Relationship between flow and incidence of thrombosis in polytetrafluoroethylene vascular grafts in free microvascular flaps in lambs

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ABSTRACT

We have done an experimental study in lambs in which we investigated the influence of flow rate on free microvascular flaps using polytetrafluoroethylene (PTFE) vascular grafts. We set up five surgical groups in which blood flow was progressively increased through the PTFE vascular graft. In group I (venous autograft) we observed just one vascular thrombosis which was located at the site of the anastomosis. In group II (PTFE 3 x 10 mm) all the microvascular flaps became necrosed after the third postoperative day. In group III (PTFE 3 x 10 mm) necrosis also developed in all cases, but the anastomoses remained permeable no longer than eight days. In group IV (3 x 15 mm) the permeability in the microvascular free flaps was about 40% after 21 days, and in group V (3 x 10 mm) it reached 70%. To match graft flow rates with flap survival we did a regression analysis of flow rates for groups II, III, and V and the corresponding survival periods for the flaps. There was a clear and highly significant relationship ($r = 0.717$, $p = 0.0001$). In conclusion, it is necessary to maintain blood flow through the prosthesis at a rate higher than the thrombogenic threshold. When the flow rate in the vessels through the PTFE grafts was higher, the viability of the flaps was better. The ideal surgical technique should always be based on an arteriovenous fistula distal to the PTFE vascular graft. It is necessary to maintain blood flow through a prosthesis at a rate higher than the thrombogenic threshold.

KEY WORDS

PTFE, free flaps, flowmeter, synthetic vascular grafts, lambs, iliac flap, microsurgery.

The transference of free vascularised tissues is sometimes impaired because recipient vessels are not in good condition, either because they have been lacerated by injury or because they are affected by vascular disease. In these cases it is necessary to use autologous vascular grafts to transport blood from recipient vessels to the flap that has to be vascularised (22).

Results obtained using autologous vein grafts are satisfactory, but they require new incisions that may cause functional and aesthetic repercussions; operating time is lengthened, morbidity is increased, and the saphenous vein cannot be used in future for coronary revascularisation. Moreover, the patient's veins may not meet the ideal conditions for this procedure because of thrombophlebitis, varicose dilations, secondary obstruction and inflammation, or previous radiation. Biological alternatives and synthetic vascular grafts have been studied as possible substitutes for autologous vascular grafts. Synthetic vascular grafts made of expanded polytetrafluoroethylene (PTFE) are by far the most widespread in vascular surgery (18, 21), but they are still at the research stage in microsurgery. The permeability of a bypass is the result of a complex equation in which, apart from metabolic, technical, and pharmacological factors, haemodynamic factors also have an important role (4, 6), including the capacity of the donor artery; graft length; blood velocity; characteristics and calibre of the graft (10); competitive flow in the distal area; peripheral resistance; and porosity (12).

The substitution of synthetic vascular grafts for small calibre arteries has been limited by short and long term thrombosis. Short term occlusions are produced by the formation of a platelet thrombus. Long term ones are secondary to development of intimal hyperplasia, which gradually closes the vessel's lumen (3, 17). Lately it has been shown that intima hyperplasia may also be caused by the outer structure of the PTFE (23).

The aim of this study was to show that it is possible to improve permeability rates in the short term in synthetic vascular micrografts, and to achieve vascularisation of free flaps for long enough to allow complete scar formation. To achieve this, we set up five groups in which the blood flow in the prostheses was progressively increased (increasing perfusion pressure and decreasing distal peripheral resistance in the graft with an arteriovenous fistula), to prevent thrombosis.

MATERIAL AND METHODS

A total of 63 lambs (Ovis aries) were studied divided into five groups. We designed a fasciocutaneous microvascular flap from the lumbar region based on the cutaneous branch of the deep circumflex iliac artery. Two types of vascular micrografts were used: in group I a venous autograft was obtained from the animal itself. In the other groups synthetic vascular grafts made of expanded PTFE were used. The internal diameter was 3 mm; wall 0.39 mm thick; 10 cm long for groups II, III, and V; and 15 cm for group IV.

