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The pedicled venous flap. An experimental study

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SUMMARY. **Musculocutaneous pedicled venous flaps on the dorsum of rats survived at a statistically significantly higher rate than musculocutaneous composite grafts ($p < 0.01$). With a Silastic® sheet (Dow Corning) beneath, both composite grafts and pedicled venous flaps necrosed. When a Silastic® sheet with holes in it to allow some revascularisation from the bed was placed beneath, the survival rate was significantly better than with a complete Silastic® sheet ($p < 0.01$). These results demonstrate that pedicled venous flap survival depends both on the draining vein, and revascularisation from the underlying bed.**

We previously demonstrated experimentally that the venous drainage of a musculocutaneous flap, 4 × 6 cm in size, was of importance in preventing flap necrosis and allowing successful flap take (Fukui *et al.*, 1985). In that experiment, the bilateral iliolumbar veins were used as the draining veins of the flap. It was questioned whether this flap model actually functioned as a pedicled venous flap, because the bilateral iliolumbar veins are connected to each other at the centre of the flap. Another experimental model was therefore produced. Only a single lumbar or iliolumbar vein was used as the draining vein in the following experiment.

Materials and methods

Fifty-four Fischer male rats (96 grafts and 120 flaps) weighing 350–400 g were used, and ether inhalation anaesthesia was administered. Four 2 × 3 cm musculocutaneous flaps were produced on the dorsum of each rat. Two of the flaps were made 3 cm proximal to the tail and another two were made 1 cm more proximal to these. These four flaps in rats are supplied by the lumbar or iliolumbar neurovascular bundles (Fig. 1). The animals were divided into 6 groups (Fig. 2). Dissection of the neurovascular bundle was performed under an operating microscope.

Group A (12 rats, 48 grafts): As a control the lumbar and iliolumbar neurovascular pedicles of the flaps were coagulated using a bipolar coagulator and cut, the flaps therefore being musculocutaneous composite grafts.

Group B (12 rats, 48 flaps): The lumbar and iliolumbar arteries, nerves and perivenous areolar tissue were cut after coagulation, preserving only the lumbar and iliolumbar veins as the draining veins. These flaps are therefore a pedicled venous flap model.

Group AT (6 rats, 24 grafts): The same method as in Group A was used and a thin Silastic® sheet was placed between the musculocutaneous grafts and the underlying bed, to prevent any revascularisation.

Group BT (6 rats, 24 flaps): The same method as in Group B was used and a Silastic® sheet was placed beneath.

Group AP (6 rats, 24 grafts): The same method as in Group A was used and a thin Silastic® sheet with 20 pores, each 3 mm in diameter, was placed beneath the grafts and the underlying bed, to allow some revascularisation.

Group BP (12 rats, 48 flaps): The same method as in Group B was used and a similar Silastic® sheet with pores was placed beneath.

The four musculocutaneous grafts or flaps were sutured back into their original sites and the rats were kept in an air-conditioned room and given the necessary food and water.

The viability of the grafts or flaps was assessed at 14 days postoperatively by four methods:

A. Macroscopic examination

Viability was determined by assessing graft or flap colour, texture, and degree of swelling.

B. Fluorescein test

Fluorescein (50 mg/kg, Alcon Laboratory Inc.) was injected into the femoral vein, and after 30 min the flap/graft was photographed using a Kodak Wratten filter (No. 15) on the camera lens and another filter (No. 32) on the strobic light.

In all groups, a fluorescein test was done routinely for each group at 0, 4, 5, 10, 14 and 28 postoperative days (Table 1).

