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The significance of cytological examination on reperfusion in rat small intestinal transplantation

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Abstract We examined the cytology of the exudate in preserved intestinal grafts on reperfusion and compared it with the histological findings in rat small intestinal transplantation. The jejunal graft was harvested from the Lewis rat and was preserved in University of Wisconsin solution for 6, 12, 24 and 48 h at 4 °C (n=6, in each group) and was then syngeneically transplanted. On reperfusion, the exudate was collected and studied cytologically. Full thickness biopsies were performed at the end of the preservation and at 30 min after reperfusion for histological examination. Histological examination after reperfusion showed that the crypt layer was preserved until 24 h. However, it was destroyed by 48 h preservation. The cytological findings correlated with the depth of tissue injury shown histologically. The degeneration of villus epithelial cells, the decrease in the content of mucin in both the goblet cells as well as villus cells, and the appearance of crypt cells are all considered to be signs of poor graft viability. Cytological examination is therefore recommended as an effective, non-invasive and real-time method for evaluating graft viability just after reperfusion in small intestinal transplantation.

Key words Small intestinal transplantation · Graft viability · Cytology · Preservation · Crypt cell

Introduction

Small bowel transplantation has been successfully performed in various animal models and recently in humans using powerful immunosuppressive agents [4, 7, 9, 23]. The small bowel is one of the most susceptible organs to ischemic and reperfusion injury because it contains a large amount of xanthine dehydrogenase-oxidase [2]. In order to be able to procure organs from a wider area, the establishment of a method for simple cold storage as well as the determination of parameters for estimating graft viability are considered to be important. We previously reported that the rat jejunum could be preserved in good physiological condition for up to 24 h before transplantation using simple cold storage in University of Wisconsin solution (UW) [21]. There are several reports concerning effective organ preservation techniques [11, 19, 24] and parameters for graft viability [5, 6, 10, 15, 16], but no useful real-time methods to assess graft viability have yet been established. The mucosal layer of the intestine is the area most susceptible to damage during ischemia [3]. On reperfusion, part of the mucosal layer is denuded due to ischemia and reperfusion injury and turns into exudate. We examined the properties of such exudate in the graft just after reperfusion cytologically, and then compared the results with the histological findings, in order to identify potential variables for the evaluation of graft viability after reperfusion.

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Materials and methods

Male Lewis rats, weighing from 250 g to 350 g, were fasted overnight and then anesthetized by an intrapertioneal injection of sodium pentobarbital (40 mg/kg body weight) and atropine sulphate (0.1 mg/kg body weight). Chloral hydrate (160 mg/kg body weight) was added by intravenous injection when necessary. The operation was done according to the modified Monchik and Russell method [14, 20]. Briefly, under operative microscopy, a 25 cm segment of the jejunum was isolated on a pedicle of the aorta and portal vein from the donor. The intestinal lumen was cleaned with lactated Ringer's solution (LR) containing 1 mg/ml amikacin sulphate, and the aorta of the graft was flushed with iced LR containing 10 U/ml heparin. Both the lumen and artery of the graft were subsequently flushed with cooled UW (which was purchased from DuPont Pharmaceuticals (Wilmington, Delaware, USA)), and then it was stored in UW for 6, 12, 24 and 48 h at 4 °C (n=6, in each group). The intestinal isograft was revascularized by performing end-to-side anastomoses of the aorta and portal vein to the recipient's abdominal aorta and inferior vena cava. Both ends of the graft were exteriorized through the abdominal wall. After reperfusion, the graft lumen was filled with the exudate, and then the exudate was flushed out by saline and smeared onto a glass slide. Three kinds of staining were performed; Papanicolaou (PAP) [17], periodic acid-Schiff (PAS) [13] and Gi-

Table 1 The morphological criteria to differentiate between villus epithelial cells and crypt cells

	Villus epithelial cells	Crypt cells
Structure	Sheet-like pattern Goblet cell intermingled	Rossette pattern Goblet cell (–)
Cytoplasm	Relatively large cytoplasm	Large nuclear: cytoplasmic ratio
Mucin	Marginal mucin (+)	Marginal mucin (–)
Microvilli	+	–
Nucleus	Fine granular	Coarse

emsa [1]. The criteria to identify the villus epithelial cells, the crypt cells and the goblet cells was based on morphology. The goblet cells were easily identified by their large content of mucin shown by PAP and PAS. The morphological characteristics of villus epithelial cells and crypt cells are shown in Table 1.

