KAWASAKI MED. J. VOL. 15 : NO. 3 · 4, 123-132 (1989)

Preservation Temperature and Replantation of an Amputated Ear in the Rabbit

Toru HASEGAWA, Yoshiki YAMANO, Akihiko INUFUSA, Kenzo FUSE and Ryo WATANABE

Department of Orthopaedic Surgery,
Kawasaki Medical School, Kurashiki 701-01, Japan

Accepted for publication on December 21, 1989

ABSTRACT. Using the amputated ear of a rabbit, the influence of the preservation temperature and duration of ischemia of the amputated ear on successful replantation was experimentally studied. Satisfactory replantation was achieved after preservation at a relatively low temperature (4 to 10°C), even after ischemia for as long as 48 hours. At ordinary temperatures (27°C), however, ischemia for 6 hours appeared to be the upper limit for successful replantation.

Key words : amputation — preservation temperature — replantation

In the replantation of amputated digits, the degree of injury, operative technique and post-operative complications represent important problems for successful replantation. Similarly, the duration of ischemia of the amputated digits is related to the quality of blood circulation after replantation. The duration of ischemia of the amputated digit is directly related to the post-operative course and the chance of success. When ischemia lasts for several hours, the factor most influencing success is the temperature during ischemia. In clinical application, the duration of preservation and temperature during preservation are inseparable, like other problems.

In the present animal study, the effect of the duration of circulatory interruption and the preservation temperature of an amputated ear on the success of its replantation was evaluated.

MATERIALS AND METHODS

Ear lobes of adult rabbits were amputated and kept at 4°C, 10°C and 27°C for 6, 12, 24 and 48 hours, at the end of which each ear was replanted. More after 24 hours, the ear lobe was studied histologically and the arterial and venous intima were evaluated using electron microscopy. Some amputated specimens were left for 48 hours at 4°C and 10°C before the replantation. At 24 hours and 1 week after replantation, the condition of the ear was evaluated (Table 1).

長谷川徹，山野慶樹，犬房秋彦，布施謙三，渡辺　良

TABLE 1. Materials

	6 hrs	12 hrs	24 hrs	48 hrs
4°C	n=2	n=2	n=2	n=2, n=2*
10°C	n=2	n=2	n=2	n=2, n=2*
27°C	n=2	n=2	n=2	n=2

* investigation after one week of replantation.

RESULTS

Macroscopic Findings

In animals in which the amputated ear was preserved for 6 hours at 4°C, 10°C or 27°C before replantation, the blood flow was favorable with only mild edema (Fig. 1a).

