THE RESPONSE OF VENOUS VALVULAR ENDOTHELIUM TO AUTOTRANSPLANTATION AND IN VITRO PRESERVATION

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The response of venous valvular endothelium to autotransplantation and in vitro preservation

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Intraoperative preservation techniques in popular use produced endothelial damage in venous valves of dogs. This damage was evident with only 20 minutes of preservation. Severe endothelial damage was also evident after surgical autologous transfer of valve-bearing vein segments. The damage evolved over a period of days. At 28 days there was essentially complete desquamation of the valve apparatus with exposure of the collagen fibrils to the bloodstream. Complete reendothelialization had occurred by 4 months. The clinical implications of these findings are discussed.

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THERE IS INCREASING interest in reconstructive venous valve surgery.[6] Patients with venous reflux appear to benefit from direct vein valvuloplasty.[4] It has been pointed out recently that deep venous insufficiency may be present in many patients as a primary condition without prior incidence of deep venous thrombosis as the causative mechanism.[8] A valve apparatus that is suitable for direct repair is often present in patients with deep venous reflux. In instances of long-standing reflux with repeated or massive deep venous thrombosis, a reparable valve may not be present because of destruction during recanalization. Some of these patients' diseases have been successfully managed by transfer of a valve-bearing axillary vein segment to the femoral vein.[8, 10] Late functional failure of these valves is a significant complication of this procedure.[8] The mechanism and cause of this late failure are not understood. In an effort to clarify this problem, the behavior of venous valvular endothelium in response to in vitro preservation techniques and autotransplantation was studied in dogs.

MATERIAL AND METHODS

Adult mongrel dogs that weighed 12 to 20 kg were used.

In vitro preservation. Valve-bearing femoral vein segments were excised from the hind limbs and mounted on a specially designed perfusion apparatus (Fig. 1). It was possible to perfuse the vein segments at 4° C and 3 to 5 cm H_2O pressure in the apparatus by use of different solutions for varying periods of time (Table I). Sixteen valve segments were perfused.

Autotransplantation. In 16 dogs a valve-containing segment of the right femoral vein was exposed under pentobarbital (Nembutal) anesthetic. Under systemic heparinization (1000 U), the segment was excised and transposed to the left femoral vein. A segment of the left femoral vein had been excised in preparation for the anastomosis. Interrupted suture technique with 6-0 Prolene was used. Intraoperative heparinization was not reversed, but chronic anticoagulation was not used. The transferred valve segments were excised after varying intervals and examined under light and scanning electron microscopes.

Scanning electron microscopy (SEM). Valves were excised from valve-bearing segments after fixation with glutaraldehyde and prepared by the method of critical point drying. Micrographs at magnifications of 1000× and 2000× were obtained.

RESULTS

Architecture of the venous valve. When it was viewed with a light microscope, the structure of a normal venous valve appeared to consist of a collagen connective tissue core covered by a layer of endothelium (Fig. 2). Beneath the endothelium on the caudad, or inner, surface of the valve cusp, there was an

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Table I. Perfusion

No. of dogs	*Solution*	*Time (min)*
3	Heparinized saline 4° C	20
4	Heparinized saline 4° C	60
3	Heparinized blood 4° C	20
6	Nonperfused control	0

extensive network of elastic fibers that was continuous with those of the tunica intima. The cephalad, or sinus, side of the valve cusp had few or no elastic fibers. No muscle fibers extended into the valve structure. Blood vessels, when they were present, were confined to the base of the cusp adjacent to the vein wall.

In vitro preservation. Irrespective of the type of perfusate that was used, endothelial changes were apparent by as early as 20 minutes after preservation time (Table I). Amorphous change of the cells and cellular edema developed in the endothelium of valves that were perfused in cold heparinized saline for 20 minutes. Endothelium from those valves that were perfused with cold saline for 60 minutes developed more severe amorphous changes, marked cellular edema, widening of intracellular spaces, cellular indentation, and endothelial loss (Fig. 3). Those valves that were perfused with cold heparinized whole blood for 20 minutes developed mild cellular edema and amorphous change (Fig. 4). Qualitatively, the endothelium was damaged least by perfusion with cold heparinized whole blood.

