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Observations on experimental flow-through venous flaps

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SUMMARY. **The aim of this work was to compare the survival of an arteriovenous island flap with the survival of an island flap with a flow-through venous supply. Our experimental studies were performed on 95 Wistar rats randomised into six groups: Group 1: Indian ink injection of flow-through venous flaps with capillary network; Group 2: control group deprived of vascularisation; Group 3: control group with arteriovenous supply; Group 4: flow-through venous flaps of group 1; Group 5: epigastric flow-through venous flaps with a main venous trunk; Group 6: histological examination of flow-through venous flaps. The survival of flaps was monitored by direct examination, histological examination, capillaroscopy, and laser Doppler. Three out of 50 flow-through venous flaps survived. There was a statistically significant difference in the delay of clinical necrosis between the composite non vascularised free grafts (2.8 ± 1.2 days) and the flow-through venous flaps (4.1 ± 1.3 to 4.9 ± 1.1 days depending on the type of flap). A 20% decreased venous blood flow was observed in the flow-through venous flaps.**

The reconstruction of tissue, simple or composite, lost through trauma includes an array of procedures to provide cover. Arteriovenous island flaps may be criticised for their sacrifice of a major arterial axis and for their limitation by the vasculature and anatomy of the donor site. In an attempt to tackle these various problems and drawbacks, the development of so-called venous flaps has addressed itself to two objectives:

— to avoid the sacrifice of a major vascular axis while ensuring maximal blood supply to the flap and an adequate arc of rotation;

— to increase the range and number of available donor sites subject to the variation in venous patterns in humans.

Single pedicled venous flaps have been used clinically[1,2] and in animal work.[3,4] As recently showed by Noreldin *et al.*,[4] the viability of those flaps seems to be related in part to preservation of perivenous areolar tissue. Flow-through venous flaps are less reliable[5] except when the blood flow is increased.[6,7] The survival of arterialised venous flaps[8] or prefabricated arterialised flaps using vein grafts is more frequently observed.[9]

Reports of these cases have identified a number of questions: Do venous flaps really exist as flaps vascularised solely by a venous system? Do they not survive rather on an arterial microcirculation, which exists in the perivenous fat, with direct or reversed flow? Do venous flaps survive as thick grafts revascularised from the bed and edges? Are they reliable wherever the donor site is located?

The aim of this work is to compare the survival of arteriovenous island flaps with the survival of island flaps with a flow-though venous supply.

Material and methods

Our studies were performed on 95 Wistar rats of an average weight of 200 g. Each rat was anaesthetised with a mixture of ketamine and chlorpromazine (10 ml of ketamine (50 mg/ml) + 1.5 ml of chlorpromazine (5 mg/ml): 0.6 ml intramuscular injection for 200 g rat). Venous pedicles were dissected under magnification (Zeiss OPMI microscope). All venous flaps (see below for sites) were dissected in identical fashion: each flap was completely isolated from its bed and from adjacent skin edges. The artery, nerve and adjacent fat were resected over a distance of 0.5 to 1 cm. The vein was meticulously cleaned until it was certain that there was no accompanying arterial microcirculation. The flaps were sutured back into their donor sites. We did not perform microanastomosis either in veins or arteries to avoid modification of venous pressure and failure due to thrombosis.

The rats were randomised into six groups: group 1 consisted of 10 rats which served as two experimental models of large flaps including a capillary venous network sited between two venous systems. The thoraco-abdominal flap (five rats) was sited between the axillary venous system and the ipsilateral epigastric venous system (Fig. 1A). The transverse abdominal flap (five rats) was sited transversely between left and right epigastric venous systems (Fig. 1B). The epigastric vein of all 10 rats was cannulated and injected with Indian ink; this demonstrated the presence of a capillary network connecting the two venous systems.