Fig. 1a

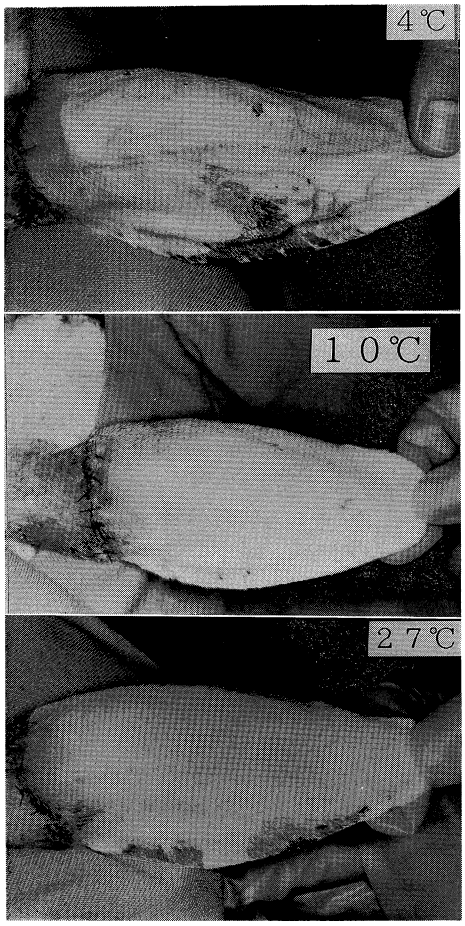

Fig. 1b

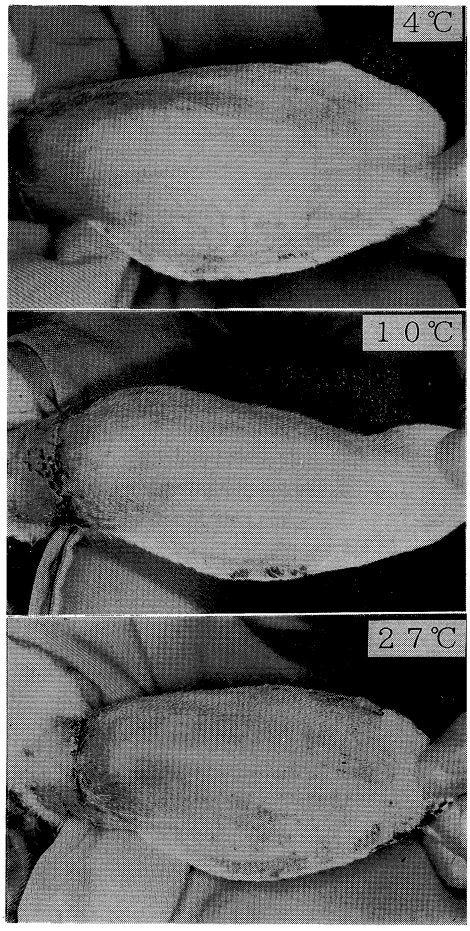

Fig. 1. Macroscopic appearances after replantation
a. Group preserved for 6 hours
b. Group preserved for 12 hours

In animals in which specimens were replanted after 12 hours of preservation at 4°C and 10°C, mild macroscopic edema was noted, but the blood flow was favorable. However, the use of specimens preserved at 27°C for 12 hours resulted in pronounced edema and erythema, indicating inadequate blood flow (Fig. 1b).

The use of specimens preserved at 4°C and 10°C for 24 hours resulted in mild edema, but the blood flow was favorable. The use of specimens preserved at 27°C for 24 hours, however, resulted in a marked macroscopic edema and erythema with interruption of blood flow (Fig. 1c).

In animals in which specimens preserved for 48 hours were used, moderate edema was noted 24 hours after replantation of ears preserved at 4°C or 10°C, although blood flow was maintained. When a preservation temperature of 27°C was employed, however, blood flow was interrupted 24 hours after replantation with progressive necrosis from the periphery (Fig. 1d). In animals replanted