Autotransplantation. Autotransplanted venous valves that were examined at 24 hours had developed widening of the intracellular spaces, edema, and amorphous changes (Fig. 5). Vein valves that were examined from 48 hours up to 28 days revealed evolving cellular damage that resulted in marked cellular loss and exposure of basement membrane and collagen fibrils (Figs. 6 and 7). Valves that were examined 4 months after autotransplantation had normal endothelium (Fig. 8). There was one instance of thrombosis in 15 autotransplants. Valvular function was not considered.

DISCUSSION

The value of reconstructive venous valve surgery for deep venous insufficiency has not been established. The interest in this area is relatively recent and only a few series have been reported.[8, 10] If the encouraging initial reports are confirmed, a large body of patients who are currently managed by tedious or unsatisfactory traditional methods may benefit from this new approach. Even though the response of nonvalvular venous endothelium to preservation and transposition to the arterial system has been studied extensively, no such information is available regarding venous valvular endothelium and transposition in the venous system. Because of differences in blood supply and architecture, the behavior of nonvalvular endothelium[11] could not be extrapolated to valvular endothelium. The current study was designed to address these questions. Venous valvular endothelium was shown to be quite sensitive to preservation techniques and autotransplantation. Damage was evident as early as 20 minutes after excision and in vitro preservation. Cold heparinized blood produced less damage than cold normal saline. The process of

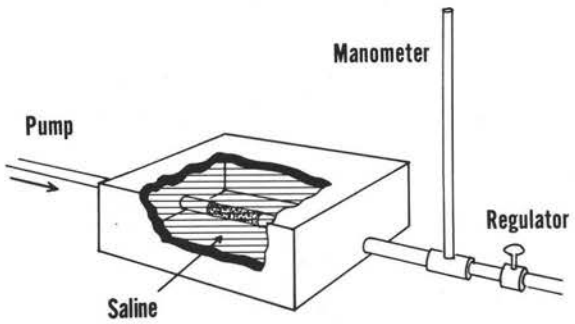

Fig. 1. Diagram of perfusion apparatus with vein in place *(stippled)*.

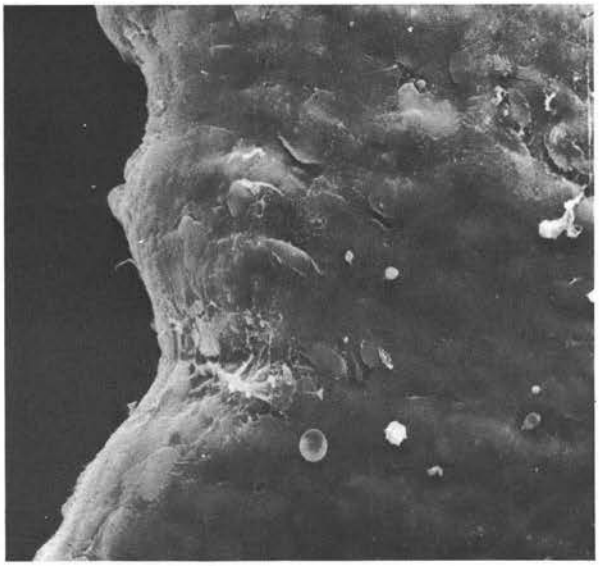

Fig. 2. Normal venous valvular endothelium. (SEM; original magnification ×1000.)