Full thickness biopsies were done at the end of the preservation and at 30 min after reperfusion for the histological examination using haematoxylin and eosin (H & E) staining. In order to quantitate the extent of tissue injury, the histological findings were evaluated by Park's grading system [18]. Briefly, these are grade 0, normal mucosa; grade 1, subepithelial space; grade 2, extended subepithelial space; grade 3, epithelial lifting along villus side; grade 4, denuded villi; grade 5, loss of villus tissue; grade 6, crypt layer infarction; grade 7, transmucosal infarction; grade 8, transmural infarction. According to this grading system, the crypt layer was damaged at a level of grade 6 or more.

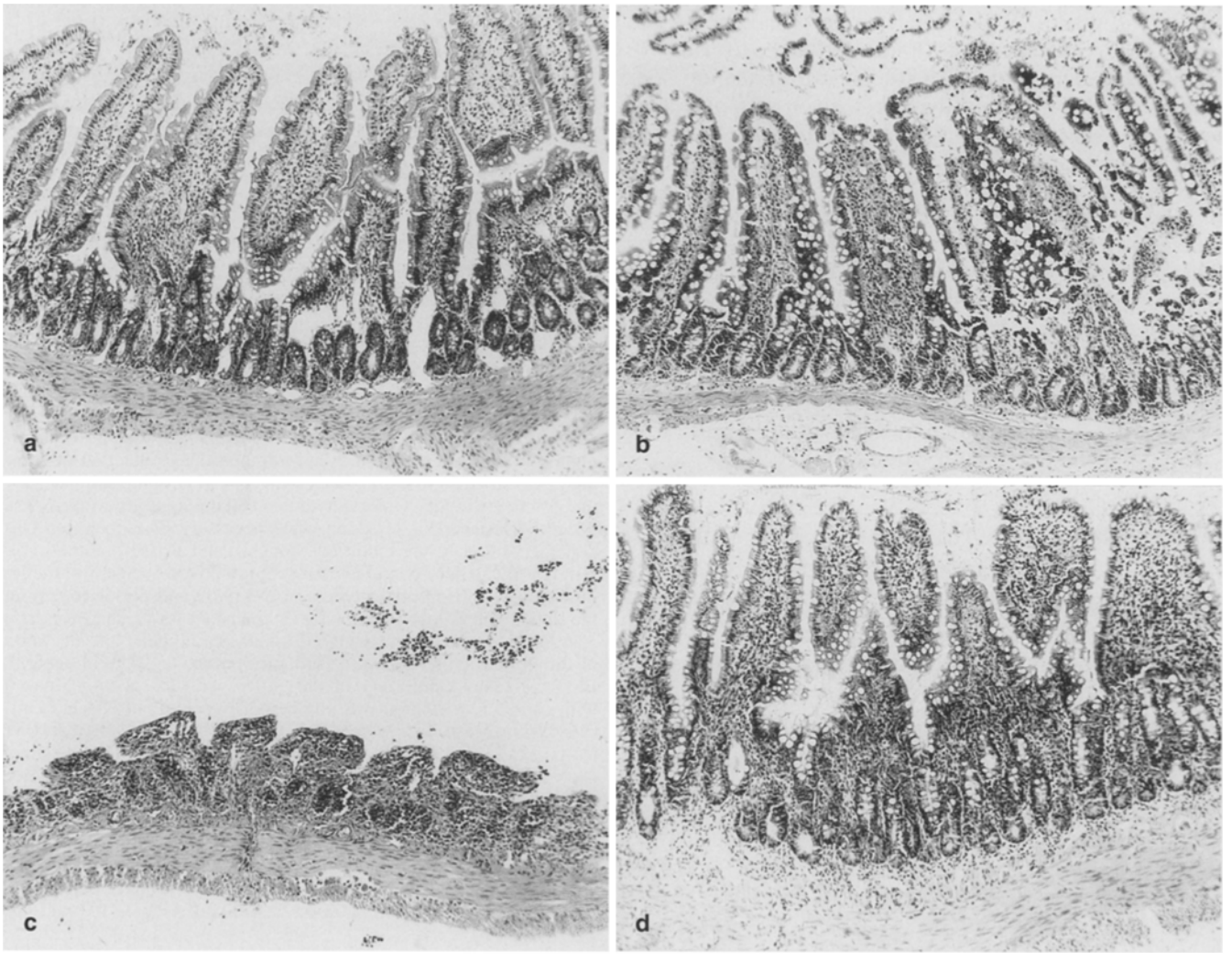
The principles of laboratory animal care (National Institutes of Health publication number 85-23, revised 1985) were adhered to in this experiment.