Fig. 1c

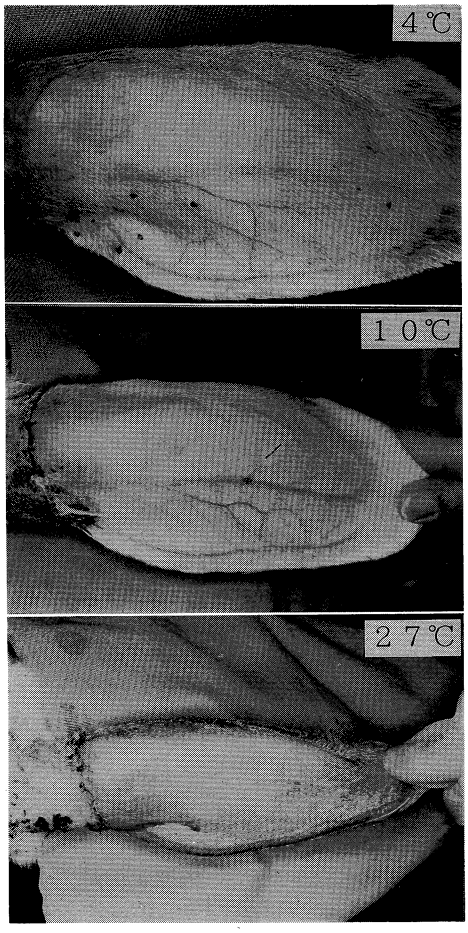

Fig. 1d

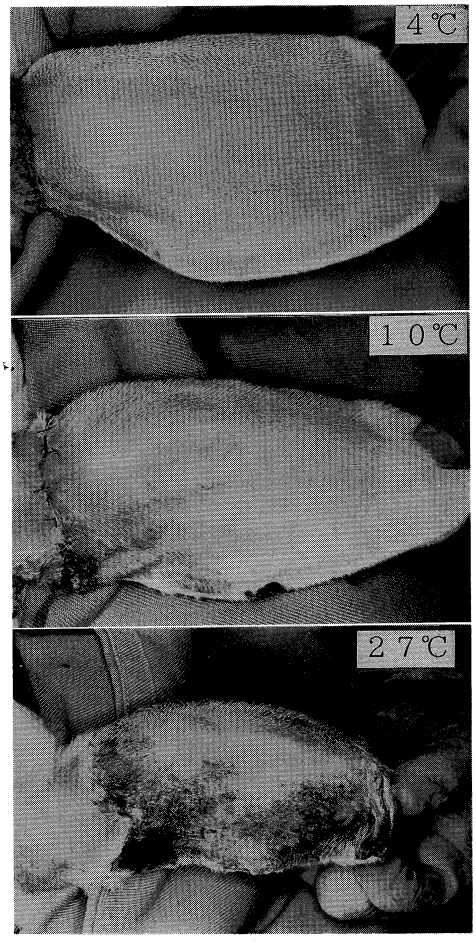

Fig. 1. Macroscopic appearances after replantation
c. Group preserved for 24 hours
d. Group preserved for 48 hours

with tissue preserved at 4°C and 10°C, favorable circulation was re-established after 1 week with improvement of the edema despite mild erythema. Macroscopically, no remarkable difference was noted between at tissue preserved 4°C and at 10°C (Fig. 2).

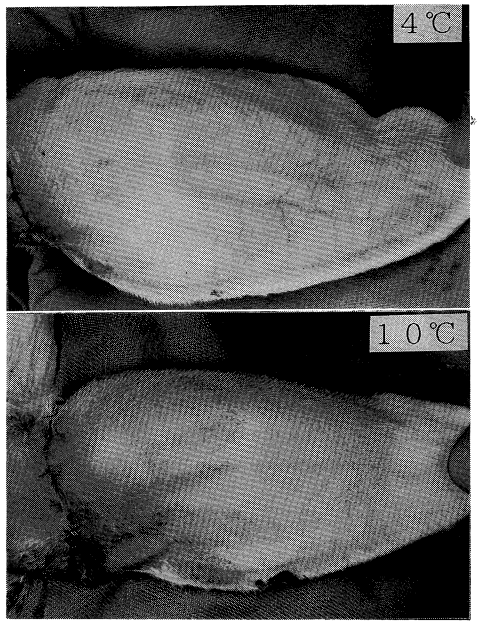

Fig. 2. Macroscopic appearances 1 week after replantation of tissue preserved for 48 hours

Histological Findings

In the group with replantation after preservation for 6 hours, mild interstitial edema was noted after preservation at 4°C, 10°C, and 27°C. It increased in severity as the temperature increased (Fig. 3a).

In the group with replantation after preservation for 12 hours, the interstitial edema was more pronounced than after preservation for 6 hours. Especially after preservation at 27°C, the edema was pronounced, with an irregular arrangement of the interstitial cells (Fig. 3b).

In the group with replantation after preservation for 24 hours at 4°C and 10°C, edema of the interstitium was noted, without an irregularity in the cellular arrangement. Preservation at 27°C, however, resulted in a pronounced irregularity of the interstitium, and thrombus formation, mainly in the venules (Fig. 3c).

In the group replanted after preservation for 48 hours at 4°C and 10°C, edema was pronounced at 24 hours, but improved after 1 week, with no thrombus formation. No remarkable difference in the change in the interstitial cells was noted between the tissues preserved at 4°C and 10°C. In the group with replantation of tissue preserved at 27°C, necrosis of the interstitial cells was noted 24 hours after surgery, with thrombus formation and bleeding into the interstitium (Fig. 3d).