In our anticoagulation protocol we combined intraoperatively acetyl salicylic acid 250 mg/24 hours with heparin 1500 units as a bolus. In the postoperative period, heparin was replaced by acenocumarol (4 mg orally) because it was difficult to keep an intravenous line open in sheep.

Surgical techniques

The groups studied were as follows: group I: An interposition vascular graft made of autologous vein was placed in the artery of the flap. After the flap had been dissected, the artery of the flap was sectioned and a saphenous vein graft was placed in an inverted position. We made end to end anastomoses, using 9/0 nylon (Fig. 1). Group II: An interposition PTFE vascular graft was placed in the artery of the flap. After the flap had been dissected, a PTFE vascular graft (10 cm long and 3 mm diameter) was placed in the artery of the flap (Fig. 1). Group III: An interposition PTFE vascular graft was placed from the femoral artery to the artery of the flap. The PTFE graft was anastomosed end to side to the femoral artery. The other end of the synthetic vascular graft was anastomosed end to end to the artery of the flap. For the femoral artery, continuous 7/0 nylon was used. For the artery of the flap we used interrupted 9/0 nylon (Fig. 1). Group IV: A PTFE interposition vascular graft was placed from the femoral artery to the femoral vein, and a latero-terminal anastomoses made from the PTFE vascular graft to the artery of the flap. An arteriovenous fistula was fashioned by anastomosing the PTFE vascular graft from the femoral artery to the femoral vein. An end to side anastomosis was made on the artery while an end to end anastomosis was made on the vein, both with 7/0 nylon. The artery of the flap was anastomosed end to side in the mid point of the PTFE bypass (Fig. 1). Group V: A PTFE interposition vascular graft was placed from the femoral artery to an artery from which the artery of the flap originated. The PTFE vascular graft was anastomosed end to side to the femoral artery and end to end to an arterial branch from which the artery of the flap originated. From this arterial branch an arteriovenous fistula was made to a vein (Figs. 2 and 3).

Evaluation of results

To assess the effectiveness of each surgical technique we studied the following variables: blood flow through the vascular graft and through the artery of the flap; survival of flap; and site of thrombosis when it appeared. Function was monitored and histological studies made.

Flow rate measurement (15 minutes after concluding anastomoses). Flow rate measurements were taken intraoperatively in all surgical models at the end of the operation with a 5 mm probe connected to Cliniflow II, Model FM 701D Electromagnetic Blood Flowmeter (Carolina Medical Electronics, Inc.) transducer. Results were collected in ml/minute. Two reference points were taken at the exit of the PTFE vascular graft and at the artery of the flap to assess the flow that reached the flap with each surgical technique: the first at the exit of the PTFE vascular graft; the second at the artery of the flap.

A descriptive statistical analysis of the sample was then carried out (arithmetic mean, SD, and SEM of the sample) as well as a paired comparative statistical analysis of the flow rates obtained for each group. Given that 10 sample elements/group was at the limit of the statistical norm, non-parametric, unpaired, comparative studies were made using the Mann Whitney test.

Clinical control (daily). Flaps were monitored daily by colour and temperature control of the flap with respect to the surrounding cutaneous regions, a common technique in monitoring flaps in clinical practice.

Histological evaluation. Histological studies of the vessels were made in all groups to enable us to locate incipient thromboses and to see their morphology.

The samples were taken when the thrombosis of the vessel appeared and three weeks after the operation (maximum follow-up time), and they were stored in formaldehyde for subsequent histological study.

RESULTS

The results were assessed for a total of 50 lambs out of the 63 on which we operated. The remaining 13 animals were discarded for the following reasons: five animals were used for fine-tuning the surgical technique in the different groups. These animals were not assessed to avoid distortions of the results by technical errors. Another seven animals were rejected because they showed symptoms of infection. There was an animal in group IV that was not assessed because the occlusion of the graft was interpreted as a fault in the surgical technique (Table I).

Flow rate measurement

Flow within the vascular graft

The flow rates differed significantly for all groups except between groups I and II (Table II).