Results

Histology

The extent of histological injury was slight at the end of cold storage, while it extended deeply after reperfusion. Figure 1 shows the changes in the histological findings of a 24 h preserved graft. There was no histological damage on harvesting (Fig. 1a). The subepithelial space was

Fig. 1a–d Histological findings of a 24 h preserved graft [haematoxylin and eosin (H & E) stain, ×80]. Histological grading is estimated by Park's criteria [17]. **a** On harvesting; grade 0. **b** At the end of cold preservation; grade 2. **c** After reperfusion; grade 5. **d** Ten days after transplantation; grade 0



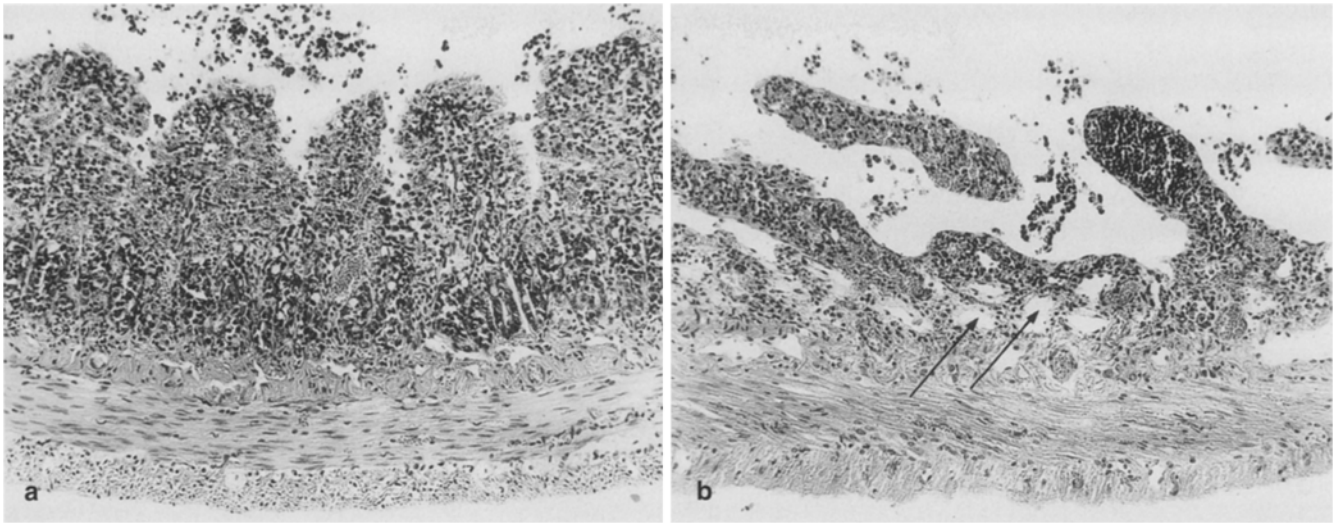


Fig. 2a, b The histological findings after reperfusion (H & E stain, ×140). **a** A 24 h preserved graft: the crypt layer is well preserved. **b** A 48 h preserved graft: the crypt layer is completely destroyed. *Arrows* show the denuded crypts