Groups 2 and 3 were control groups. Group 2 consisted of 10 rats in which we raised a left epigastric flap. The flap was dissected free and restored to its bed after division of all vascular pedicles (Fig. 2). This was intended to demonstrate that the musculo-aponeurotic bed and wound edges were incapable of nourishing the 'flap' tissue as a composite graft. Group 3 consisted of

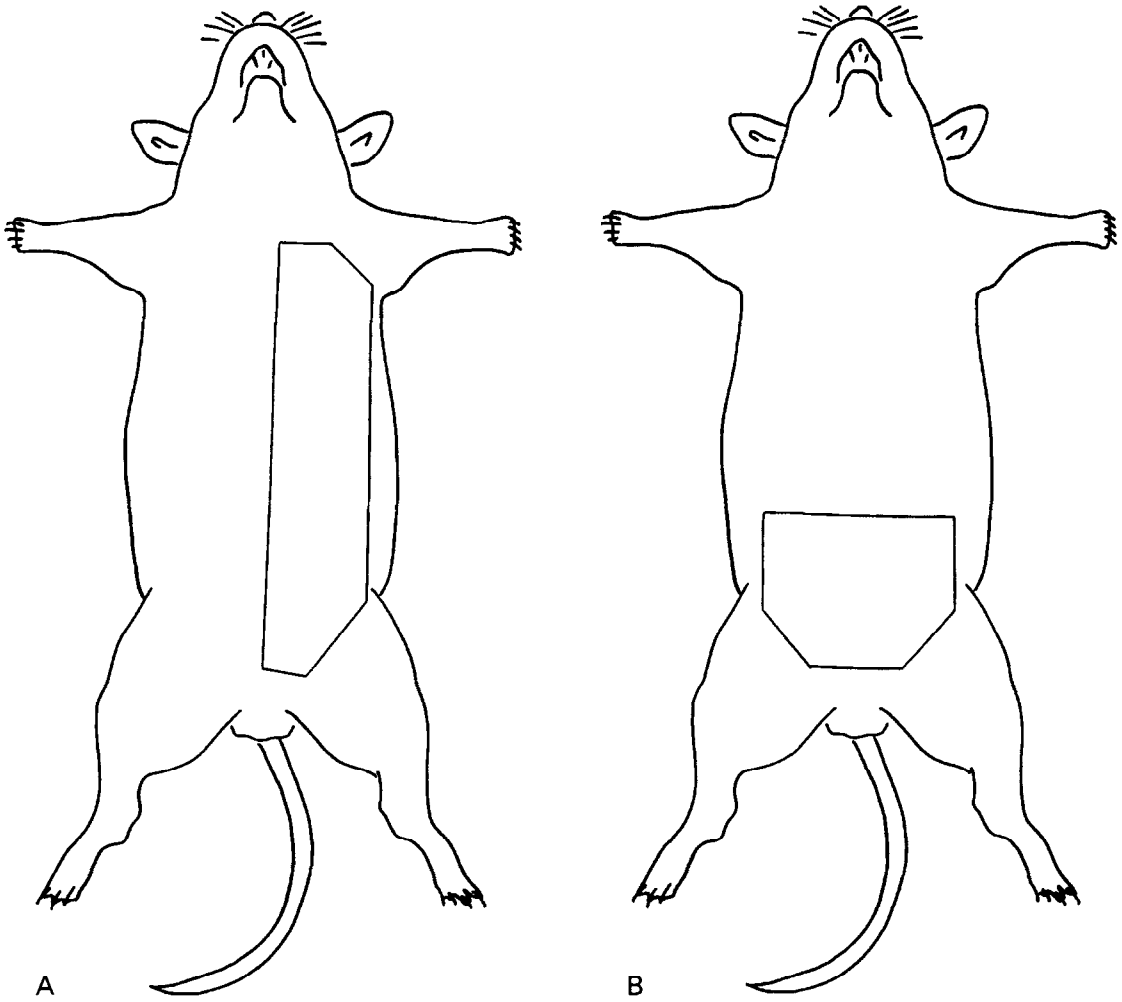

Fig. 1

Figure 1—(A) Thoraco-abdominal flap of group 1. The flap is sited on the left side of the thoraco-abdominal wall of the rat between the left epigastric vein and the left axillary vein. A capillary network connects the two venous systems: (B) Transverse abdominal flap of group 1. The flap is sited transversely on the abdominal wall between left and right epigastric veins. A capillary network connects the two venous systems.

