 60 min of reperfusion, the villi were still contracted with a sharp profile. At 6 h, there was no sign of epithelial recovery. However, at 24 h, epithelial recovery was evident with flat or short villi almost completely covered with an epithelial layer. Cuboidal cells were prominent. There was a polymorphonuclear cell infiltration in the lamina propria, submucosa, and muscle layers. At 48 h of reperfusion, the villi had not yet recovered their length and the epithelium (mostly cuboidal cells) was still fragile. The height of the crypts was still less than normal and the structures remained grossly irregular (Fig 4). Polymorphonuclear cell infiltration in the

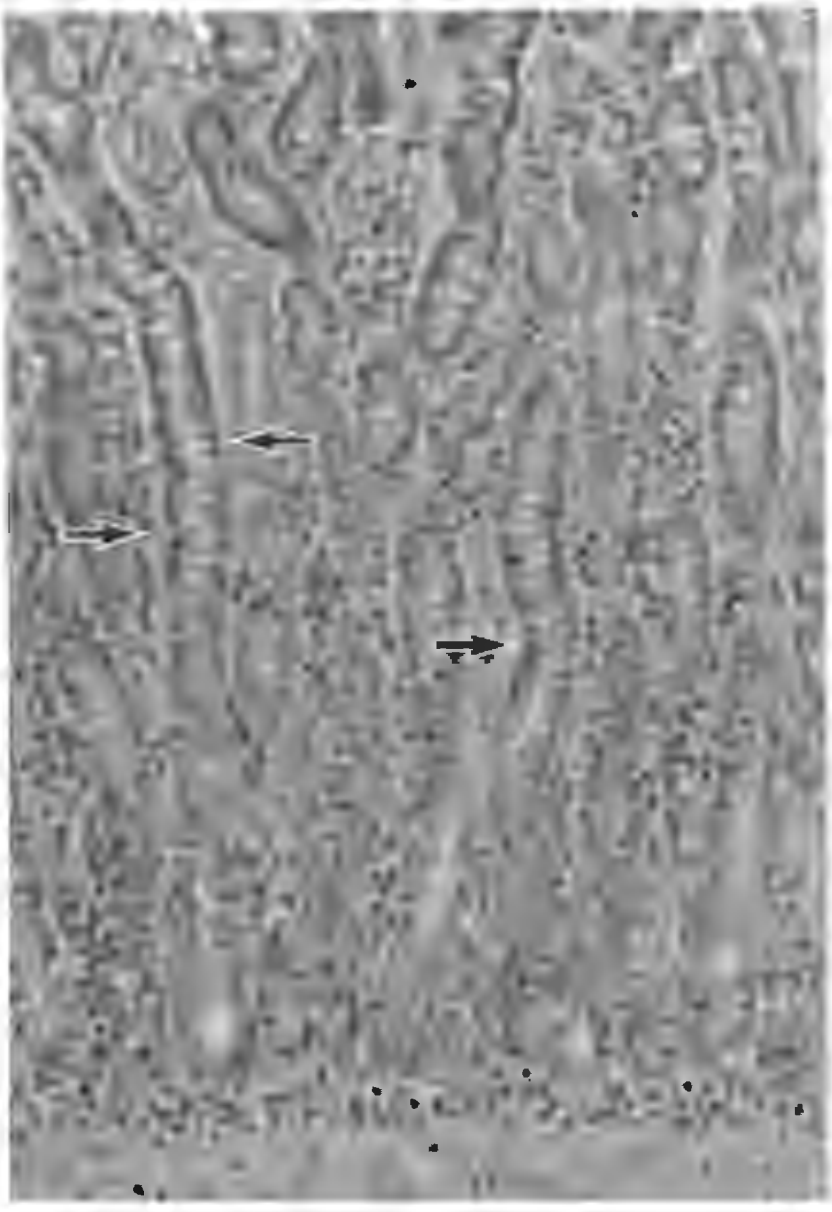

**Figure 1.** Jejunum at 6 h during cold storage. Detachment of epithelial cells is seen down to half the crypt depth. Arrows show the deepest level of epithelial cell detachment.

villi persisted and karyorrhectic nuclear debris was scattered in the mucosa. Lacteals were dilated. The recovery of mucosal architecture was significantly delayed compared with nonstored grafts.