Flow within the flap

The flow rates differed significantly between the following pairs of groups: I—III, I—V, II—III, II—V, II—IV, and IV—V (Table III).

Results of clinical monitoring

Colour and temperature of the free microvascular flap were evaluated daily. Any sign of ischaemia in the flap was interpreted as a thrombosis of the vascular graft and this was corroborated surgically in all groups.

In group I there was one vascular thrombosis located at the site of the distal anastomosis (90% permeability). In group II all the microvascular flaps became necrosed after the third postoperative day. In group III all the microvascular flaps also became necrosed although they remained permeable no longer than eight days. In group IV, the permeability in the microvascular free flaps was about 40% after 21 days. In group V the permeability rate reached 70% (Table IV). The pedicle of the flaps in groups IV and V remained permeable 14 and 16 days, respectively (Table V).

To match graft flow rates with flap survival a regression test was carried out between flow rates for groups II, III, and V and their corresponding survival periods for the flap. There was a highly significant relationship ($p = 0.0001$, $r = 0.717$) (Fig. 4).

Histological findings

Microscopically, the most delicate point was located at the distal anastomosis of the PTFE interposition vascular graft. When the vascular graft became occluded, this was not the result of fibrous platelet deposition in the lumen, but rather the formation of a platelet thrombus at the level of the distal anastomosis. Curiously, in groups II and V, where flow increased at the same rate both at the proximal and distal ends, thrombosis was always produced at the latter (Fig. 5a, b).

DISCUSSION

PTFE as a vascular graft

Among synthetic vascular grafts, PTFE offers the best results in cardiovascular surgery which was why we chose it for this study. However, with the smaller calibre thromboses have been described in the short and long term (16). Despite this, the PTFE microvascular graft has been used in clinical practice (11). PTFE is marketed by several manufacturers and can be obtained in diameters ranging from 1 mm to 12 mm. For our study we chose PTFE grafts with an internal diameter of 3 mm and a length of 10 and 15 cm.

Anticoagulation method

We based the anticoagulant protocol on the work by Barry et al. (1) who reported considerable improvement in permeability of synthetic vascular grafts with aspirin and heparin respectively.

Following previous studies, anticoagulant treatment improves viability of synthetic vascular grafts. This was corroborated by Barry et al. in 1981 who obtained 0% permeability in a PTFE micrograft in a rat carotid artery which increased to 50% after heparin was given (1). The postoperative use of low molecular weight heparin seems to inhibit intimal hyperplasia, and this also has interesting effects on patency and cellular coverage (25).

Flow rate through the vascular graft

In our study, four different surgical techniques were designed with the intention of increasing flow through a PTFE vascular graft. The two main factors that influenced the increase of flow in the graft were: anastomoses of the proximal end of the vascular graft

to the femoral artery (because of an increase in perfusion pressure), and a decrease in peripheral resistance caused by an arteriovenous fistula in the distal end of the PTFE vascular graft.

Flow rate was measured at the distal end of the vascular graft. Flow rates obtained in groups II and III were lower than 100 ml/minute; in all cases the grafts thrombosed. The flow in group IV always remained above 300 ml/minute and all grafts were permeable. In group V mean values of 174.4 ml/minute were obtained, with 30% thromboses in this group. These figures point to a direct relationship between blood flow through the vascular graft and a tendency towards thrombosis: the greater the flow, the less the thrombosis.

To achieve high flow in the vascular graft, an arteriovenous fistula was created at the dista] level in groups IV and V. This idea was first devised in 1979, when Kunlin and Kunlin (9) suggested the temporary use of an arteriovenous fistula to improve permeability in synthetic vascular grafts in veins. In 1984, Gloviczki et al. (7) used an arteriovenous fistula distal to the synthetic vascular graft to improve permeability of PTFE vascular grafts placed in the vena cavas of dogs. Permeability improved from 25% to 75%. The same technique was applied to microsurgery by Cuadros and Hughes James in 1986 (5), who created an arteriovenous fistula at the proximal end of a PTFE vascular graft with a diameter of 1.5 mm, placed as a venous substitute, which improved results from 0 to 100%.