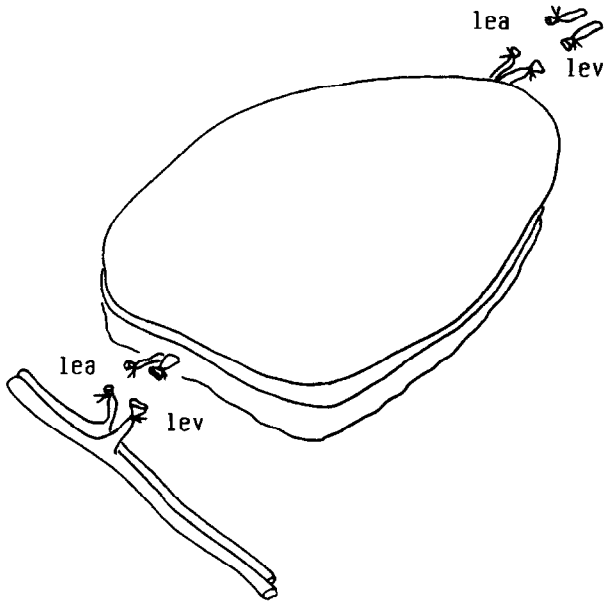

Fig. 2

Figure 2—Left epigastric flap of group 2. The flap was dissected free and restored to its bed after division of the pedicle. (lev: left epigastric vein, lea: left epigastric artery).

10 flaps (five thoraco-abdominal and five transverse abdominal flaps) raised in identical fashion to those in group 1. In each flap, the left epigastric artery and vein were left undivided (Fig. 3A, B). In the five thoraco-abdominal flaps, the left axillary artery was divided and the left axillary vein was left undivided. In the five transverse abdominal flaps, the right epigastric artery was divided and the right epigastric vein was left undivided. These groups were intended to demonstrate that the flaps as we designed them were reliable and could survive with an intact arteriovenous system.

Group 4 consisted of 20 flaps (10 thoraco-abdominal and 10 transverse abdominal flaps) raised and left attached only by a vein at either end (Fig. 4A, B). This was intended to produce a flow-through capillary venous flap perfused by a solely venous flow in a capillary venous network. The dimensions of these flaps were dictated by the location of the venous pedicles. The thoraco-abdominal flaps measured 2×5 cm while the transverse abdominal flaps measured 3×5 cm.

Group 5 consisted of 30 left flow-through venous epigastric flaps with only the inferior epigastric vein entering and exiting the flap as one main venous trunk uninterrupted by a capillary network (Fig. 5). The left