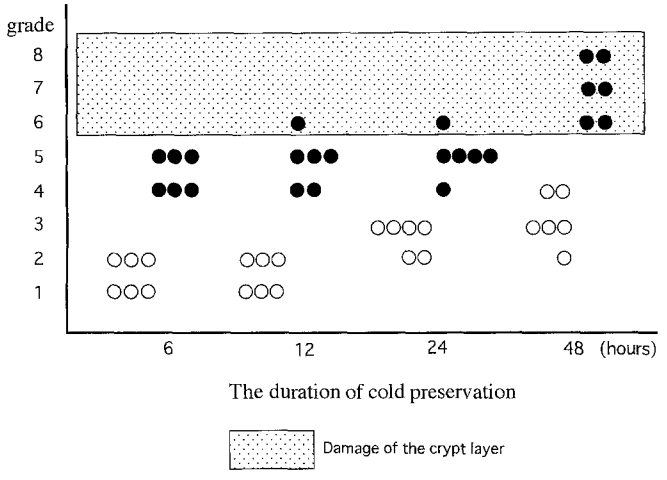
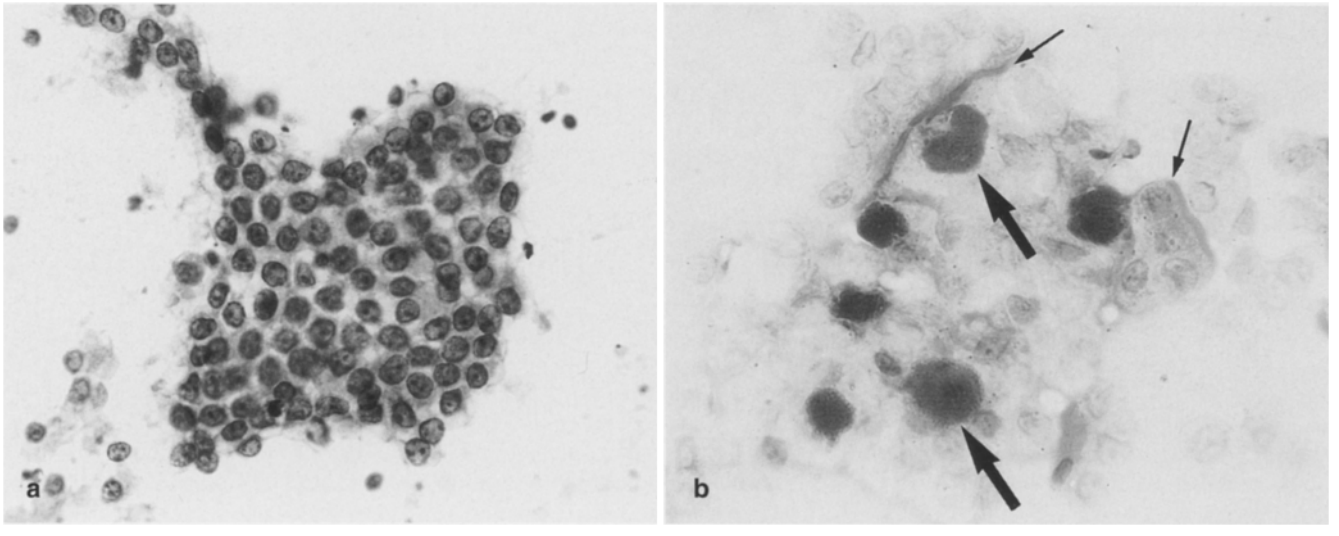