In this study we obtained permeabilities of 0% at 21 days in groups II and III, 70% in group V, and 100% in group IV for a 3 mm diameter, 10 cm long PTFE vascular graft. It seems that the limit for thrombosis of the vascular graft is between 100 ml/minute and 200 ml/minute for group IV. Below these values, there was always thrombosis and above them permeability approaches 100%, while the flap necrosed in 60% of cases. We think that this could be explained by the thrombosis found in the area of the anastomoses of the PTFE vascular graft with the artery of the flap. This fact can probably be explained by the fluid dynamic theory, according to which the current follows in laminar form. This means that the blood that is in contact with the vessel wall moves at a slower rate than the column of blood located in the centre of the vessel (2). Blood flow rate along vessel walls does not therefore surpass the thrombogenic flow rate threshold of the laterotermínal anastomoses whereas the central column of blood in the end to end anastomoses does. We think that other factors such as the turbulence caused by the end to side anastomoses also had a major role in the thrombosis of group IV.

Other factors that may influence thrombosis are alterations secondary to the surgical trauma, ischaemia, desiccation, pH, and temperature changes undergone by the arterial wall during the anastomoses (2).

Flow rate through the artery of the flap

Flap flow rate measurements were taken in the artery of the flap. These flow rates depended on pressures existing in the vascular graft.

The highest values were obtained in group III. However, thrombosis developed in all animals in this group. We interpret this fact as being due to the capacity for absorbing the flow on the part of the vascular graft being lower than the thrombogenic flow rate threshold for the 3 mm diameter PTFE vascular graft. An arteriovenous fistula distal to the graft was therefore needed to increase blood flow.

Rao et al. (19) showed that the peripheral vessel resistance of a flap has a blood flow absorption limit that cannot be increased regardless of how much arterial pressure is augmented in the vessel pedicle. In flaps with a greater surface or in muscle flaps in which there are more arteriovenous connections, less peripheral resistance may be found, as well as more flow through the PTFE vascular graft, so that a distal arteriovenous fistula is not needed.

Mean flap survival increased gradually with the different surgical techniques used. The shortest period for which a PTFE vascular graft remained permeable was three hours and the longest three weeks, when the study was ended.

There is no doubt that the results obtained with autologous venous grafts are better than those with PTFE vascular grafts. However, they have certain limitations. The use of PTFE vascular grafts with the techniques used in groups IV and V offer permeability of the primary pedicle at 14 and 16 days, respectively. During this time, vessel connections have formed to feed part of the free flap, which means that the covering function will be achieved through partial scar formation in the flap.

Histological study

We think that the thrombosis may be influenced by several procoagulating factors that meet at the distal anastomoses. Sutures may damage the endothelium, and that encourages the formation of small thrombosis at the level of the anastomoses, both in the proximal and distal ends (8). This phenomenon and the activation of platelet adhesiveness in the area of the PTFE vascular graft, make the anastomoses particularly prone to production of a platelet thrombus (2). Endothelial lesions caused by surgical trauma were described by Melka et al. as early as 1979 (14), and this was later corroborated in 1981 by Lidman and Daniel (13), who also showed partial necrosis of the media and the adventitia.

An explanation of how this always occurs distally can be found in the balance between prostaglandin I_2 (PGI_2) and thromboxane A_2 (TXA_2) (3). PGI_2 is the greatest platelet aggregation inhibitor and vasodilator known. It is segregated only by endothelial cells. TXA_2 is the most effective procoagulant factor known and it is segregated by the platelets. In the proximal anastomoses, secretion of PGI_2 by endothelial cells proximal to the anastomoses avoids formation of a thrombus. In the distal anastomoses, the platelets are activated by contact with PTFE, so TXA_2 secretion is activated (24) and a thrombus is formed because the endothelium has been denuded by the surgical trauma. The endothelial cells that are capable of producing PGI_2 are distal to the anastomosis and the action of this hormone can be neutralised by the washing effect. The same conclusions were reached by Van der Lei et al. (26) in 1989, in a study on the carotid artery of a rabbit. Bush et al. (3) confirmed the divergence between TXA_2 and PGI_2 in

the distal anastomosis, comparing a control group with a dissected artery with a group that had a PTFE vascular graft.