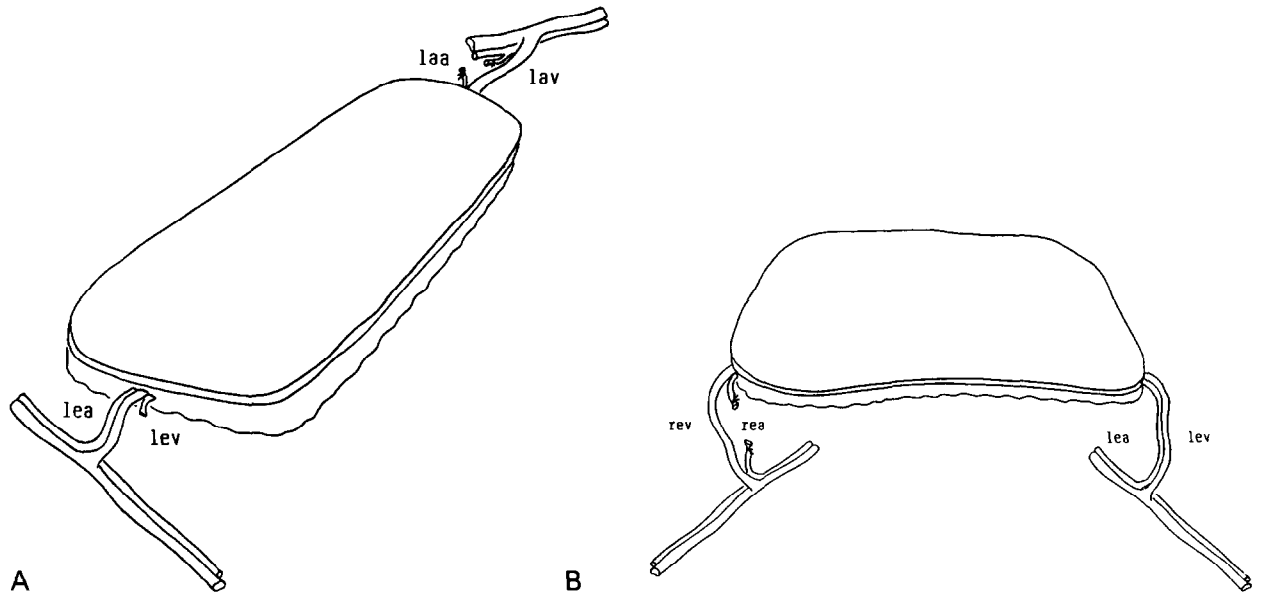

Fig. 3

Figure 3—(A) Thoraco-abdominal flap of group 3. The left epigastric vein (lev) and artery (lea) were preserved like the axillary vein (lav). The left axillary artery (laa) was ligated and resected. (The unligated cut structure is a nerve.) (B) Transverse abdominal flap of group 3. The left epigastric vein (lev) and artery (lea) were preserved like the right epigastric vein (rev). The right epigastric artery (rea) was ligated and resected.

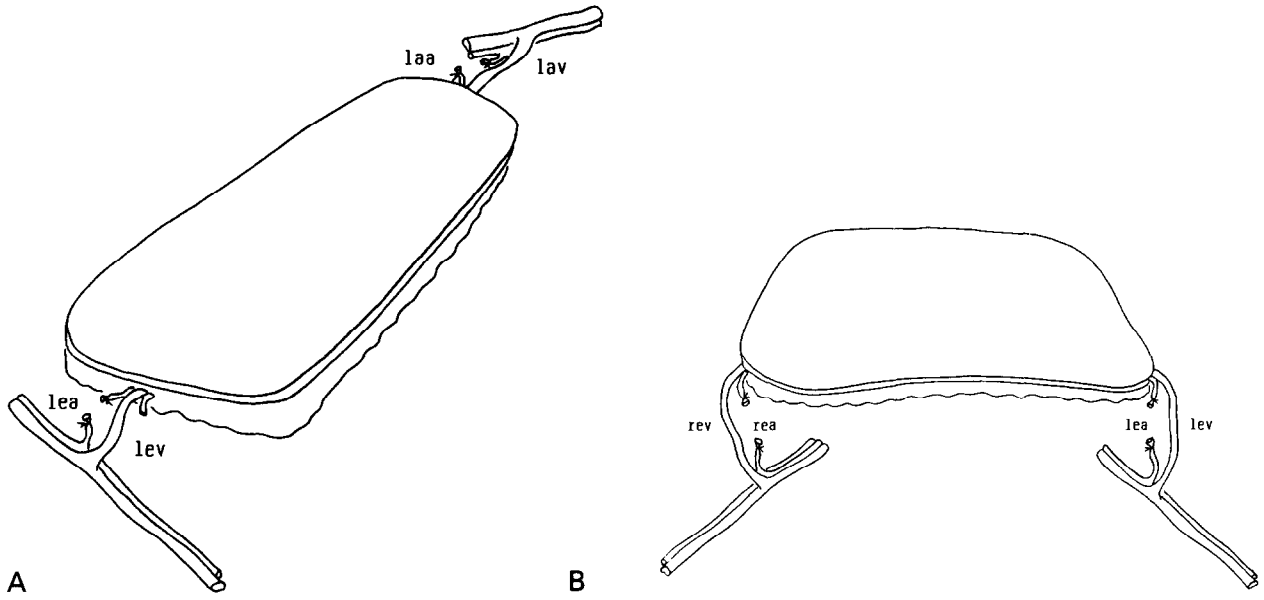

Fig. 4

Figure 4—(A) Thoraco-abdominal flow-through venous flap of group 4. The left epigastric (lev) and axillary (lav) veins were preserved. The arteries (lea: left epigastric artery, laa: left axillary artery) were ligated and resected. (The unligated cut structure is a nerve.) (B) Transverse abdominal flow-through venous flap of group 4. The epigastric veins (lev: left epigastric vein, rev: right epigastric vein) were preserved. The epigastric arteries (lea: left epigastric artery, rea: right epigastric artery) were resected.

epigastric artery was divided at both extremities of the flap. We varied the dimensions of flaps: 10 measured 4×4 cm, 10 measured 4×3 cm and 10 measured 3×2 cm. This variation in size was intended to establish the minimum volume of tissue required to ensure survival for a given dimension of vein.

