THE EXPERIMENTAL USE OF HETEROLOGOUS UMBILICAL VEIN GRAFTS AS AORTIC SUBSTITUTES

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Although arterial homografts have been successfully used in the replacement or bypassing of diseased arteries, long-term observations have produced disquieting reports of progressive graft degeneration in the form of calcification, atherosclerosis, aneurysmal dilatation and rupture. In the peripheral arteries, homografts have been associated with a disappointingly high incidence of late occlusion. In a search for other suitable biological substitutes autogenous and homologous vein grafts, autologous tissue tubes fashioned from fascia, skin, rectus sheath and pericardium have been tried. Of these, the autologous vein graft is regarded by Dale (1959), Hitchcock et al (1959), Jones and Dale (1958), Linton (1959), Nyhus and Harkins (1957) amongst others as the graft material of choice for peripheral arterial substitutes. Venous grafts in the aorta have been found unduly susceptible to aneurysmal dilatation. Experiments with heterologous and homologous vein grafts have moreover met with poor results.

The report of Voorhees and Blakemore (1952) on the successful experimental use of tubes tailored from a plastic fabric, 'Vinyon-N', aroused widespread and intense interest in plastic prostheses and eventually led to the development of seamless, crimped plastic prostheses of Dacron or Teflon. They have been found eminently satisfactory in aortic replacements but opinion differs with regard to their place in peripheral arterial surgery. DeBakey et al (1958) have used Dacron tubes extensively and with great success but other workers (Linton, 1959; Dale and Mavor, 1959; Foster et al, 1960; Dale and Niguidula, 1959; Julian et al, 1959; Hohf et al, 1958; Edwards, 1960) have not found them superior to autologous vein grafts or even arterial homografts. These synthetic grafts are as well extremely expensive and therefore not suitable for widespread use in some parts of the world

Although homologous and heterologous vein grafts have had no advocates, we have thought it worthwhile to investigate in the experimental animal the possible value of umbilical vein grafts as an arterial substitute. The rationale for using this source of vascular substitute was: (1) its easy supply in fresh umbilical cords; (2) the tough fibrous amniotic covering of the umbilical cord which reinforces the vein itself; and (3) the alleged minimal antigenicity of foetal tissue or tissue from a new-born animal.

METHOD

Human umbilical cords were collected immediately after delivery under clean but not sterile conditions and placed in sterile saline containing one mega unit Penicillin and one gram Streptomycin per 500 ml. of saline. Both fresh and alcohol-preserved cord grafts were used. The latter were preserved in absolute alcohol and reconstituted in normal saline at room temperature for half an hour before implantation.

Adult mongrel dogs, ranging from 10-15 Kgm. in weight were anaesthesised with pentobarbitone. Through a midline incision, the abdominal aorta was approached transperitoneally and the portion between the renal arteries and the trifurcation isolated. Usually one or two pairs of lumbar branches had to be divided between ligatures but the inferior mesenteric artery was left intact. A short segment, 0.5-1.0 cm. long, was excised between Potts ductus clamps and a 2.0-2.5 cm. segment of umbilical vein graft implanted, using a continuous over-and-over suture of 5-0 arterial silk for the anastomoses. On the graft side, the sutures passed through the combined thickness of the amniotic covering of the cord and the vein wall. Despite the disparity between the thicknesses of the graft and host vessels, it was possible to ensure a smooth anastomosis by making sure that every stitch passed through the intima of the umbilical vein.

On completion of the anastomoses, the distal clamp was released first followed shortly by the proximal clamp. Bleeding from the suture lines was characteristically minimal and controlled by direct pressure. Heparin was not utilized. The graft could be seen and felt pulsating and usually looked dilated after release of the clamps because of the greater bulk of the surrounding tissues of the umbilical cord (Fig. 1). Immediately after operation the dogs were given a single dose of one mega unit Penicillin and one gram Stretptomycin. The animals were followed for up to 3 months.

The grafts were studied by direct and histological examination when the animals died or

were sacrificed. Arteriography was carried out in one dog at the time of sacrifice.