Fig. 3 The histological grading of the graft at the end of cold preservation (○) and at 30 min after reperfusion (●)

seen at the end of cold preservation (Fig. 1b). After reperfusion, the villi were almost completely denuded, while the crypt layer was preserved (Fig. 1c). These denuded cells turned into exudate on reperfusion and thereafter were examined cytologically. The villi regenerated and returned almost to normal after 10 days of transplantation (Fig. 1d). The histological injuries at the end of cold storage of 6, 12, 24 and 48 h ranged between grade 1 and 2, grade 1 and 2, grade 2 and 3, and grade 2 and 4, respectively. The crypt layer was preserved even after 48 h of cold preservation. The histological damage extended

Fig. 4a, b The cytology of a 6 h preserved graft. **a** Clusters of viable villus epithelial cells show a sheet like appearance [Papanicolaou (PAP) stain ×600]. **b** Marginal mucin (*small arrow*), which represents the microvillus structures, is found on the surface of the villus epithelial cells. Goblet cells (*large arrow*) rich in mucin are intermingled in the clusters of villus epithelial cells (periodic acid-Schiff stain ×600)



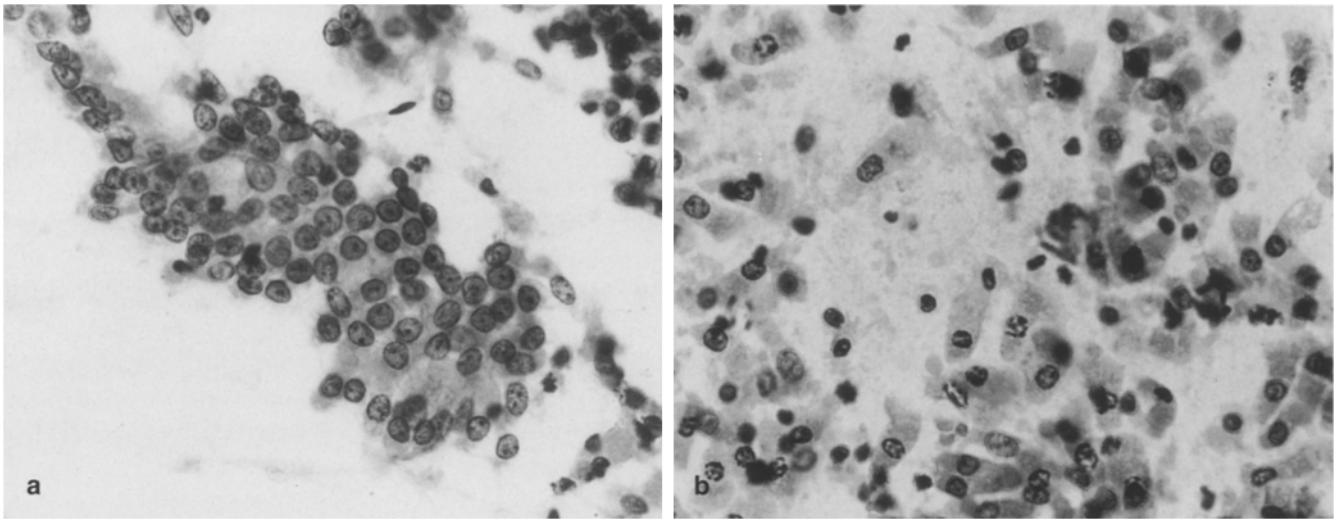


Fig. 5a, b The cytology of a 24 h preserved graft. **a** Some of the villus epithelial cells are viable (PAP stain ×600). **b** The villus epithelial cells are observed to have degenerated in other areas (PAP stain ×600)

deeply after reperfusion. The crypt cells were preserved until 24 h, while they were destroyed in the 48 h group (Fig. 2). The histological grades at both the end of cold preservation and after reperfusion are summarized in Figure 3.