Fig. 3a

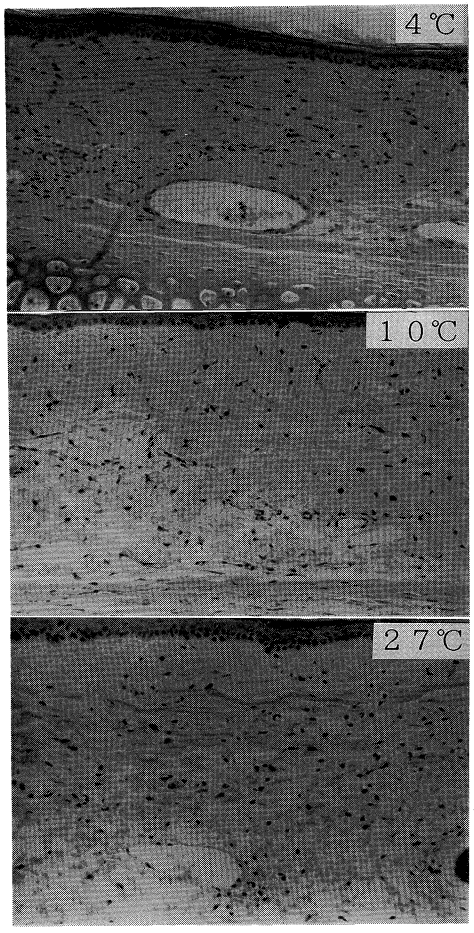

Fig. 3b

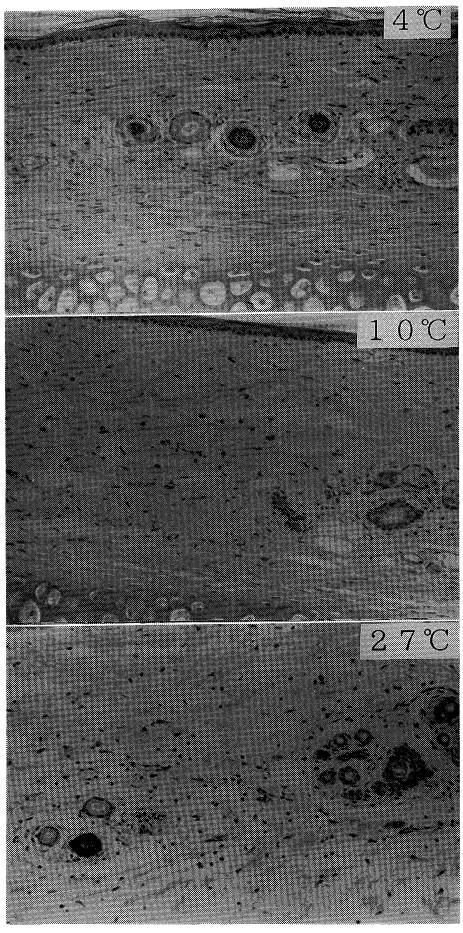

Fig. 3. Histological findings (×100)
a. Group preserved for 6 hours
b. Group preserved for 12 hours

Scanning Electron Microscopic Findings

In the groups with replantation of tissue preserved for 6 hours at 4°C, 10°C and 27°C, venous endothelial cells showed mild degeneration and fallout to similar degrees (Fig. 4). No remarkable changes, however, were noted in the arterial endothelial cells.

In the group with replantation of tissue preserved for 12 hours at 4°C and 10°C, degeneration of venous endothelial cells was found to a degree similar to that in the replanted tissue preserved for 6 hours. Again, no remarkable changes were noted in the arterial endothelial cells. In the tissue preserved at 27°C, however, degeneration and fallout of the venous endothelial cells had become pronounced, and degeneration was also noted in the arterial endothelial cells (Fig. 5).

In the group with replantation of tissue preserved for 24 hours at 4°C and 10°C, degeneration and loss of venous endothelial cells were more pronounced than that in the group with replantation after 12 hours of preservation,