C. Microangiography

Five rats (20 grafts) in Group A, 10 (40 flaps) in Group B, 5 (20 grafts) in Group AT, 5 (20 flaps) in Group BT, 5 (20 grafts) in Group AP and 10 (40 flaps) in Group BP were killed for microangiography and the four grafts or flaps were harvested for histological examination simultaneously. One rat in Group A, 3 in

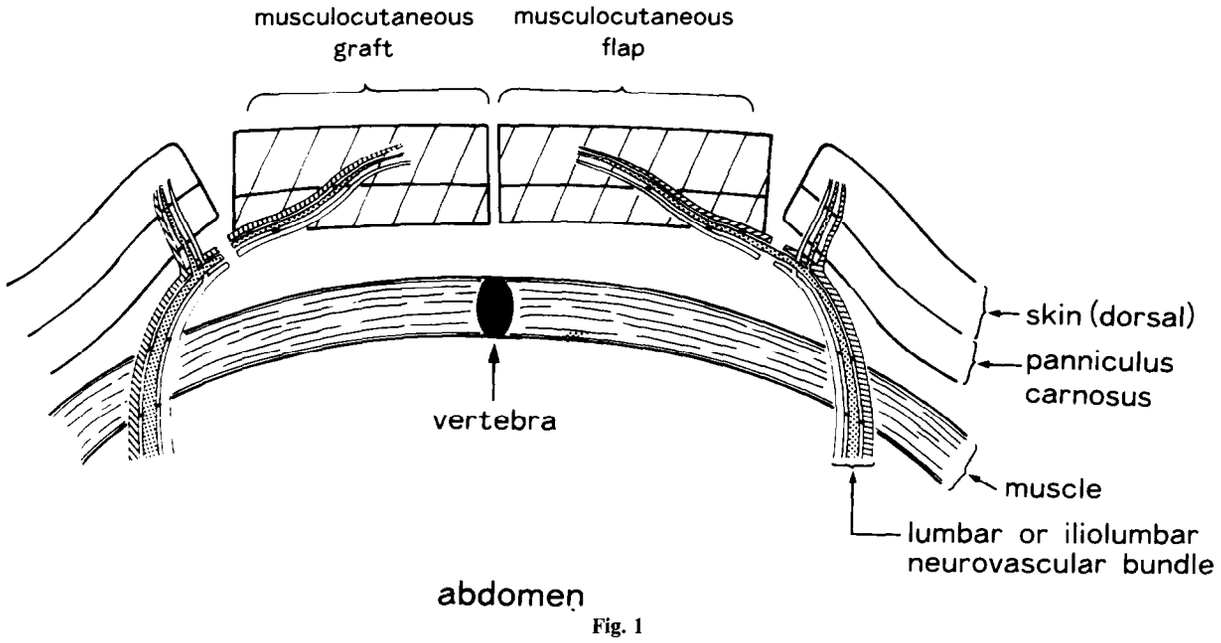

Fig. 1

Figure 1—Transverse sections of the musculocutaneous graft and flap model.

Group B, 1 in Groups AT, BT and AP and 4 in Group BP (total 11 rats) were killed for these examinations within 14 days; these rats were not included in the flap survival results (Table 1).

30 ml of 20% micropaque containing 5% gelatin was injected into the ascending aorta of each rat at a physiologic arterial pressure of 110 mmHg. The rats were placed in a refrigerator for 30 min to allow the gelatin to harden. An area of skin which included the flaps/grafts about 9 × 6 cm was harvested.

In the Silastic® groups, the sheet was contained within the harvested tissue. A thin membrane existed between the underlying bed and Silastic® sheet after 10 postoperative days. This membrane contained some vessels which came from the underlying bed, and this membrane was included in the harvested tissue. Microangiography was performed using soft X-rays (Softex CMS, Type 2).

D. Histological examination

Tissue, 3 × 1 cm in size, was harvested from the lateral edge of the graft or flap after microangiography. After fixation with 10% neutral buffered formalin, tissues were examined histologically by means of haematoxylin and eosin staining.

Additional tissue was obtained from two rats each (8 grafts or flaps) of Groups A, B and BP, and 1 rat each (4 grafts or flaps) of Groups AT, BT and AP (total 9 rats) at other postoperative days for histological examination only; these rats were not contained in the results (Table 1).

Removing 20 rats (11 rats for microangiography and 9 for histological examination), the remaining 34 rats (68 grafts and 68 flaps) were observed for 28 days after the operation. Assessment of survival or necrosis was made on the 14th day. Statistical analysis was done using the Mann–Whitney test (U-test).