Group 6 consisted of 15 left flow-through venous epigastric flaps of 4×3 cm in 15 rats, raised in identical fashion to those in group 5, which were randomised in five groups to be killed and examined histologically at 24 h, 48 h, three days, four days and five days post-operatively. The entire flap was subjected to standard histological examination.

The progress and survival of flaps were monitored by direct observation, histological examination (Table 1) and capillaroscopy (Table 2). Venous flow was monitored by means of a laser doppler (Table 2). Flaps were observed each day, when skin colour and suppleness as well as hair growth were recorded. We also noted intervals between surgery and established clinical necrosis, partial or total. Necrosed flaps were removed and examined histologically. At the time that

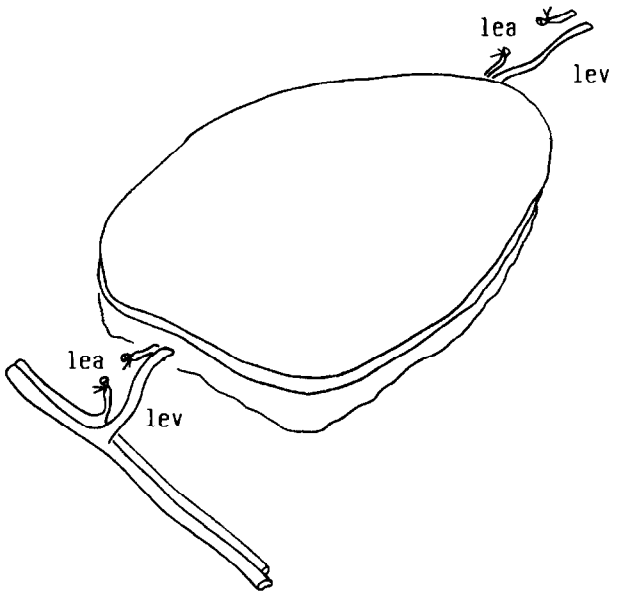

Fig. 5

Figure 5—Left epigastric flow-through venous flap of group 5. The epigastric vein (lev) was preserved crossing through the flap. The epigastric artery (lea) was resected on both ends of the flap.

flaps were removed for histological examination the veins were observed for fullness. Histological features of note included the state of necrosis of the epidermis, of skin adnexae and of skin corrugator muscle fibres, the state of dilatation, filling and thrombosis of the arterioles and venules and of the main pedicle.

Most of the flaps of groups 3, 4 and 5 were assessed once by capillaroscopy and by laser-Doppler (P.F2-Periflux-Perimed-Sueden). After electrical and biological zero calibration, the laser light-emitting probe was applied without pressure to the skin of the abdominal wall of the rat. After the steady state was reached, the basal laser-Doppler flow was recorded for five min. Laser-Doppler examination enabled verification of the patency of the capillary network or the vein but gave no indication of direction of flow.

Capillaroscopy of the skin was performed using a NACHET slit lamp biomicroscope and viewed at *20 to *80 magnification under illumination. To view the capillary network, a drop of high-viscosity immersion oil was placed on the skin to overcome the effects of surface irregularities. In 10 left epigastric venous flaps capillaroscopy was combined with fluorescein injection in a vein of the rat tail. In cases of alteration of the wall of the vessel, the fluorescein is seen outside the vessel. In each rat we performed this assessment both on the operated side and the non-operated side at a corresponding point for comparison.

Student's t test and variance analysis were applied to the recorded figures for the time it took for the onset of clinical necrosis in the flow-through venous flaps (groups 4 and 5) and in the flaps totally deprived of a blood vascularisation (group 2). We compared the three flap types in group 5 with variance analysis, and then each subgroup of 10 flaps of group 5 to group 2 with the Student's t test. Variance analysis was not used to compare flaps of group 2 or the three sizes of flap in group 5. We did not use the Student's t test to compare group 2 and the global average duration of survival of the 30 flaps of group 5.

Capillaroscopic and laser-Doppler data could not be subjected to statistical analysis since it was non- or semi-quantitative.

Table 2 Distribution of the flaps for the laser-doppler and capillaroscopy examination in relation to the group and to the postoperative day. (NA = Flaps not assessed).