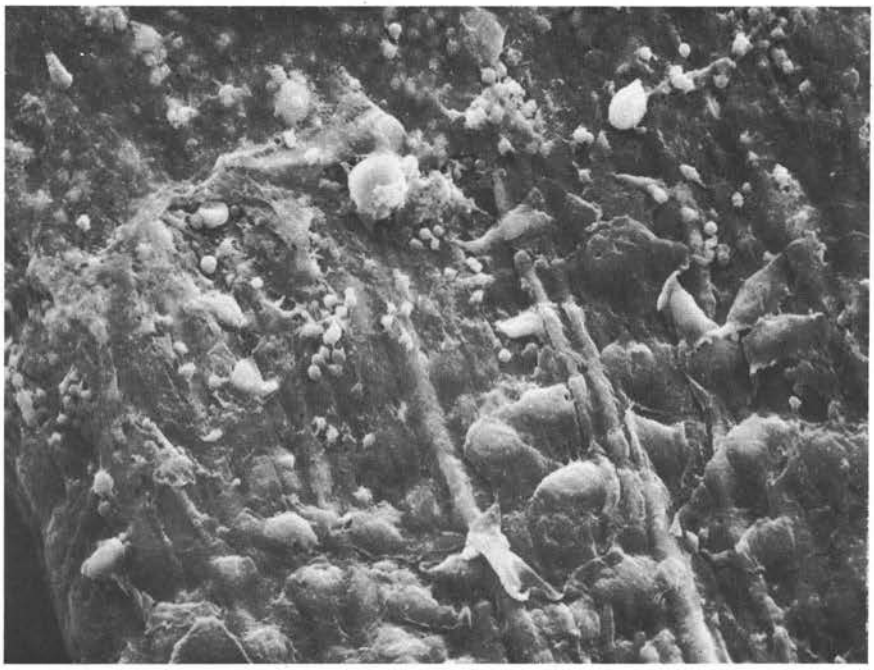

Fig. 3. Valve endothelium after 60 minutes of perfusion with heparinized saline. Demonstrates complete desquamation of endothelium. (SEM; original magnification ×1000.)

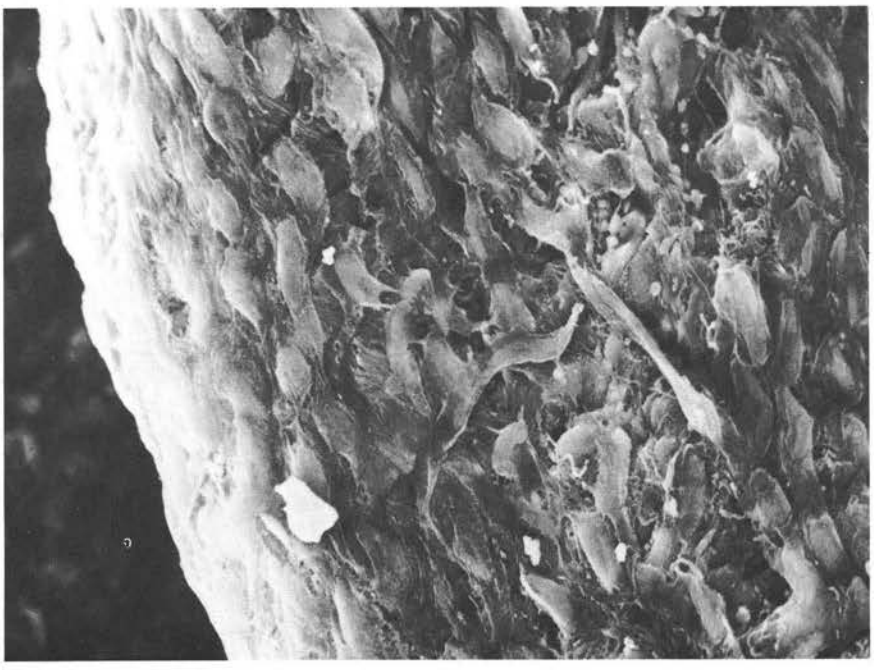

Fig. 4. Valve edge after perfusion with heparinized whole blood for 20 minutes. Reveals mild cellular edema, widening of intercellular spaces, and some desquamation. (SEM; original magnification ×1000.)

Fig. 5. Autotransplanted valves examined 24 hours after surgery. Reveals widening of intercellular spaces and some loss of cellular morphology. (SEM; original magnification ×1000.)