Results (Table 2)

1. Macroscopic examination

In Group A, 25 out of 36 grafts showed complete necrosis. The graft appeared to be markedly oedematous for the first 7 days, later gradually becoming dry and hard, and finally shrinking. The other 11 grafts survived.

In Group B, 24 out of 28 flaps survived. The flaps did not show any obvious oedema during the first 7 days; then, superficial necrosis was seen over the whole flap in all cases after 10 days. This became an eschar, which separated leaving the deeper parts of the flap visible during the second or third week after the operation. The remaining 4 flaps showed complete necrosis.

In Groups AT, BT and AP, 16 grafts or flaps showed complete necrosis.

In Group BP, 12 out of 24 flaps partially survived. However, superficial necrosis was seen in all cases after 10 days and behaved similarly to Group B. The remaining 12 flaps showed complete necrosis.

The survival rate in Group B (pedicled venous flaps) was statistically significantly higher ($p < 0.01$) than Group A (composite grafts). Preserving a pedicle vein in Group BP gave statistically significantly higher survival ($p < 0.01$), when compared to Group AP. This group (with a pored Silastic® sheet) had statistically significantly higher survival ($p < 0.01$) than Group BT (with a pedicle vein but no pores in the Silastic® sheet).

2. Fluorescein test

In Group A, the 25 necrotic grafts showed no fluorescence at any time. The eleven surviving grafts first exhibited fluorescence on the 4th day after the operation.