*Recovery of mucosal structure (groups 1–3).* Serial mucosal biopsies up to 5

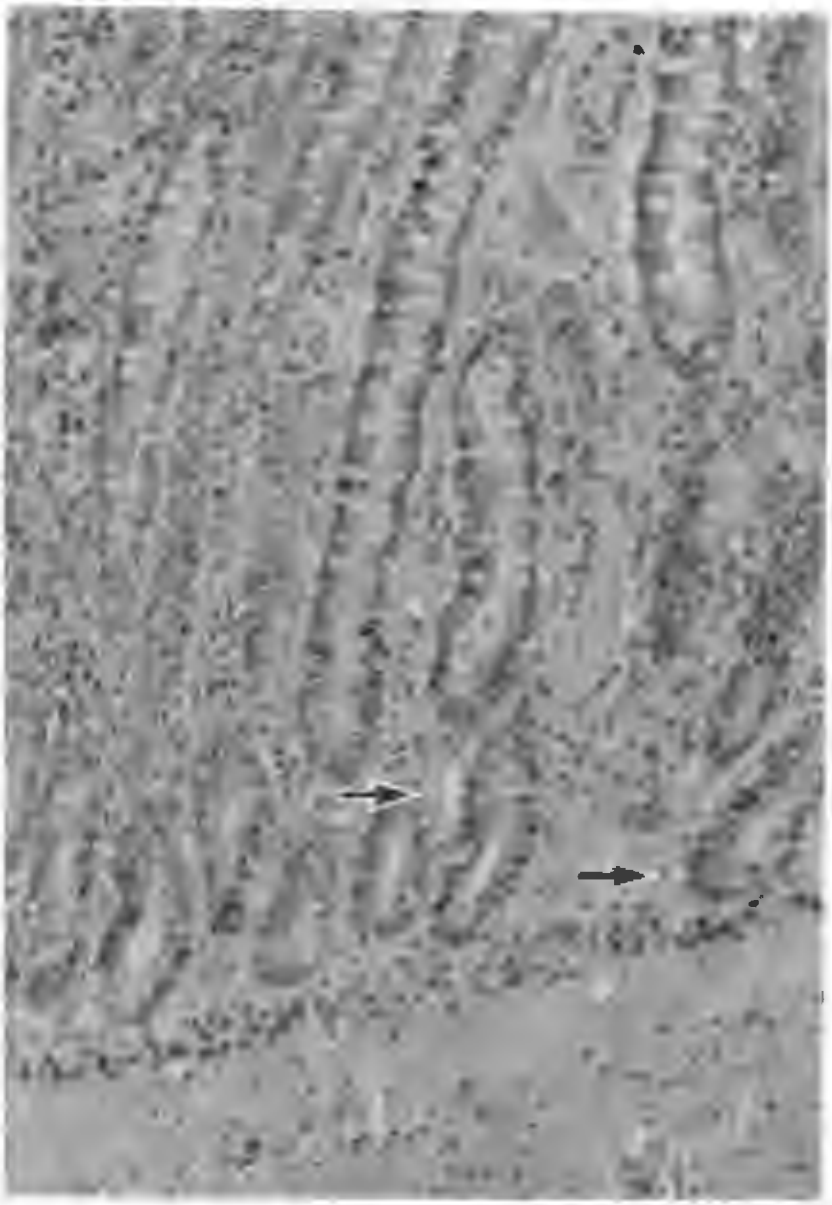

**Figure 2.** Jejunum at 12 h during cold storage. Scattered detachment of epithelial cells (arrows) at deep portions of crypts can be seen.