The cliniflow method

For taking measurements of the blood flow at the vascular graft and at the artery of the flap, we used an electromagnetic flowmeter. The qualitative presentation of blood flow waveforms from vessels presents a few problems when using the electromagnetic flow probe.

Zero line stability is affected by interference, gating effects, motion artefacts, eddy currents, and insulation defects. Variations in sensitivity occur as a result of interference, gating defects, amplifier input impedance, and poor wall contact. Sensitivity will also be reduced if the vessel walls are electrically conductive because of shunting of part of the induced current. Serious effects on zero line stability and sensitivity can be experienced in flowmeters if different types of magnetic field excitation are used simultaneously. The magnitude of this artefacts depends on the distance and angle between the probes: a separation at least 10 cm is required. If the same types of flowmeters are used their magnetic fields should be synchronously driven.

The exact blood flow value depends on the anatomical location, vessel size, and the state of the cardiovascular system at the time of measurement, so a relatively large range of blood flow values is found in the same vessel in different patients (20).

The most suitable diameter of cuff-type probes is about 10% smaller than the outside diameter of the blood vessel to be monitored.

These should be calibrated with values of the packed cell volume within the reference range. An increased packed cell volume results in a reduced flow signal and a reduction produces an increase in the flow signal (27).

Magnetic field variations along the flow axis also reduce the signal from the flow head by allowing circulating currents to flow within and outside the flow head. The transducer must be long enough to avoid this, or the effect taken into account in calibration (15).

CONCLUSIONS

To vascularise a free microvascular flap with a PTFE microvascular graft, it should be necessary to create an arteriovenous fistula distal to the vascular graft to increase the flow above the thrombogenic threshold.

It is necessary to maintain blood flow through a prosthesis at a rate higher than the thrombogenic threshold.

The most controversial point in PTFE micrografts is the distal anastomosis. Thrombosis in the immediate postoperative period is due to formation of a platelet thrombus at this level.

Endothelial lesions produced at the distal anastomosis by operatives injuries is a factor which favours thrombosis.

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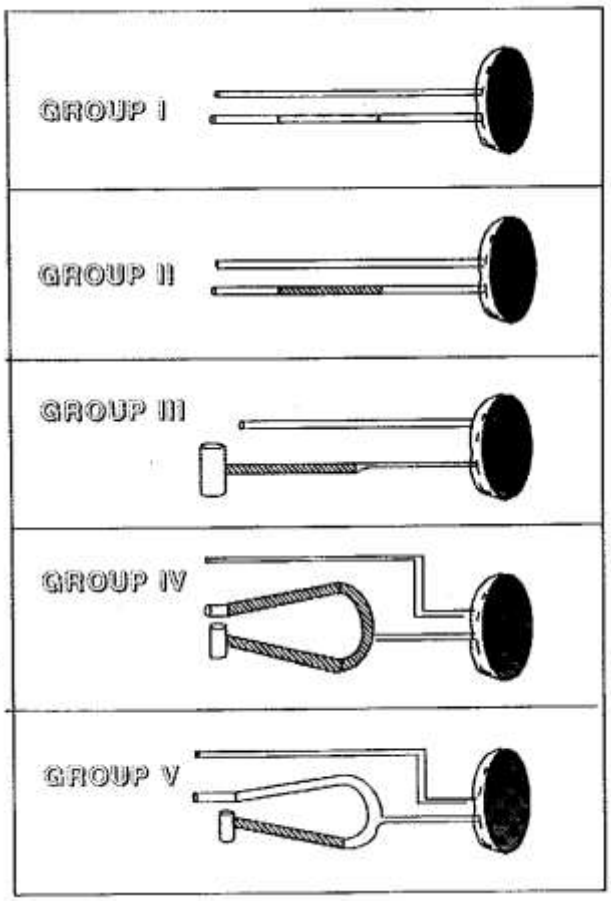

Figure 1. Diagram of the five microflaps designed for this study.