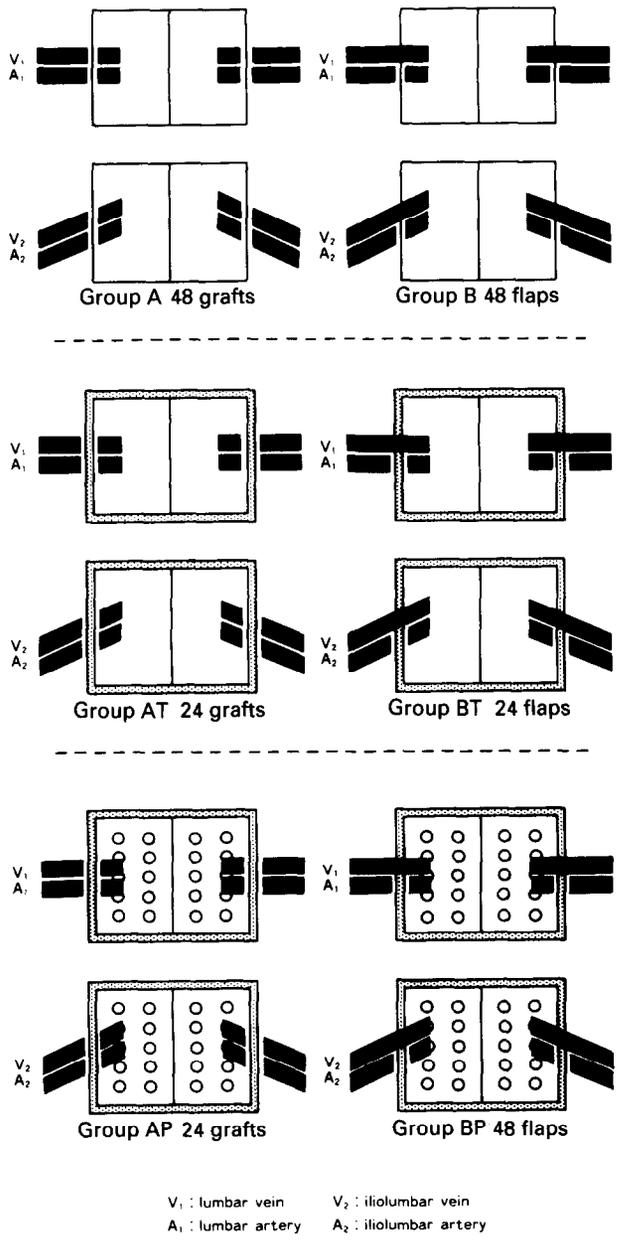

Fig. 2

Figure 2—Experimental groups. Group A: Composite grafts. The lumbar and iliolumbar neurovascular pedicles of the flaps were cut. Group B: Pedicled venous flaps. The lumbar and iliolumbar arteries, nerves and perivenous areolar tissues were cut, preserving only the lumbar and iliolumbar veins. Group AT: The same model as in Group A, but a Silastic® sheet was placed between the grafts and underlying bed to prevent any revascularisation. Group BT: The same model as in Group B, with a Silastic®sheet placed between the flaps and underlying bed, was used. Group AP: The same model as in Group A with a Silastic® sheet with 20 pores, each 3 mm in diameter, placed between the grafts and underlying bed, to allow some revascularisation. Group BP: The same model as in Group B with a porous Silastic® sheet placed between the flaps and underlying bed.

In Group B, the 24 surviving flaps showed only weak fluorescence on the 4th day. Normal fluorescence was seen after the superficial eschar sloughed off. The four necrotic flaps showed no fluorescence at any time.

In Groups AT, BT and AP, the 16 necrotic grafts showed no fluorescence at any time.

In Group BP, the twelve partially surviving flaps showed fluorescence on the 4th or 5th day after the operation. Twelve necrotic flaps showed no fluorescence at any time.

3. Microangiography (Fig. 3A–F)

In Group A, the necrotic grafts showed no vessels. The surviving grafts showed a few vessels.

In Group B, the surviving flaps showed no vessels on the 3rd day after the operation; on the 4th day, the preserved lumbar or iliolumbar vein and flap vasculature was demonstrated. Six days after the operation, hypervascularity was seen, which continued until the twentieth day.

No vessels were seen in Group AT.

In Group BT, vessels were seen, but these were located beneath the Silastic® sheet and not contained in the flap.

No vessels were seen in Group AP.

In Group BP, the surviving flaps showed the pedicle lumbar or iliolumbar vein and the flap vasculature on the 7th day after operation.

4. Histological findings

In Group A, the necrotic grafts showed epidermal degeneration with hyaline degeneration of the connective tissue of the dermis. The surviving grafts showed thinning of the epidermal layer; the connective tissue of dermis showed degeneration on the 3rd postoperative day. Sweat glands and hair follicles disappeared. On the 7th day, degeneration had progressed and some sloughing off of epidermal cells was seen. The connective tissue of the dermis showed hyaline degeneration. On the 12th day, the epidermal layer thickened, and the connective tissue of the dermis also thickened but did not yet exhibit a normal structure. Sweat glands and hair follicles were seen again. Finally the epidermis and dermal layers showed normal patterns.

In Group B, the surviving flaps showed epidermal degeneration and the connective tissue of the dermis became thin 3 days after the operation. Sweat glands and hair follicles did not disappear. On the 5th day, a squamous epithelial layer was observed; no remarkable change was seen in the connective tissue. On the 6th day, the epidermal layer and connective tissue thickened. By the 11th day, the epidermis and dermis showed normal patterns. The findings of the necrotic flaps were the same as the necrotic grafts in Group A.

In Groups AT, BT and AP, the histological findings were the same as the necrotic grafts in Group A.

In Group BP, the histological findings of the surviving flaps were the same as surviving flaps in Group B, and the necrotic flaps were the same as necrotic grafts in Group A.

Discussion

In our previous report (Fukui *et al.*, 1985), microangiography did not show the preserving vein or flap

Table 1 The days on which the various assessments were carried out in the different groups

Group	*A*	*B*	*AT* *BT* *AP*	*BP*
Fluorescein test	0, 4, 5, 10 14 28 (maximum) sacrificed day	0, 4, 5, 10 14 28 (maximum) sacrificed day	0, 4, 5, 10 14 28 (maximum) sacrificed day	0, 4, 5, 10 14 28 (maximum) sacrificed day
Microangiography	7 15, 20 (5 rats) 25, 28	3, 4, 6 15, 18, 20, 22, 24, 26, 28	7 15, 18 21, 28	4, 6, 7, 10 15, 18, 20 22, 25, 28
Histological examination	3, 7 12, 15, 20 25, 28	3, 4, 5, 6 11, 15, 18, 201 22, 24, 26, 28	3, 7 12, 15, 18 21, 28	3, 4, 5, 6, 7, 10 15, 18, 20 22, 25, 28