days from 3 groups (groups 1–3) showed earlier recovery of the length of the villi in nonstored grafts than in stored grafts (Table 2). The trend of recovery of nonstored grafts was similar between auto- and allotransplants before the fifth day. Shorter appearance of villi in allografts on the fifth day (group 2) may be due to

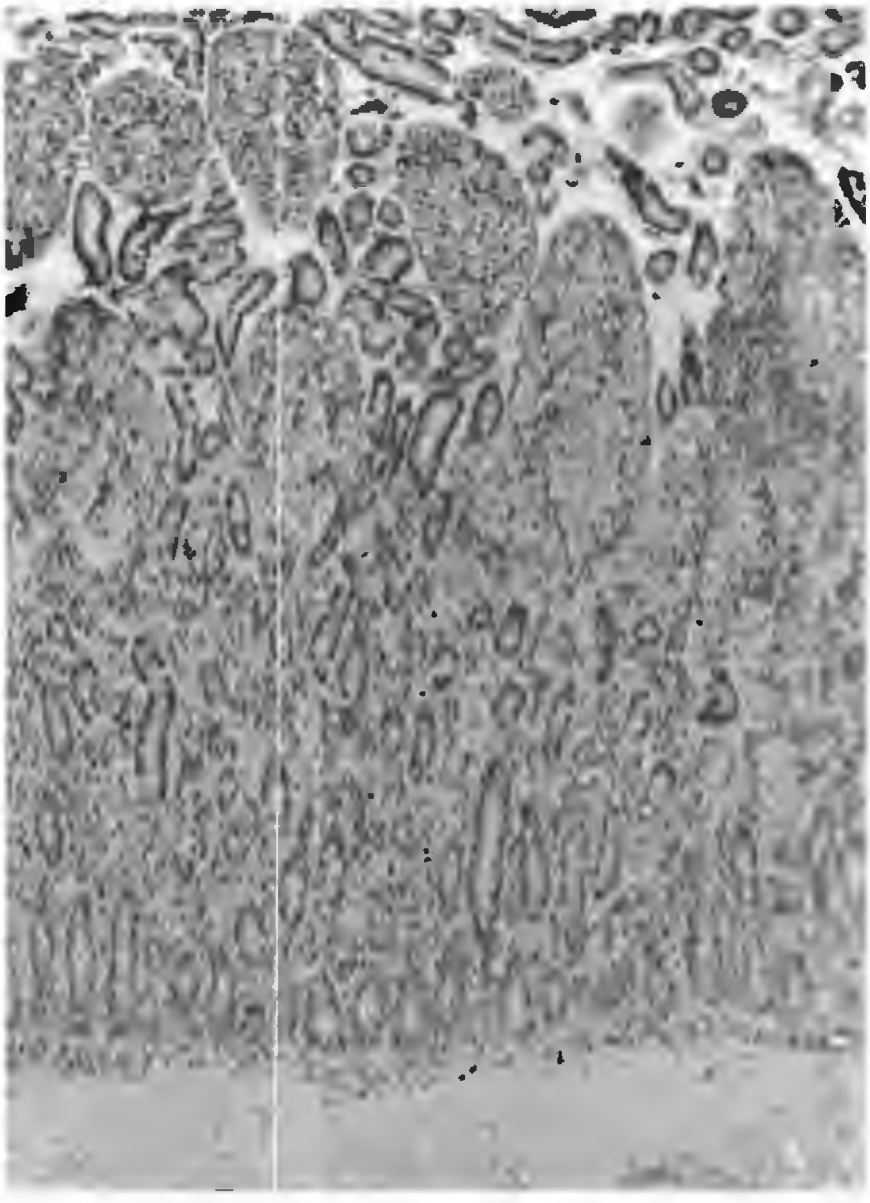

**Figure 3.** Jejunum 5 min after reperfusion following 24 h cold storage. The upper half of crypt structure is disorganized. There is significant extravasation into both the lamina propria and bowel lumen.

immunological reaction. In stored grafts (group 3) already evincing storage injury, recovery of the villi architecture was presumably jeopardized further by the onset of immunological reaction. Interstitial edema and dilatation of lacteals were common findings among all three groups during the observation period.