	Group 1	*Group 2*	*Group 3*	*Group 4*	*Group 5*	*Group 6*
	10	10	10	20	30	15
Day 1	0	0	0	0	0	0
Day 2	0	0	2	4	4	0
Day 3	0	0	3	5	7	0
Day 4	0	0	2	4	7	0
Day 5	0	0	1	4	4	0
NA	10	10	2	3	8	15

Results

In group 1 ink flowed freely between the two venous systems in both the thoraco-abdominal and transverse abdominal types of flap. It was easy to demonstrate the communicating capillary network between the two epigastric venous systems and between epigastric and axillary systems.

All of the flaps (composite grafts) in group 2 necrosed completely within 2.8 days ($\pm$1.2 days). All of the flaps in group 3 survived totally.

Of the 10 transverse flaps in group 4, nine necrosed after an average of 4.1 days ($\pm$1.3 days). One flap survived in parts. The 10 thoraco-abdominal flaps

Table 1 Distribution of the flaps for the histological examination in relation to the group and to the postoperative day. (NA = Flaps not assessed).

	Group 1 *10 rats*	*Group 2* *10 rats*	*Group 3* *10 rats*	*Group 4* *20 rats*	*Group 5* *30 rats*	*Group 6* *15 rats*
Day 1	0	0	0	0	0	3
Day 2	0	2 necrosed	0	0	0	3
Day 3	0	4 necrosed	0	2 necrosed	1 necrosed	3
Day 4	0	3 necrosed	0	11 necrosed	9 necrosed	3
Day 5	0	1 necrosed	0	3 necrosed	11 necrosed	3
Day 6	0	0	10	1 necrosed	4 necrosed	0
NA	10	0	0	3	5	0

necrosed totally within an average of 4.4 days (±1.1 days).

In group 5, the 10 flaps measuring 4 × 4 cm necrosed totally within 4.2 days (±1.2 days) while the 10 flaps measuring 4 × 3 cm necrosed totally within 4.6 days (±1.3 days). Of the 10 flaps measuring 3 × 2 cm, eight necrosed completely within 4.9 days (±1.1 days). Two thirds of the surface area of the remaining two flaps necrosed. At the time of removal of each necrosed flap the veins were seen to be filled with non-thrombosed blood.

There was a statistically significant difference between duration of survival for groups 2 and 4 ($t = 2.203$; $p < 0.01$). There was also a significant difference in survival between groups 2 and 5, which varied with the dimension of flap in group 5:

— for flaps measuring 4 × 4 cm: $t = 2.849$; $p < 0.02$
— for flaps measuring 4 × 3 cm: $t = 3.051$; $p < 0.01$
— for flaps measuring 3 × 2 cm: $t = 3.690$; $p < 0.01$.

The differences in duration of survival between groups 4 and 5 were not significant. The differences in duration of survival between the three types of flaps in group 5 were not significant.

Histological examination of those flaps in which the arteriovenous vascularisation had been preserved (group 3) showed a fibroleucocytic exudate around the margins of the wound. The appearances of the epidermal layer and of the skin adnexae were unaltered. There was significant oedema, and an inflammatory exudate containing macrophages and mast cells, as well as vascular congestion in the subcutaneous fat. In the interface between flap and bed similar changes occurred with an inflammatory exudate rich in macrophages, interstitial oedema and vascular congestion. Both arteries and veins were dilated or normal calibre and full of non-thrombosed blood.

In flow-through venous flaps, either capillary or without a capillary bed (groups 4 and 5), the first signs of necrosis occurred in the skin adnexae, sparing intradermal muscle fibres and epidermis. The arteries were seen to be empty and collapsed. Veins were dilated and filled with non-thrombosed blood. At 48 h, two thirds of flaps showed histological features of ischaemic necrosis. Apocrine glands in the skin showed signs of vacuolating degeneration and acidophilic necrosis. The deep aspect of the flap and the adjacent bed were both markedly oedematous with a fibrinoleucocytic exudate. The capillary network was congested. The arteries were collapsed and empty, while the veins were widely dilated and full of non-thrombosed blood. At 72 h, one third of the flaps showed necrosis: muscle fibres in the skin were infarcted, all apocrine glands were totally necrosed and there was a patchy necrosis of the epidermis. At 4 days, the dermis and subcutaneous layers of the flap were completely necrosed and showed a dense inflammatory response. By the fifth day necrosis included all structures.