Fig. 3c

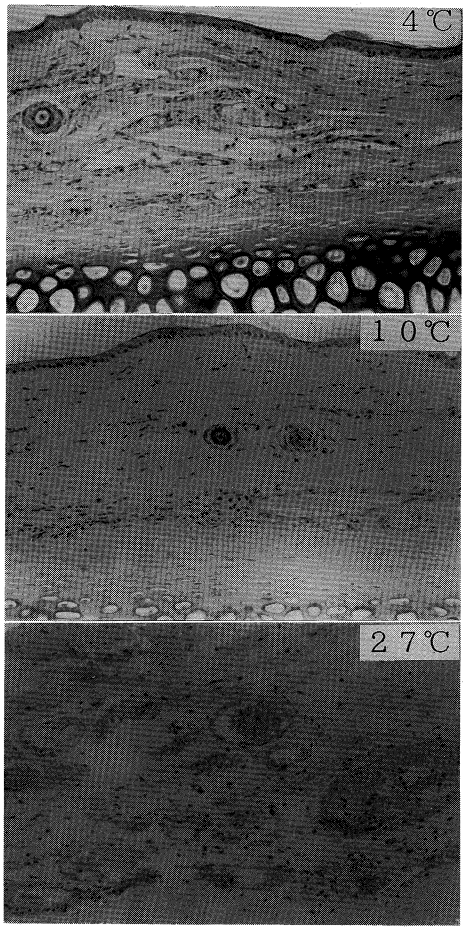

Fig. 3d

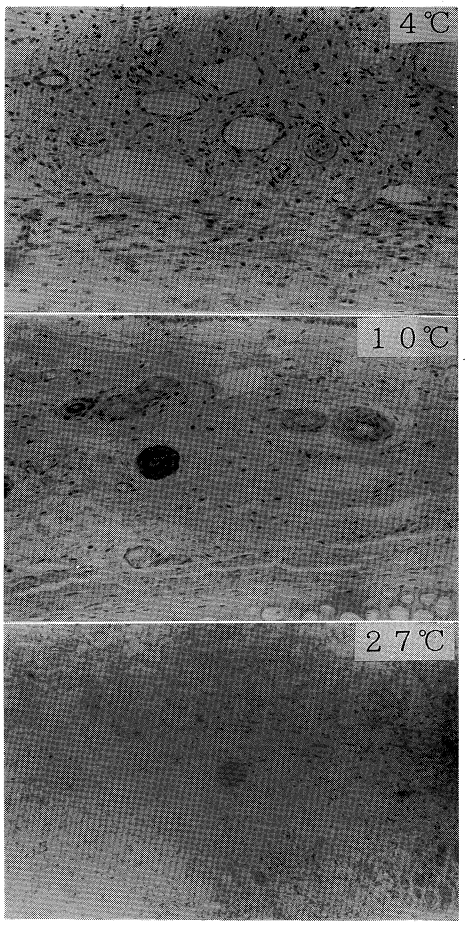

Fig. 3. Histological findings (×100)
c. Group preserved for 24 hours
d. Group preserved for 48 hours

with degeneration and loss of some of the arterial endothelial cells. In the group with replantation after preservation at 27°C, the venous endothelial cells underwent extensive degeneration and loss, along with a similar pronounced degeneration of the arterial endothelial cells. Vascular folds were flattened, suggesting a decrease in elasticity (Fig. 6).

In the group receiving tissue preserved for 48 hours at 4°C, 10°C and 27°C, marked degeneration and loss of the vascular endothelium were noted both in the artery and vein 24 hours after the replantation, with exposure of the elastic fibers. As the temperature of preservation increased, the degree of degeneration also increased (Fig. 7). At 1 week after the replantation of the tissue preserved at 4°C and 10°C, the exposed elastic fibers in the artery and vein were covered by fibrin (Fig. 8). Especially in the vein, the normal endothelial cells were covered with many small microprocesses (Fig. 9). The degree of these changes did not differ between tissues preserved at 4°C and 10°C.