Cytology

The shape of the nucleus and the cytosol was well shown by PAP staining, while PAS staining clearly demonstrated the distribution and the property of the mucin. Giemsa staining was not appropriate to examine the properties of the mucosal epithelial cells in the intestine.

The results of the cytological examination in the 6 h preservation group was as follows: viable villus epithelial cells were seen with fine granular nuclei, and demonstrated a sheet-like appearance in PAP staining (Fig. 4a). The marginal mucin of the epithelial cells was well preserved and goblet cells rich in mucin intermingled in the sheets of epithelial cells in PAS staining (Fig. 4b). In the 12 h preservation group, the villus epithelial cells became partially degenerate. However, the sheet-like pattern was preserved. The mucin structure of the epithelium and goblet cells also remained. In the 24 h preservation group, a large proportion of the villus epithelial cells were observed to have degenerated. However, the villous structures still remained in some areas (Fig. 5). The amount of marginal mucin in the epithelial cells and mucin in the goblet cells decreased. Clusters of crypt cells were seen in one of six rats in this group. The characteristics of the crypt cells included a large nuclear: cytoplasmic ratio, coarse nuclei and the formation of rossette

Fig. 6a, b The cytology of a 48 h preserved graft. **a** Most of the villus cells and goblet cells are necrotic. Clusters of crypt cells (*arrows*) are dominant (PAP stain ×300). **b** The crypt cells show a large nuclear: cytoplasmic ratio, coarse nuclei and form rossette structures (PAP stain ×600)

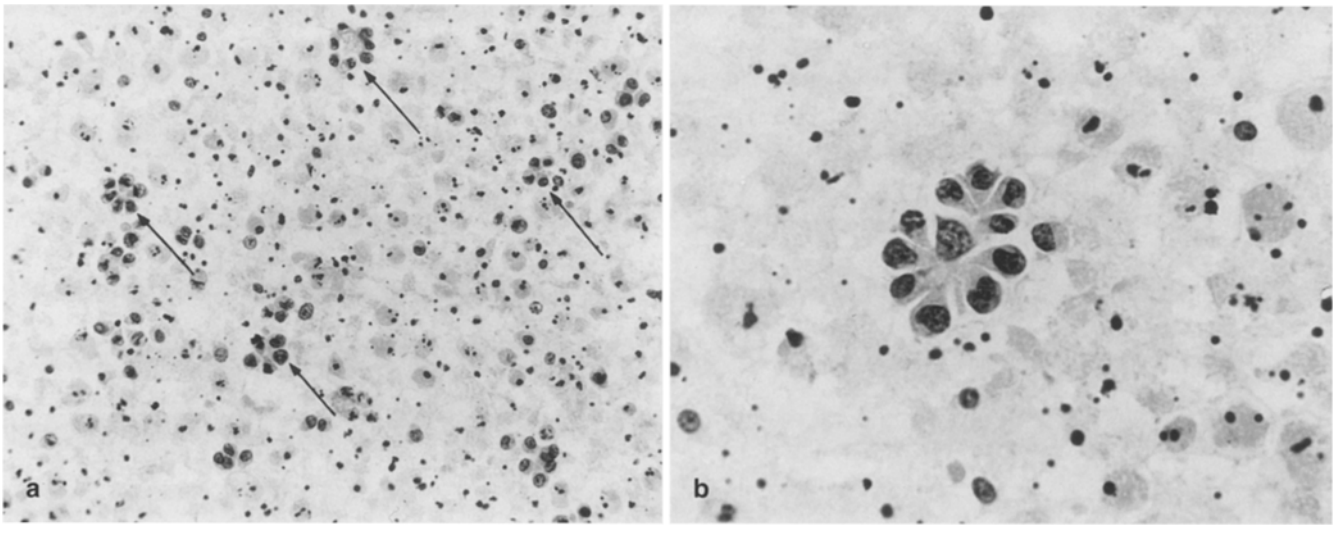


Table 2 The cytological findings of exudate on reperfusion after various periods of cold preservation in University of Wisconsin solution