RESULTS

Aortic replacements with heterologous human umbilical veins were carried out in 15 dogs; 4 fresh grafts and 11 preserved in alcohol were thus studied. Pulsation was felt in the graft at completion of the anastomosis in all but one (dog 3) in which technical difficulties resulted in prolonged aortic occlusion leading to immediate graft thrombosis.

Six dogs died within the first post-operative week; 2 from haemorrhage, due to disruption of the anastomoses; 2 from abdominal wound dehiscence; 1 from massive thrombosis of the graft; and the last from a complication from anaesthesia.

TABLE I

Deaths in the first post-operative week.

Total Number of Dogs grafted: 15.	Deaths: 6
Causes:	
Haemorrhage from suture line:	2
Massive graft thrombosis:	1
Abdominal wound dehiscence:	2
Anaesthesia:	1

Of the 15 aortic heterografts, 12 were available for long-term study. The results are summarised in Table II.

Only 1 heterograft remained patent for more than 2 months, but had undergone significant dilatation. A glistening pearly-white translucent membrane extended across the suture line from the host aorta at either end for 3-4 mm. The remainder of the graft was lined with a red fibrinous layer (Fig. 2). In the remaining 11 grafts, thrombus formation had caused complete occlusion in 3, partial occlusion in 6 and mural thrombi in 2. No significant dilatation was found in those grafts which had mural thrombi but were still patent (Fig. 3). Those grafts completely or partially occluded were not dilated.

All the grafts in animals that survived for more than one week were densely adherent to surrounding structures and in particular to the inferior vena cava (Fig. 4). One graft (dog 13) was adherent to a multilocular cyst with a thick dense fibrous wall and containing brownish fluid (Fig. 5). No direct communication could be established between the cyst and the graft lumen. It was thought that this cyst had resulted from a haematoma around the graft caused by leakage from the suture line. The graft itself was completely occluded by thrombus (Fig. 6).

HISTOLOGICAL EXAMINATION

Longitudinal paraffin sections of the grafts were stained with haematoxylin and eosin and by Verhoeff's method for elastic fibres.

Marked necrosis was a prominent feature in all the grafts examined and nuclear fragments were only rarely present. A chronic lymphocytic inflammatory infiltrate was present in both the adventitial layer and along the anastomotic sites. Thrombi adherent to the internal surface were present to varying degree in all cases.

TABLE II

Period Implanted	Number	FAILURES			Patent
		Complete thrombosis	Partial	Murah Thrombi	
2 weeks and less	5	1	3	1	—
2 weeks to 1 month	2	1	—	1	—
1 month to 2 months	2	1	1	—	—
2 months to 3 months	3	—	2	—	1
TOTAL	12	3	6	2	1

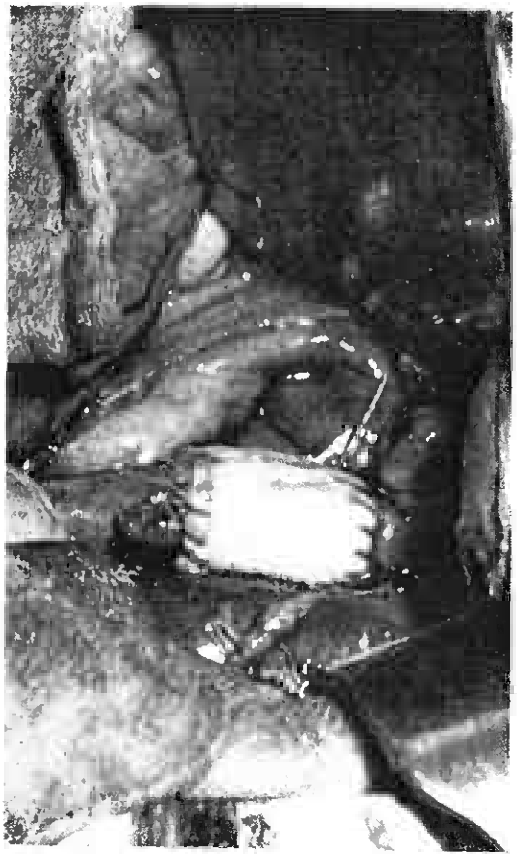

Fig. 1. Umbilical vein graft immediately after implantation. The disparity between diameter of the graft and that of the host aorta is clearly shown.