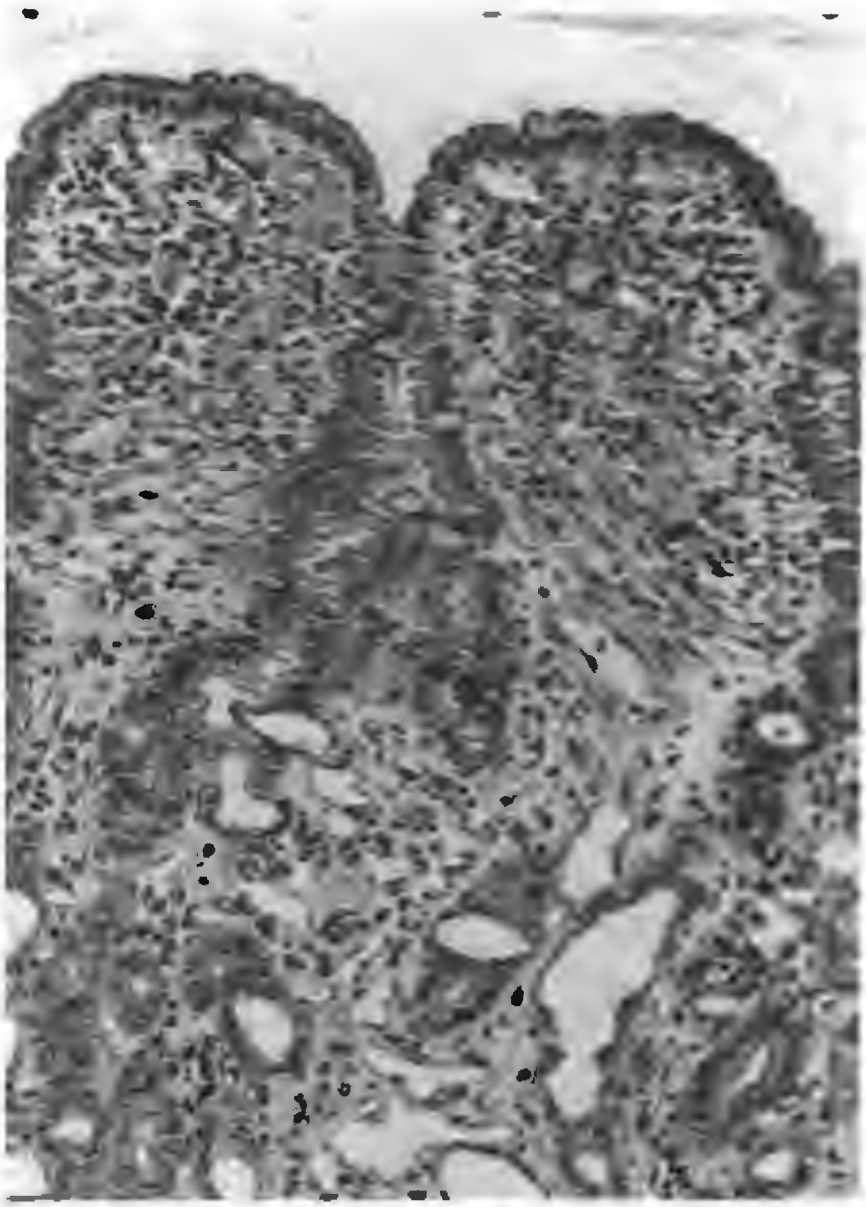

**Figure 4.** (*a*) Jejunum (group 4) 48 h after reperfusion (transplantation) following 24 h cold storage. Significant polymorphonuclear cell infiltration is seen in the lamina propria. Cuboidal cells are predominant on the very short villi. Crypt structure is grossly abnormal.