The Doppler laser findings showed that flaps with an intact arteriovenous vascularisation (group 3) had a 20% greater blood flow than the contralateral control side. The flow-through venous flaps (groups 4 and 5) had a 35% inferior blood flow to that of the contralateral control side. There was no difference regarding flaps of group 4 or flaps of group 5. These values were very rough approximations because anaesthesia caused a permanent tremor in the rats and thus interfered with data collection.

Capillaroscopy of the flow-through venous flaps (groups 4 and 5) showed that the capillary network was present and dilated without sign of extravasation. There were, however, fewer capillaries overall, by comparison with the contralateral side. There was no difference between flaps of group 4 or 5. Capillaroscopy of the flaps with an arteriovenous vascularisation (group 3) showed that the capillary network was not different from that in the contralateral control side. Fluoroscopic capillaroscopy provided no additional information; the absence of fluorescein free in the tissues confirmed the absence of ischaemic lesions of the capillaries.

Discussion

Within the confines of our experiment we were unable to obtain survival of flow-through venous flaps. We succeeded in demonstrating a flow of blood coursing throughout the flap exclusively within the venous system. This flow was, however, inferior to that within normal skin and to that in flaps with an arteriovenous system. This flow was sufficient to prevent capillary lesions and ensured some prolongation of survival of these flaps by comparison with that for composite grafts deprived of all blood supply. It appeared from our studies that the smaller the flap, the better the survival, but this tendency was not statistically significant. In practical terms, despite this trend, all flow-through venous flaps with or without capillary network showed necrosis.

There is sufficient oxygen in venous blood to maintain adequate oxygenation of tissues, provided this oxygen is made available at capillary level.[10] It is therefore also theoretically possible to nourish a flap exclusively on venous blood. Skin necrosis results not only from tissue ischaemia but also from a dysfunction of the microcirculation.[11] Even in ischaemic tissue, blood bypasses the capillary beds, travelling through arteriovenous shunts caused to dilate by the ischaemia. The result is poor tissue nutrition. Vascular tone is restored by the tenth to the 14th day, but by then the flap must have survived through the critical period.

In 1985, Baek[10] put forward the theory that venous flaps survive on oxygenation provided by alternating flow and stagnation in the capillary bed. Given the fact that venous blood contains enough oxygen to nourish tissue, Baek has postulated the concept of an intermittent flux with periods of stasis in the capillary network, during which exchange occurs. The work of Amarante[12] has confirmed that flaps with an exclusively venous vascularisation can survive, but they need an entry and an exit vein.

Sasa[5] has postulated that rearterialisation is encouraged by an efficient run-off via venous drainage. Even without demonstrable flow from veins to the capillary network, the venous capillary network seems

able to nourish tissues adequately.[5] Another theory to explain flap survival centres on vascular communications via the flap edges and bed. Arterial flow is re-established by the third day and capillary flow must soon follow, with a rapid resumption of normal nutrient exchange.

Flow-through venous flaps have been successfully used as pedicled or free flaps in dogs' thighs.[10,12] Sasa *et al.*[5] were not successful with saphenous flow-through venous flaps, but they observed necrosis in only 2 of eight cephalic venous flaps. They concluded that a healthy bed seems to promote vascularisation. Chow *et al.*[6] were successful in 25% of flow-through venous flaps when increasing the venous blood flow with end-to-end anastomosis between the distal stump of the femoral vein and the epigastric vein. This could explain the failure of our flow-through venous flaps models where the blood flow was not increased.

Successful clinical cases have been reported.[1,2,7,12,13] In a number of cases it is unclear whether the venous pedicles were raised to include surrounding fat which could have contained an arterial microcirculation. The experimental work of Noreldin *et al.*[3] has confirmed the importance of the perivenous areolar tissue in perfusion of the skin island in the rat inferior epigastric venous flap. Honda[7] has used skin and subcutaneous tissue as venous carriers for digital replants including dorsal skin loss, with success in two out of five cases. In effect, this was equivalent to a free transfer of a flap with an exclusively venous vascularisation. The survival of those flaps was related to the augmentation of blood flow and pressure in a replanted digit. This explanation seems to be supported by Chow *et al.*[6]

In experimental conditions on rats and without augmentation of blood flow, the survival of exclusively flow-through venous flaps was not obtained despite the venous blood flow coursing throughout the flap. The survival observed in clinical reports could be explained by the conjunction of arterial microcirculation preservation in perivenous fat, augmentation of blood flow and pressure, and by exchanges with the recipient bed.

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