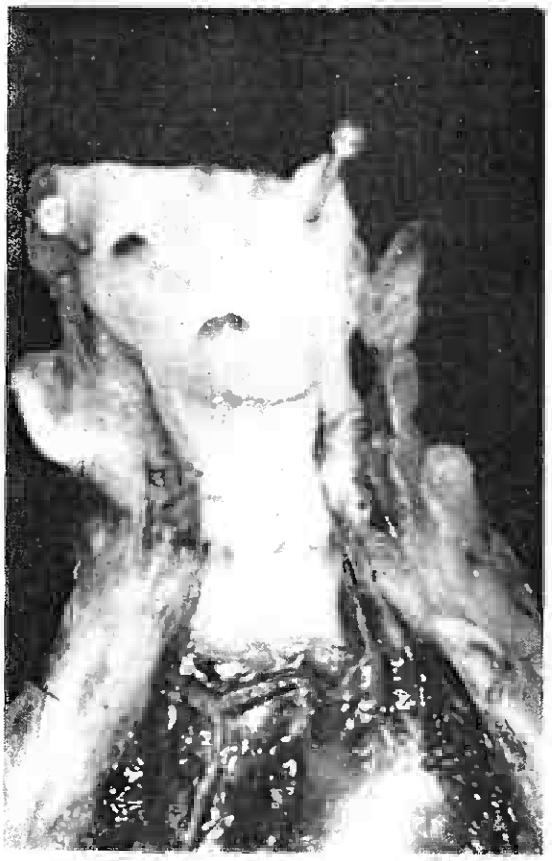

Fig. 3. The upper part of a partially patent graft. The suture line and the adjacent 5-6 mm. of the graft are lined by endothelium. A thick layer of thrombus lines the rest of the intimal surface.

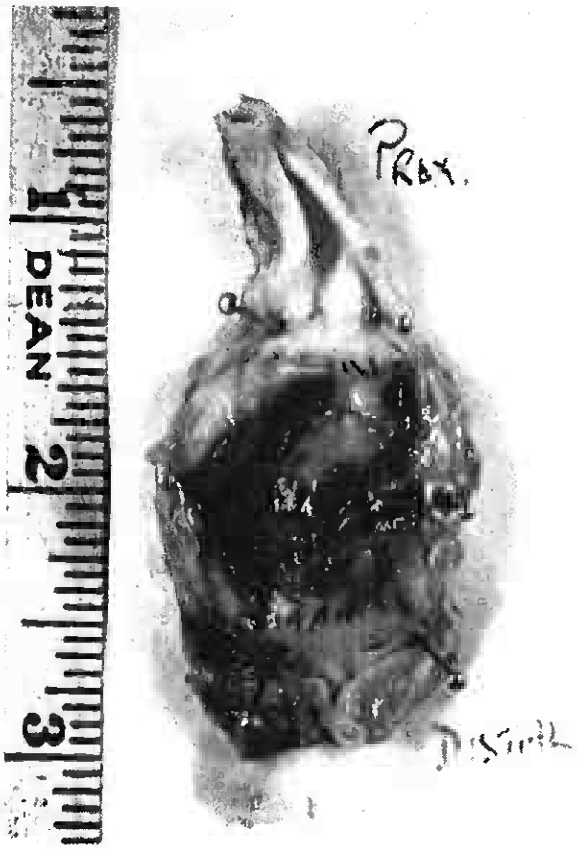

Fig. 2. Graft No. 14. The suture lines are covered by a smooth glistening translucent layer of endothelium. The rest of the graft surface is lined by a thin layer of red fibrinous thrombus. There is marked dilatation of the graft.