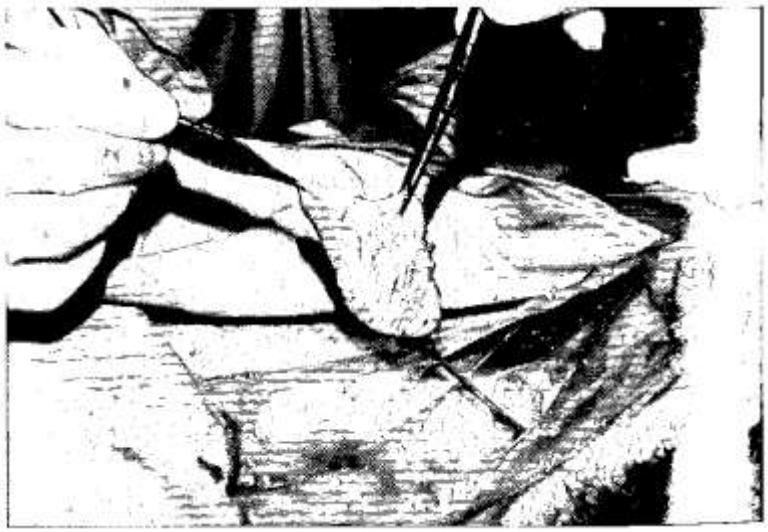

Figure 2. Fasciocutaneous flap vascularised through the cutaneous branch of the deep circumflex iliac artery.

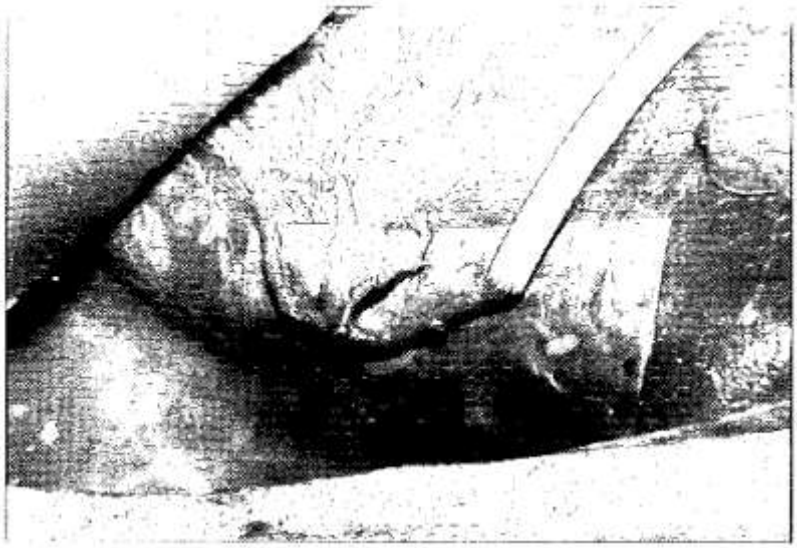

Figure 3. (Group V). PTFE vascular graft from the femoral artery to an artery from which originates the artery of the flap (at the fork).

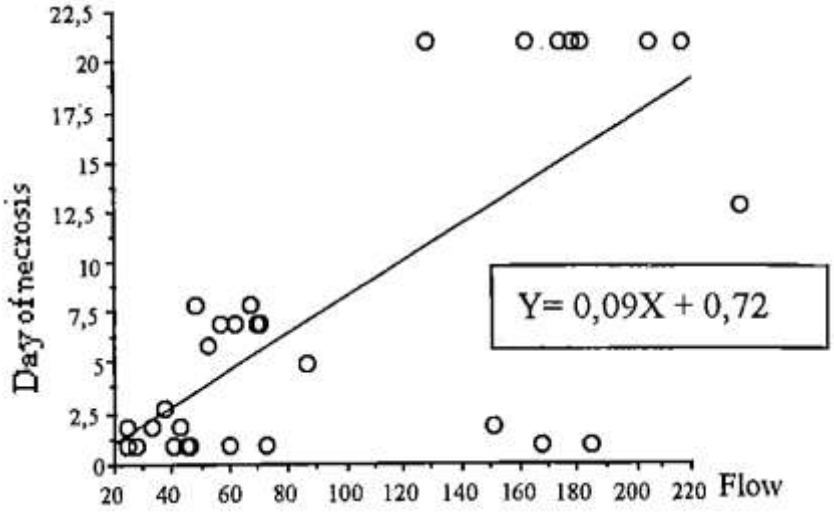

Figure 4. Linear regression between flow rates obtained and observed skin necroses.