—— : only for histological examination

Table 2 The survival figures of the flaps and grafts in the different groups

Type	*Number*	*Survived*	*Partial necrosis*	*Complete necrosis*
Group A	36[a]	11	0	25
Group B	28[a]	24	0	4
Group AT	16	0	0	16
Group BT	16[c]	0	0	16
Group AP	16[b]	0	0	16
Group BP	24[b,c]	0	12	12

The other 20 rats were sacrificed within two weeks for microangiographic study, and histological examination.
MannWhitney Test
a: $p < 0.01$ (Group A vs. Group B)
b: $p < 0.01$ (Group AP vs. Group BP)
c: $p < 0.01$ (Group BT vs. Group BP)

vasculature within 3 days. It occurred to us that this may be because micropaque would have to reach the flap retrogradely and only after neovascularisation from the recipient bed by 4 days postoperatively could micropaque reach the flap through neovascularised vessels. In this experiment, the same results were obtained.

The process of flap survival of the pedicled venous flaps was similar to our previous study (Fukui *et al.*, 1985). When revascularisation from the recipient bed was interrupted by inserting a Silastic® sheet between the grafts or flaps and the recipient bed, neither composite grafts nor pedicled venous flaps survived. When some revascularisation was allowed via a pored Silastic® sheet, composite grafts still necrosed but twelve out of 24 pedicled venous flaps partially survived. Preserving a pedicle vein in Group BP had statistically significantly higher survival ($p < 0.01$), when compared to groups AP and BT. From these results, it can be concluded that the survival of certain pedicled venous flaps depends not only on the vein but also on neovascularisation from the flap bed. The superficial necrosis seen in some of these experimental flaps, shown by macroscopic examination, fluorescence, microangiography and histology matches the initial unhealthy ischaemic appearances of some pedicled venous flaps used clinically (Chavoin *et al.*, 1987; Foucher and Norris, 1988; Fukui *et al.*, 1989). These clinical flaps may also depend on neovascularisation from their beds.

In an other experimental pedicled flap, the rat inferior epigastric flap has been used (Yuen and Leung, 1991) and in that study it was shown that a pedicled venous flap could survive even if a Silastic® sheet was placed between the flap and recipient bed. However, this flap could not survive after transfer by venous anastomosis. Using a similar model, Noreldin *et al.* (1992) reported that a pedicled venous flap preserving vein and perivenous areolar tissue could survive even if a Silastic® sheet was placed between the flap and recipient bed. However, this flap could not survive with just a vein, even if a Silastic® sheet was not placed beneath it. They stressed the importance of perivenous areolar tissue for flap survival. In our experiment, perivenous areolar tissue around the preserved draining vein was divided under magnification. The reason for different results between Yuen *et al.*, Noreldin *et al.* and our group might be due to differences in the size and components of the flap, and condition of the recipient bed.

Other factors have been suggested to explain the survival of venous flaps. We think the reasons for venous flap survival are probably initially "plasmatic imbibition" occurring; then, the plasma imbibed into the flap tissue flows out through the preserving draining vein. The pedicled flap is thus kept viable until it is revascularised from the recipient bed and surrounding tissue. Blood then comes from the recipient bed into the flaps, passing out through the draining vein(s) (Fukui *et al.*, 1991a). Venous pressure and oxygen tension appear to be important for venous flap survival clinically, for example with digital,