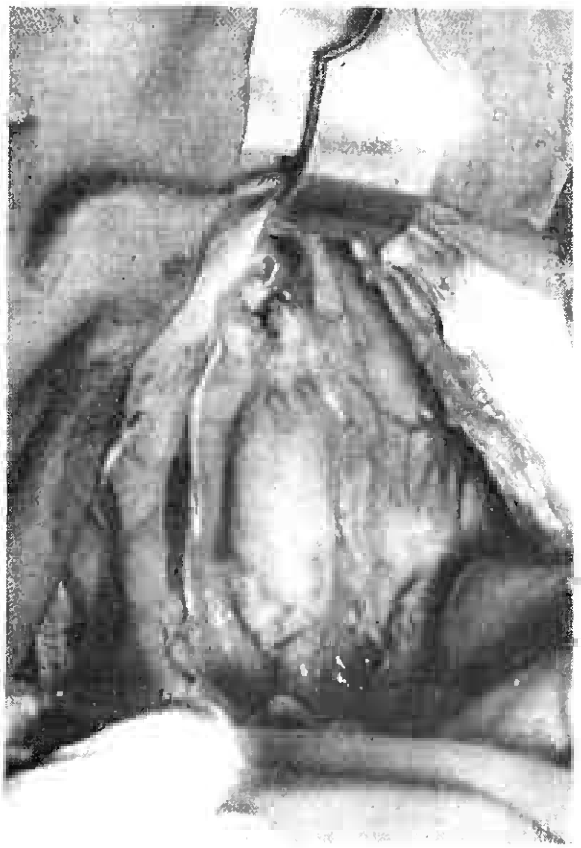

Fig. 4. Graft in situ at the time of re-exploration two months after implantation. There is dilatation of the graft which is densely adherent to adjacent structures.

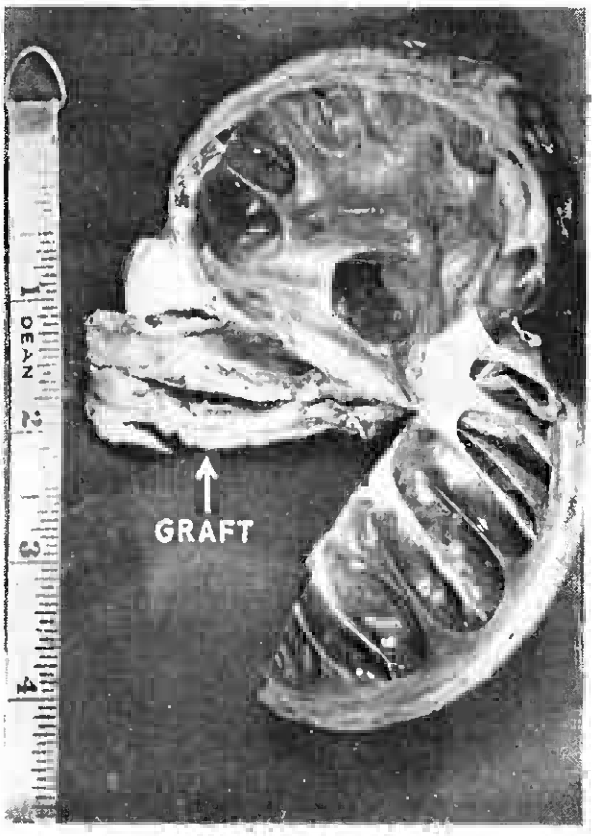

Fig. 5. Thrombosed graft attached to a multilocular cyst (see text).