## Discussion

Successful simple cold storage of the entire small bowel was reliably achieved up to 12 h and less successfully up to 24 h in our laboratory.[1] Early studies have shown that storage beyond 4–6 h requires adjuvants besides cold, such as meta-

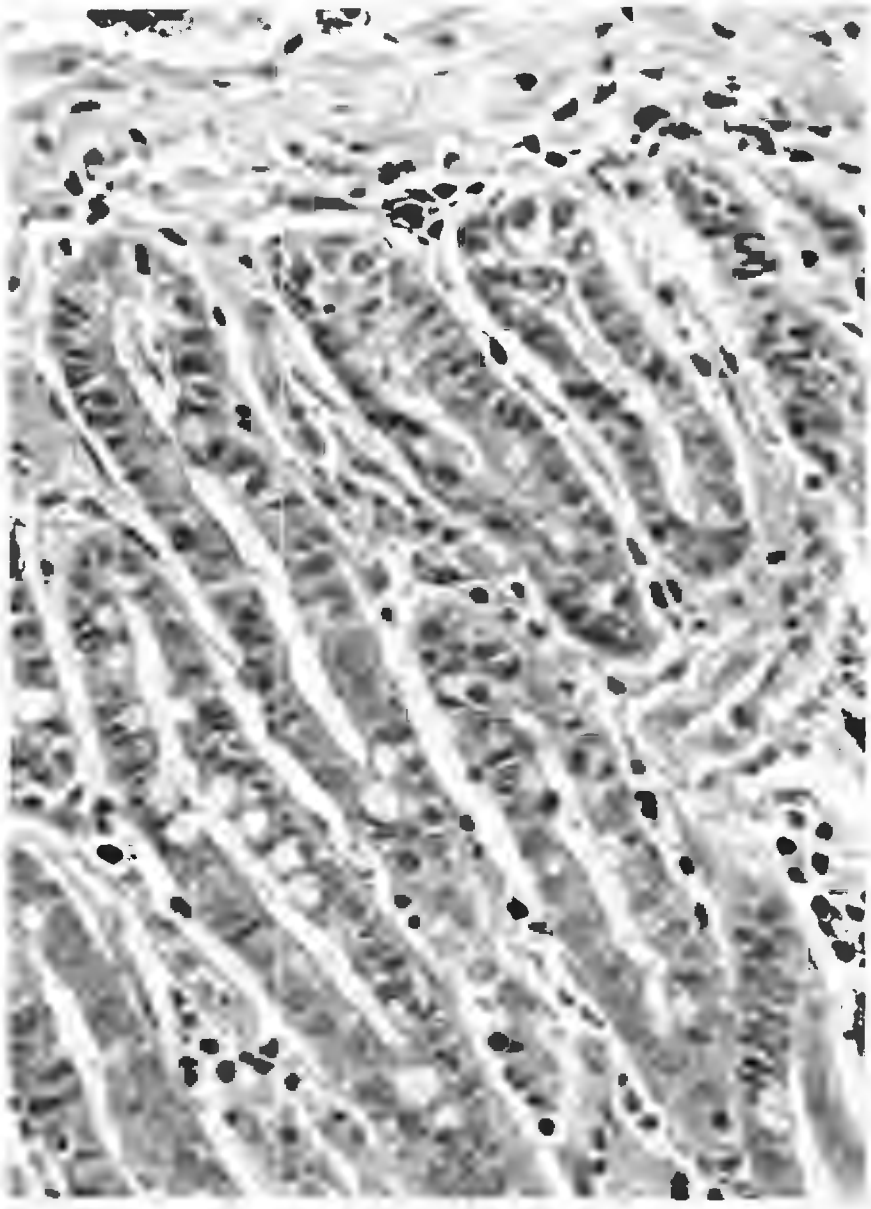

**Figure 4.** (*Continued*) (*b*) Jejunum (group 1) 48 h after reperfusion (transplantation) following 60–80 min cold ischemia. Note nearly complete recovery of crypt structure.