Findings	Time (h)			
	6	12	24	48
Villus epithelial cells:				
sheet-like arrangement	++	++	+	–
marginal mucus (microvilli)	++	++	+	–
mucus of the goblet cells	++	++	+	±
degeneration of the nucleus	–	±	+	++
Presence of crypt cells	–	–	±	++

structures. In the 48 h preservation group, most of the villus cells and goblet cells were necrotic and showed both a loss of their structures and a loss of mucin. The clusters of crypt cells were dominant and were found in all six rats in this group (Fig. 6).

The cytological findings are summarized in Table 2. As the preservation time increased, especially when it was longer than 24 h, the degeneration of the villus epithelial cells, the depletion of mucin and the presence of crypt cells all became evident.

Discussion

The small bowel is an organ highly sensitive of ischemia [12], and ischemia-reperfusion injury of the small bowel leads to the destruction of the mucosa and increases mucosal permeability, which can result in lethal effects [8]. Therefore, it is important to predict graft viability in the early period after reperfusion in small bowel transplantation. The mucosal layer of the intestine is the area most susceptible to damage during ischemia [3]. Wagner et al. reported in an histological study that the ischemic change in the mucosa was reversible unless the crypts were destroyed [25]. We previously reported that the survival of rats receiving a 24 h preserved graft in UW was good, while at 48 h survival was poor. Thus rat jejunum could be preserved in good physiological condition for up to 24 h before transplantation using simple cold storage in UW [21].

The histological findings after reperfusion in this study correlated well with the survival of rats in our previous study. The presence of a crypt layer after reperfusion is thought to be essential for graft viability. As shown in Figure 1, the mucosal damage was reversible unless the crypts were destroyed. Nevertheless, histological damage was limited to the villus epithelial layer even after 48 h of cold preservation before reperfusion. Therefore, histological study before reperfusion did not predict graft viability.

We previously reported that the recovery levels of ATP content and the energy charge in the graft at 30 min after reperfusion were reliable variables for the prediction of graft viability in rat small bowel transplantation [10]. In clinical transplantation, however, both energy metabolism and histology are considered to be too late for the recipient as a predictor of graft viability. Cytological examination, however, is a real-time method and is considered to be useful in clinical transplantation.

The mucosal layer was damaged on reperfusion, was denuded and then turned into the exudate when it could be examined cytologically. The cytological findings are thus considered to correlate with the depth of tissue injury on reperfusion. As the preservation time became longer (especially when it exceeded 24 h) the degeneration of villus epithelial cells, the depletion of mucin in goblet cells of the villus brush border and the presence of crypt cells all became evident. These findings are thus considered to be signs of poor graft viability. The presence of crypt cells on cytological examination means that the tissue damage extends deeper than the crypt layer.

There have been few reports on the indicators for graft viability after reperfusion. Mucosal glutamine activity [16] and tissue blood flow [5, 26] were shown to be useful to estimate graft viability after reperfusion. In order to avoid the transplantation of an inadequately preserved graft, better techniques are called for to evaluate viability at the end of preservation. Mueller et al. reported that the hyaluronic acid levels in the vascular effluents and purine nucleoside phosphorylase activities in the luminal effluents were valuable as predictive variables [15]. We have reported that physiological examination of nerve activity is useful in predicting graft viability prior to transplantation [22]. These parameters are thus considered to be helpful in clinical transplantation. However, further detailed investigations are still called for.

We conclude that cytological examination of the luminal exudate on reperfusion is an effective, non-invasive and real-time method for evaluating graft viability in small bowel transplantation.

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