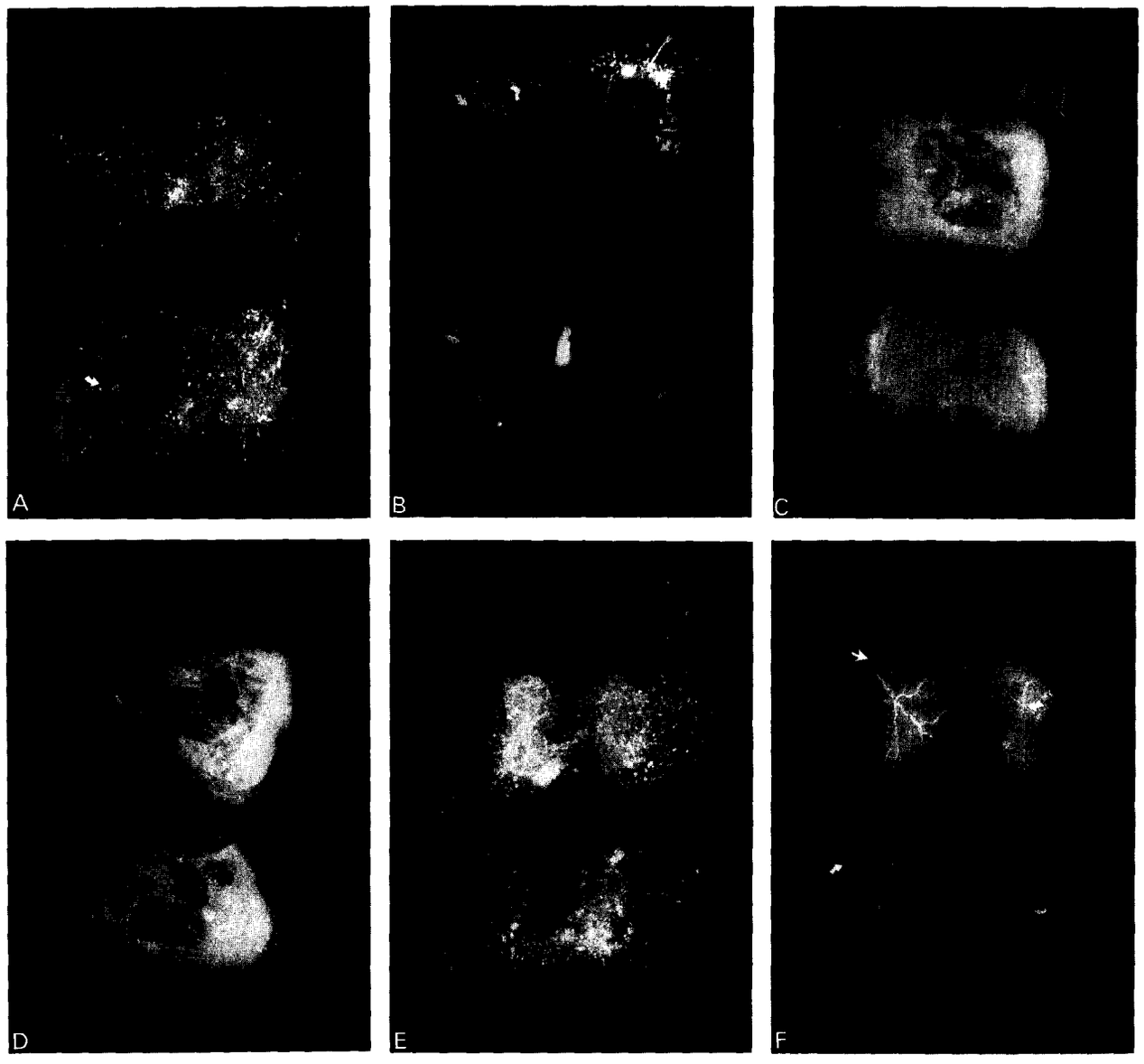

Fig. 3

Figure 3—Microangiography. (A) Group A: The necrotic grafts show no vessels, and the surviving grafts demonstrate vasculature (arrow). (B) Group B: Pedicle veins (arrows) and flap vasculature demonstrated 3 days after the operation. (C) Group AT: No vessels are seen in the grafts. (D) Group BT: Vessels were seen entering the flaps, but these were located between the Silastic® sheet and not contained in the flap. Fifteen postoperative days in this case. (E) Group AP: No vessels are seen in the grafts. (F) Group BP: The surviving flaps show the pedicle veins and flap vasculature (arrows) 7 days after the operation.

cephalic and saphenous venous flaps (Inada *et al.*, 1990; Fukui *et al.*, 1991b). The viability of these flaps may not be due only to relatively high venous pressure and oxygen tension but also to "plasmatic imbibition".

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