bolic inhibitors, hyperbaric oxygen, or continuous perfusion technique.[4–6] Elements of our techniques such as antibiotic pretreatment of donor intestine, the in situ flush procedure, and immediate intraluminal irrigation before storage were thought to be responsible for our successful simple hypothermic storage by reducing perfusion endotoxemia and other infectious complications, as well as enzymatic damage, to the unperfused bowel during storage.[7–9]

**Table 2**
Number of Animals in Which Tall Villi Were Observed in Postoperative Biopsy

| | Postoperative Day | | | | |
|---|---|---|---|---|---|
| Group | 1 | 2 | 3 | 4 | 5 |
| 1 | 2/6 | 5/6 | 5/6 | 6/6 | 6/6 |
| 2 | 3/5 | 5/5 | 3/4 | 5/5 | 1/4 |
| 3 | — | 1/7 | — | — | 0/7 |

A peculiar feature of bowel storage is the selective and progressive destruction of functioning structures with increasing duration of ischemia despite adequate cold storage. With onset of intestinal ischemia, epithelial cells are initially lost only from the villi. Infranuclear fluid accumulation with bulging of plasma and basement membrane occurs within 10 min of ischemia.[10] Epithelial cells are detached from villi with intact tight junctions. The resulting space (Gruenhagen's space) progresses from the tip of the villi downward to the crypts with increasing ischemia time. The mechanism for this epithelial desquamation is unknown.

As shown in our study, the crypts were generally spared epithelial damage with limited cold ischemia time. On the other hand, epithelial desquamation associated with structural disorganization was quite severe on reperfusion in the stored grafts. In either case, the extent of reperfusion damage was proportional to the extent of cold ischemic injury in general (Table 3). The mechanism of reperfusion injury has been elucidated recently by Granger et al[11] and Grøgaard et al.[12] The presence of polymorphonuclear cell infiltration and karyorrhectic debris indicated severity of injury in stored grafts. Submucosa and muscle layers appeared relatively resistant to ischemic injury and remained intact even in the presence of extensive mucosal loss.

**Table 3**
Aspects of Ischemic Injury in Unstored (60–80 min Cold Ischemia) and Stored (24 h) Bowel

| Stage | 60–80 min Intraoperative Cold Ischemia | 24 h Cold Storage |
|---|---|---|
| Pretransplant at end of ischemia | Longer villi | Contracted villi |
| | Minimal change in the crypt epithelium | Detachment of deeper crypt epithelium |
| Posttransplant 0–6 h | Moderate contraction of villi | Strong contraction of villi |
| | Minimal change in the crypt structure | Disorganization of the crypt structure |
| | Earlier reepithelialization | No sign of early regeneration of epithelium |
| Posttransplant 1–2 postop days | Complete epithelialization | Incomplete epithelialization |
| | Longer villi | Very short, often deformed villi |
| | Few polymorphonuclear cells | Polymorphonuclear cells |
| | No karyorrhectic debris | Karyorrhectic debris |
| | Normal crypt structure | Decreased length of crypt structure |

Reepithelialization and healing from mucosal injury are dependent on the remaining epithelial structures in the crypts.[13] Also, the time required for healing is proportional to the extent of original ischemic and reperfusion injury. In our transplantation experiments in which cold ischemia time was 60 to 80 min, complete reepithelialization had occurred by 24 h and the villi had regained the preoperative height by 48 h. In the storage group, however, restoration of villous height was not achieved before the fifth postoperative day. Recovering mucosal epithelium remained predominantly cuboidal without generating goblet cells for several days after transplantation. Even though long-term survivors after allotransplantation with stored grafts[1] showed near normal histology after a time, the gut function was inferior compared with nonstored allotransplants. This suggests the existence of irreversible functional injury in allografts damaged by severe storage and reperfusion injury.

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