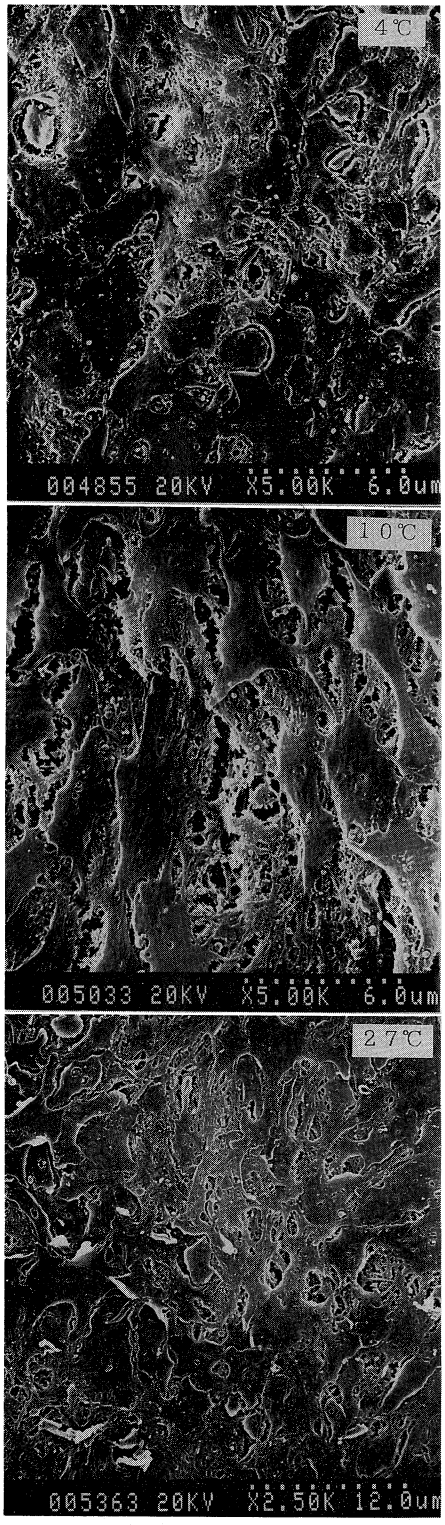

Fig. 4. Group preserved for 6 hours
Scanning electron microscopic findings of the venous inner membrane

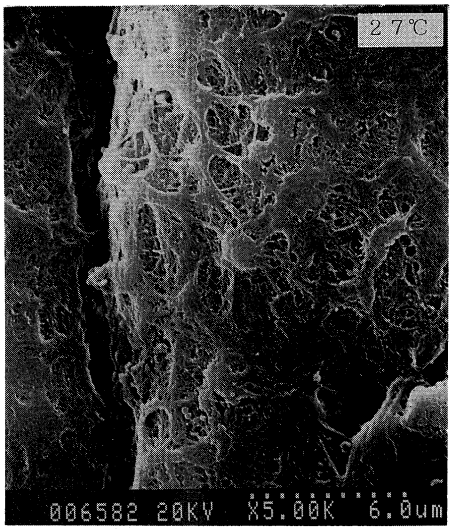

Fig. 5. Group preserved for 12 hours at 27°C
Scanning electron microscopic findings of the venous inner membrane