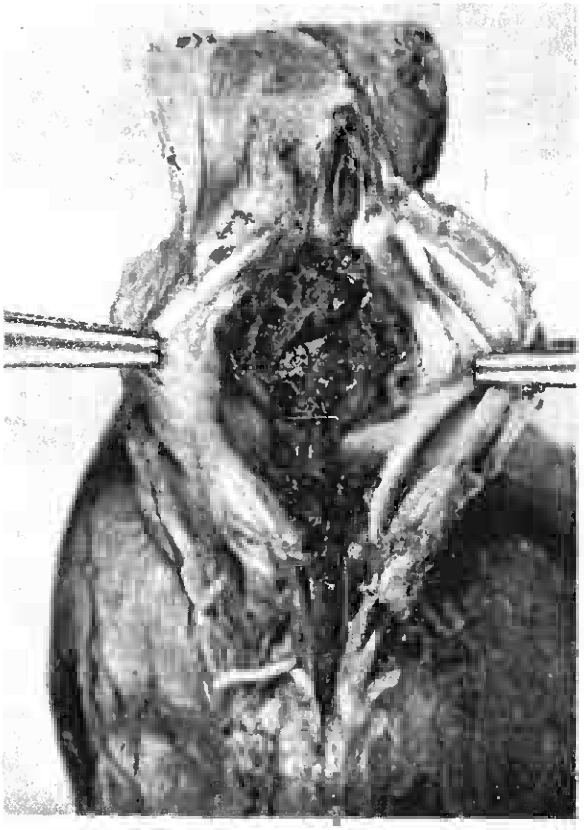

Fig. 6. Graft shown in Fig. 5. opened up to show occluding thrombus.

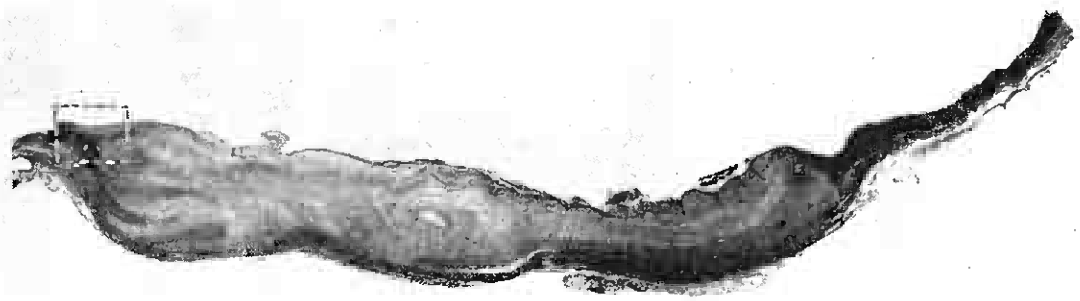

Fig. 7. Longitudinal section of the entire length of a patent umbilical vein graft. The fibrocellular intimal layer extending from host aorta at either end is seen covering the region of the suture lines and the adjacent portions of the graft surfaces.

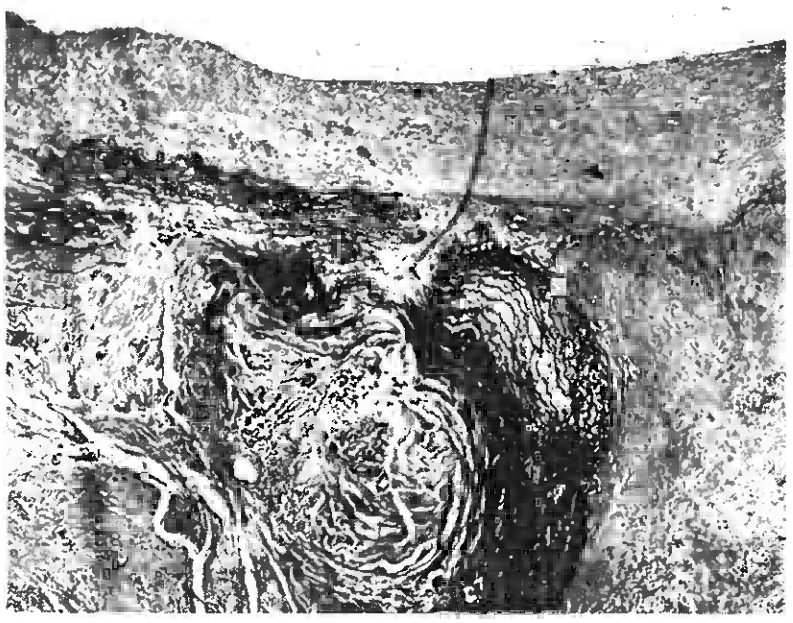

Fig. 8. Higher power view of the area marked off in Fig. 7.

Despite the necrotic changes, proliferation of host intima had led to a varying degree of endothelialisation in 6 of the heterografts. Fibrinous thrombus lined the non-endothelialised portions of the internal surface (Figs. 7 & 8).