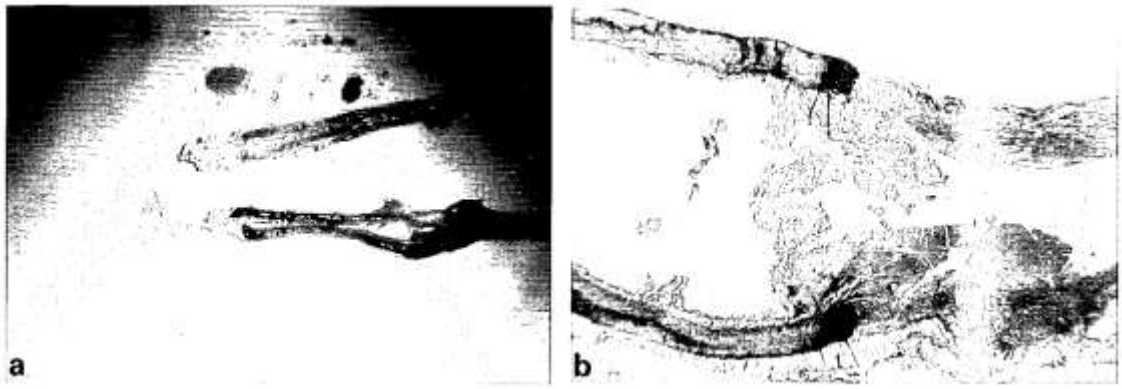

Figure 5. (a) Distal end of the PTFE graft, located in the lower part of the picture, with a platelet thrombus occluding the lumen. **(b)** Histological findings.

Table I. Animals killed, evaluated, and rejected						
	Animals operated on	**Animals evaluated**	**Animals rejected**	**Animals for surgical study**	**Technical failures**	**Infections**
Group I	11	10	1	1	0	0
Group II	13	10	3	1	0	2
Group III	13	10	3	1	0	2
Group IV	14	10	4	1	1	2
Group V	12	10	2	1	0	1
Total	63	50	13	5	1	7

Table II. Mean flow (SD, SEM) (ml/minute) of the microvascular graft in each group		
Group	**Lambs**	**Mean (SD, SEM) ml/m**
I	10	37.8 (14.3, 4.5)
II	10	35.2 (8.0, 2.5)
III	10	63.9 (11.2, 3.5)
IV	10	473.9 (98.7, 31.2)
V	10	174.4 (25.3, 8.0)

Table III. Mean flow (SD, SEM) (ml/minute) of the artery of the flap in each group		
Group	**Lambs**	**Mean (SD, SEM) ml/m**
I	10	37.8 (14.3, 4.5)
II	10	35.2 (8.0, 2.5)
III	10	63.9 (11.2, 3.5)
IV	10	40.9 (13.0, 31.2)
V	10	64.4 (11.2, 8.0)

Table IV. Days during which the flap showed no evidence of ischaemia. Numbers 1 to 10 show each animal in each group

	Animals										
Group	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Flap necrosis**
I	21	21	5	21	21	21	21	21	21	21	1
II	2	2	3	1	2	1	1	3	1	1	10
III	7	8	1	1	7	7	7	6	5	8	10
IV	1	7	7	14	21	13	21	6	21	21	6
V	1	21	1	21	2	21	21	21	21	21	3

Table V. Mean (SD) number of days before flaps necrosed in each group

Group	**Mean (SD) no. of days**
I	19.4 (5.0)
II	1.7 (0.8)
III	5.7 (2.6)
IV	13.2 (7.6)
V	15.1 (9.5)