Fig. 6

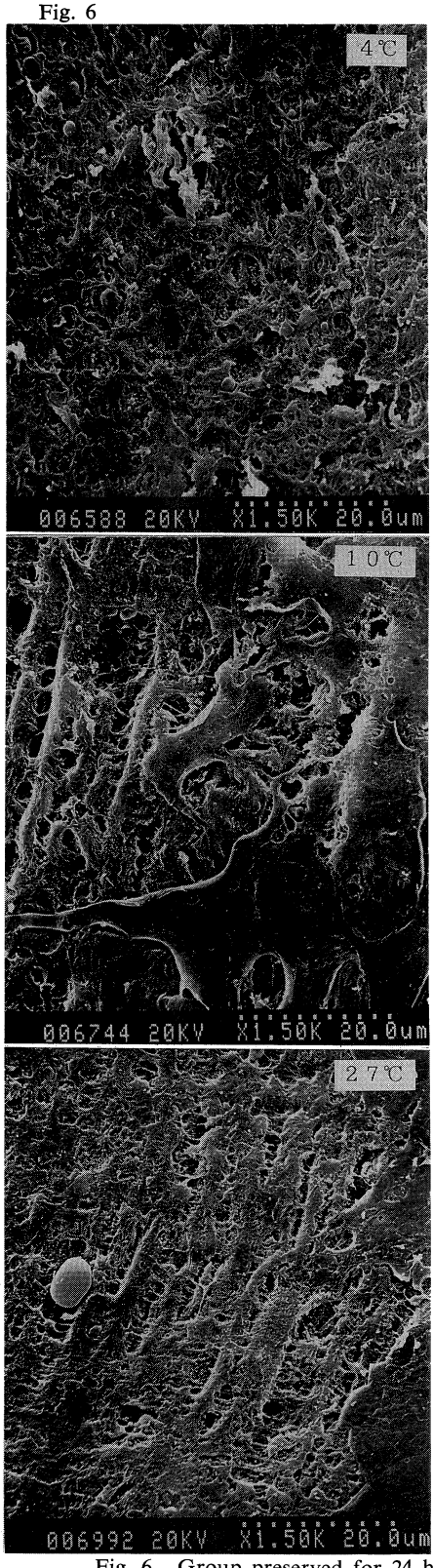

Fig. 7

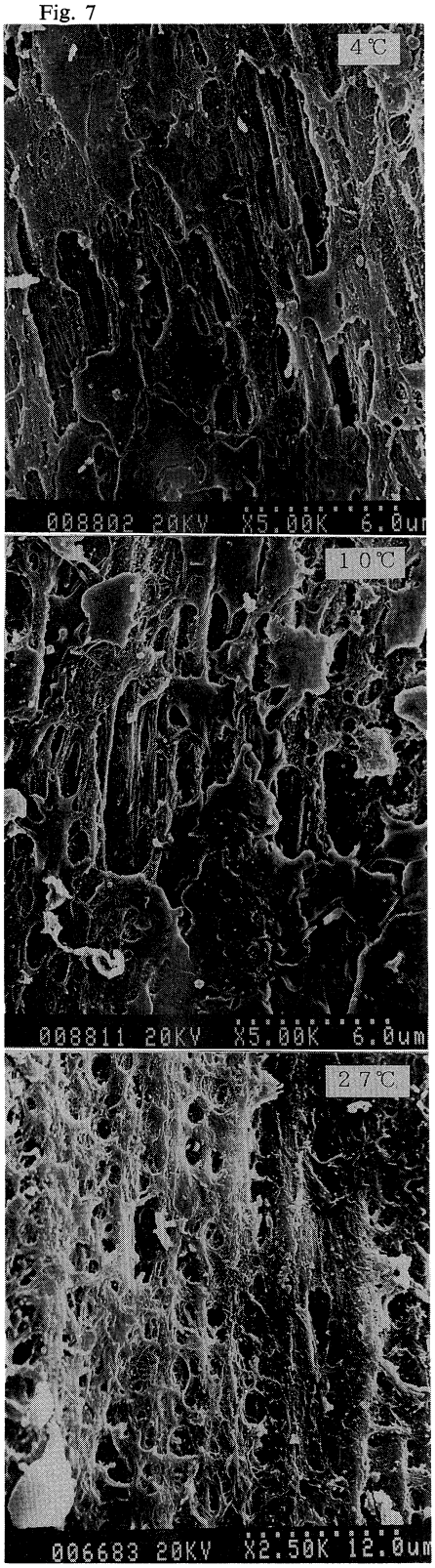

Fig. 6. Group preserved for 24 hours
Scanning electron microscopic findings of the venous inner membrane

Fig. 7. Group preserved for 48 hours
Scanning electron microscopic findings of the venous inner membrane

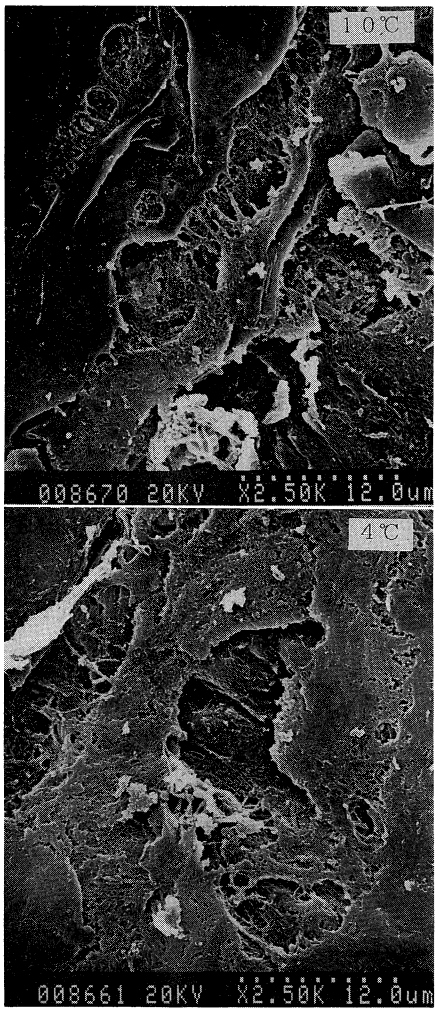

Fig. 8. Group preserved for 48 hours at 4°C and 10°C
Scanning electron microscopic findings of the venous inner membrane one week after replantation