There was well marked ingrowth of host connective tissue into the adventitial layer of the 6 grafts in which endothelialisation was demonstrable.

The host aorta demonstrated a generalised inflammatory reaction adjacent to the graft and intimal proliferation near the suture lines. The latter was most marked in those cases where the graft was endothelialised.

DISCUSSION

The results have been disappointing, for all of these umbilical vein heterografts showed either partial or complete thrombotic occlusion. In the 3 grafts that were patent, 2 had mural thrombi while the third although partially endothelialised had undergone moderate dilatation.

Thrombosis and aneurysmal dilatation have been the main causes of failure of venous grafts in the aorta and major arterial trunks. Marked dilatation of venous grafts in the abdominal and thoracic aorta have been reported by Nabatoff et al (1955), Johnson, Kirby and Hardy (1953), Jesseph et al (1958) amongst others. Attempts to reinforce the thin venous wall with Ivalon sponge (Morton and Mahoney, 1954 and 1958), fascia lata (Sako, 1951), nylon net (Vargas and Deterling, 1953), pericardium (Johnson et al, 1953. and other materials have not been successful. The tough amniotic umbilical cord sheath did not fare any better although it had remained intact in the majority of grafts. The marked necrosis which characterised all the grafts was in all probability the decisive contributory factor, and was no doubt due to the host reaction to the heterograft.

Thrombosis is a common complication of venous grafts in the peripheral arteries. Dye, Grove, Olwin and Julian (1956) in a series of 30 cases had an initial failure rate of 50% with 4 late failures. Similarly high failure rates have also been reported by Shaw and Wheelock (1955) and Hoye and Warren (1956). In a more encouraging series, Lord and Stone (1957) reported that 12 autologous vein grafts in the extremities implanted for conditions other than occlusive arterial disease remained patent for periods from 2 months to 7 years. More recently, Dale, DeWeese and Scott (1959) noted a late patency rate of 62% in autologous vein grafts in experimental animals and a very low incidence of dilatation and aneurysmal formation. Thrombosis in the peripheral arterial substitutes is no doubt due in a large measure to extensive distal arterial obliterative disease with a poor vascular "run off" and subsequent thrombosis.

In the current experiments it seems clear that a vigorous antigen-antibody reaction in the host tissues was the primary cause for graft failure. In a long-term study of externally supported venous heterografts using pigs' veins grafted into dogs, Morton and Mahoney (1958) found a very high incidence of graft failure — 48% — due to thrombosis and rupture. In a study on arterial heterografts, Creech et al (1954) found that they were especially prone to rapid destruction with resultant dilatation, aneurysmal formation and rupture. In order to minimise this reaction, Kimoto et al (1954) preserved arterial heterografts in alcohol, pure or 70%, and in a series of 22 experimental animals found no disruption in any graft, complete thrombosis only in 2, small mural thrombi in 5 and patency in 14. They suggested that alcohol through its action in the dissolution of lipo proteins and the denaturation of other proteins might render the graft tissue serologically inert.

Our experiences failed to bear this out. The marked necrosis which characterised all the grafts was evidence of a severe antigen-antibody reaction and was most probably the principal cause for the high thrombosis rate. Anzola et al (1951) using 20-24 cm. segments of umbilical cord vein grafts (preserved in Tyrode's solution) as long femoral and ilio-femoral grafts found that all thrombosed in 2 hours. Similar high failure rates have been reported more recently by Nabseth et al (1960) in a study of the use of bovine foetal arterial heterografts in dogs.

SUMMARY AND CONCLUSIONS

1. Fresh and alcohol-preserved umbilical cord vein grafts were implanted into the abdominal aorta of 15 dogs.
2. Of the 9 dogs that survived the first post-operative week, only 1 had a patent graft when sacrificed 3 months later. The rest had failed due to partial or complete thrombosis. The majority of these heterografts thrombosed within the first month.
3. The problem of the host antigen-antibody reaction to heterologous tissue had not been overcome by preservation in alcohol.

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