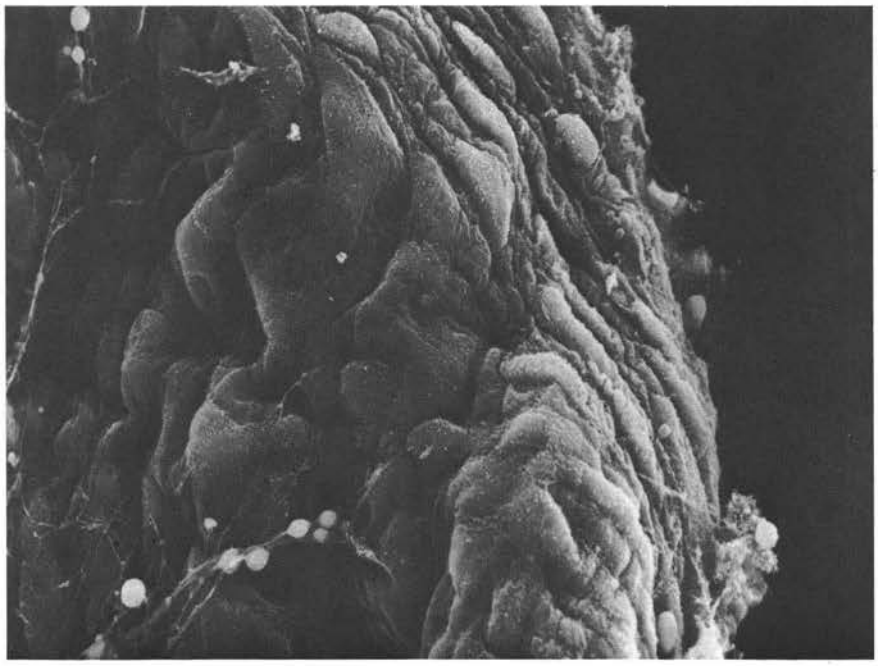

Fig. 6. Valvular endothelium examined 3 days after autotransplantation. Reveals widening of intercellular spaces, cellular edema, and small areas of desquamation. (SEM; original magnification ×1000.)

Fig. 7. Basement membrane on a vein valve 30 days after autotransplantation in which total desquamation had occurred. (SEM; original magnification ×1000.)

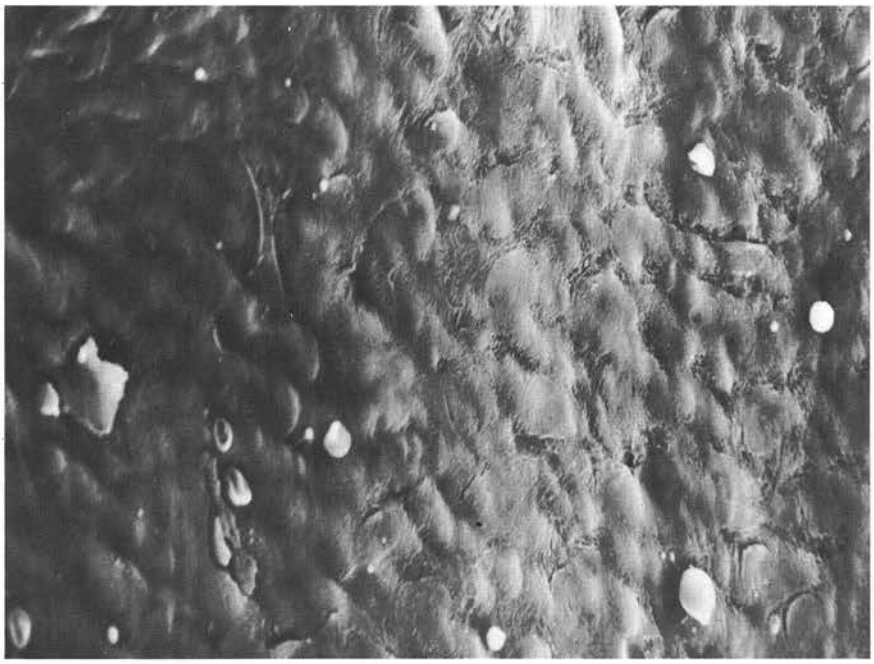

Fig. 8. Normal appearing endothelium 4 months after autotransplantation. (SEM; original magnification ×1000.)

surgical transfer of a valve-bearing segment from one area to another in the venous system also resulted in marked endothelial damage. The endothelial damage grew progressively worse up to 28 days after transposition. At this time, the collagen framework of the valve was exposed with an essentially total loss of endothelial cover. At 4 months, however, complete reendothelialization had occurred. With basement membrane and collagen fibrils exposed in a low-flow system for 4 months, the potential for thrombosis and subsequent

destruction of the valve is obvious. This finding suggests that chronic anticoagulation of up to 4 months should be employed in valve transfer procedures performed for deep venous insufficiency. Kroener and Bernstein[7] have demonstrated the improved patency that was obtained with a temporary arteriovenous fistula in vein transfer experiments in dogs. The perioperative endothelial damage that is reported in this study may be the basis for their observation.

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