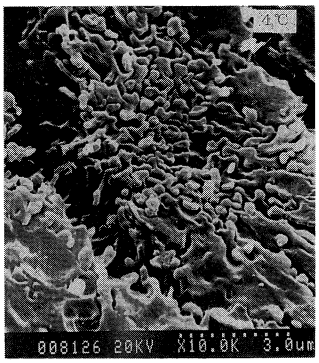

Fig. 9. Small microprocesses observed on the surface of the venous endothelial cells

DISCUSSION

May *et al.*[1] studied the effect of the state of preservation on replantation experimentally, using rabbit groin flaps after warm ischemia (body surface temperature). Successful replantation was achieved in 100% after 1 to 4 hours of ischemia, in 80% after 12 hours, but none at more than 12 hours. Lapchinsky[2] amputated the leg of a dog and achieved survival after 24 hours of preservation at 4°C, obtaining favorable function. Hayhurst *et al.*[3] also reported successful survival of the index finger in 5 of 7 monkeys. The limit of preservation for successful survival was 6 hours at room temperature (20 to 25°C) and 24 hours at lower temperatures (0 to 5°C), according to these authors. Cooley *et al.*[4] reported 100% survival using rat groin flaps after preservation for 6 hours at room temperature and for 48 hours at lower temperatures. While different animals were used, 6 to 8 hours at room temperature (20 to 25°C) and 24 to 48 hours at low temperature (0 to 5°C) appear to be the limit for tissue survival.

According to the results of the present experiment, preservation in a state of ischemia for 48 hours at 4°C and 10°C was consistent with favorable replantation, though degeneration of the vascular endothelium was somewhat more pronounced after preservation at 10°C. This would indicate a higher preservation temperature for successful replantation compared with previous reports. While the low temperature used by previous investigators was 0 to 5°C, preservation at 0°C for 1 hour caused a marked degeneration of the venous endothelial cells according to our previous experiments.[5] Stock *et al.*[6] reported the role of the collagen exposure due to vascular endothelial cell loss as one of the triggers for thrombus formation. Preservation at 0°C may represent a considerably poorer risk for the maintenance of favorable blood flow and subsequent success. For ischemia as long as 48 hours, the safe temperature assuring satisfactory survival of the skin, bone and cartilage tissue, not including muscle, appears to be 4 to 10°C.

At room temperature of 27°C, 12 hours of ischemia caused disturbances of blood flow and marked degeneration of the vascular endothelium, probably indicating the limit of preservation. As in previous reports, 6 hours of ischemia at room temperature may represent the limit for safe preservation.

In the present experiment, compared to previous reports, successful replantation was achieved even after prolonged ischemia at a high preservation temperature successfully extending the limit over the previously accepted range.

REFERENCES

1) May, J.W., Chait, L.A., O'Brien, B.Mc.C. and Hurley, J.V.: The noreflow phenomenon in experimental free flaps. Plast. Reconstr. Surg. **61** : 256–267, 1978
2) Lapchinsky, A.G.: Recent results of experimental transplantation of preserved limbs and kidneys and possible use of this technique in clinical practice. Ann. NY Acad. Sci. **87** : 539–571, 1960
3) Hayhurst, J.W., O'Brien, B.Mc.C., Ishida, H. and Baxter, T.J.: Experimental digital replantation after prolonged cooling. Hand **6** : 134–141, 1974
4) Cooley, B.C., Hansen, F.C. and Dellon, A.L.: The effect of temperature on tolerance to ischemia in experimental free flaps. J. Microsurg. **3** : 11–14, 1981
5) Hasegawa, T., Yamano, Y., Hino, Y., Okunobou, Y., Hayashi, K. and Watanabe, R.: An experimental study involving the replantation of frozen digits. Jpn. J. Plast. Reconstr. Surg. **31** : 1151–1159, 1988
6) Strock, P.E. and Manjo, G.: Microvascular changes in acutely ischemic rat muscle. Surg. Gynecol. Obstet. **129** : 1213, 1969