Morphologic change in rabbit femoral arteries induced by storage at four degrees Celsius and by subsequent reperfusion

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Purpose: **Cold-stored arteries function well as microvascular autografts, but little is known of the morphologic changes that occur in them during cold storage or of further changes during reperfusion.**
Methods: **In part A of the study, rabbit femoral arteries were stored at 4° C for up to 6 months. In part B rabbit femoral arteries were stored at 4° C for up to 6 months, inserted as end-to-end autografts into contralateral femoral arteries, and reperfused for 24 hours. Tissue was examined by histologic study, transmission and scanning electron microscopy, histochemical study, immunohistochemical study, and tissue culture.**
Results: **Cell viability declined gradually at 4° C, so that by 4 weeks no viable cells remained. However, the extracellular framework and elastic lamellae remain intact. If cold-stored arteries are reinserted as autografts for 24 hours, this accelerates breakdown of necrotic cells and reduces the thickness of the medial wall and internal elastic lamina but does not alter the extracellular framework.**
Conclusions: **Cold storage results in acellular vascular grafts with intact extracellular frameworks. After 24 hours reperfusion there is no major change to the extracellular framework. (J VASC SURG 1995;22:769-79.)**

Currently the only satisfactory replacement for blood vessels of the size commonly anastomosed in replantation surgery is an autologous graft, usually a vein. For a variety of reasons these are not always available, and an alternative prosthesis would be of considerable clinical value. A number of possible sources, including polytetrafluoroethylene,[1,2] glutaraldehyde-tanned vessels,[3,4] bioabsorbable polyurethane grafts,[5,6] freeze-dried vessels,[7] fresh allografts,[8] endothelial cell (EC)–seeded synthetic grafts,[9] and cryopreserved arteries,[10] have been examined. Several of these prostheses have been shown to remain patent when short lengths are inserted into large-diameter, high-flow blood vessels, but none so far examined has remained patent consistently when used to bridge gaps in vessels with the caliber and flow characteristics of those involved in clinical microsurgery.

An interest in the fate of vessels in an amputated body part stored in cold[11] led us to investigate cold-stored small arteries as a source of microvascular grafts. Gray et al.[12] found that rabbit femoral arteries wrapped in saline solution–moistened gauze and stored at 4° C for up to 10 weeks had a patency rate of more than 80% 3 weeks after their reinsertion as autografts into a divided femoral artery and described the histologic appearance of cold-stored grafts 3 to 12 weeks after reimplantation.

Gray et al.[12] did not examine either the progressive changes that occur during cold storage of rabbit femoral arteries before their insertion as a graft or the early reactive changes that follow reinsertion. This study is a histologic, electron microscopic, tissue culture, and immunohistochemical study of these aspects of cold-stored arterial grafts.

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MATERIAL AND METHODS

A total of 120 rabbits was used. Animal care complied with the "Principles of Laboratory Animal Care" (formulated by the National Society for Medical Research) and the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No.86-23, revised 1985). In part A of the study, rabbit femoral arteries were stored at 4° C for various periods before examination; in Part B arteries stored as in part A were reimplanted into the contralateral leg of the same rabbit and reperfused for 24 hours before examination.

Harvest of the femoral arterial graft (parts A and B). Femoral artery grafts of 3.5 to 4.0 cm in length were harvested from both legs in part A (vessels cold stored in vitro) and from the left side only in part B (reperfused vessels) and irrigated under low pressure with heparinized saline solution (10 U/ml). Part A animals were then killed, and part B animals were resuscitated.

Cold storage. The graft was placed on a saline solution–moistened sponge in a sterile container and was stored at 4° C for either 24 hours, 1, 4, or 10 weeks, or 6 months. No preservative or other additive was used. At the end of the storage period, the graft was allowed to return to room temperature and was processed as described below. Approximately 10 grafts were examined at each time point. A control group (0 hours cold storage) was also examined.

Part B: graft reinsertion and removal. After completion of the appropriate period of cold storage, the graft was brought to room temperature over 1 hour and irrigated with heparinized saline solution. A 2 to 2.5 cm segment of the contralateral femoral artery was excised, and the graft was inserted by use of approximately 10 10/0 nylon sutures per anastomosis. After testing the anastomosis for patency, the wound was closed, and the rabbit was resuscitated. Twenty-four hours later the graft was exposed and tested for patency, and the graft and 1 cm of artery proximal and distal to it were excised and processed as described below.

Fixation of grafts and adjacent artery. Grafts for histologic study were fixed by immersion in buffered formal saline solution; those for histochemical and immunohistochemical studies were fixed by immersion in liquid N_2 and stored at $-70°$ C; those for electron microscopy were perfusion-fixed with heparinized Hank's balanced salt solution followed by Karnovsky's fixative.[13] After 24 hours in Karnovsky's fixative 2 mm lengths were cut from the graft for transmission electron microscopy (TEM) and 5 mm lengths for scanning electron microscopy (SEM).

Histologic study. One graft per group was used for histologic study. After fixation grafts were embedded in paraffin, 5 μm sections cut and stained with either Verhoeff-van Gieson stain or hematoxylin-eosin.

Electron microscopy

TEM. Four grafts per group were used for electron microscopy. Karnovsky-fixed tissue was postfixed in osmium tetroxide (1% wt/vol), dehydrated in graded acetone, and embedded in Epon/Araldite (LADD Research Industries, Burlington, Vt.). One-micrometer sections were stained with methylene blue, areas of interest were identified, and 80 nm sections were cut, stained with Reynold's lead citrate and uranyl acetate, and viewed in a CM10 electron microscope (Philips, Eindhoven, Holland).

SEM. Karnovsky-fixed tissue was postfixed in osmium tetroxide (1% wt/vol), dehydrated in graded ethylene glycol followed by graded cellosolve (May and Baker Ltd., Essex, United Kingdom), critical point–dried, gold-coated by a "cool" sputter coater (JEOL, Tokyo, Japan) and viewed in a JSM 35 scanning electron microscope (JEOL).

Immunohistochemistry. Three grafts in each group were reserved for immunohistochemical study. Five micrometer–thick cryosections were cut and fixed with 100% acetone at 4° C for 10 minutes. Sections were stained by use of the indirect immunostaining technique. The monoclonal primary antibody was either mouse anti-human α smooth muscle actin (dilution 1/60, DAKO, Carpinteria, Calif.), or mouse anti-human vimentin (dilution 1/60, DAKO), and the secondary antibody was goat anti-mouse immunoglobulin G conjugated with fluorescein isothiocyanate probe (dilution 1/70; Serotec, Oxford, United Kingdom). Positive controls were fresh artery, and negative controls were tissue incubated without the primary antibody. Sections were viewed in a fluorescence microscope, semiquantitatively scored, and photographed.

Nitroblue tetrazolium (NBT) staining for reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase. The three grafts from the group used for immunohistochemical study were also used for NBT staining. The NBT-NADH stain is a histochemical test for cell viability.[14] Reducing enzymes (or diaphorases) of NADH, of which NADH dehydrogenase is the most common, reduces NBT to form an insoluble blue precipitate. Cryostat sections were cut and, after fixation in acetone, incubated for 30 minutes in TRIS ph 7.1 (0.2 mol/L)

buffered salt solution containing 0.025% wt/vol NBT, 0.2% wt/vol NADH (reduced form), and 0.1% wt/vol magnesium chloride (a cocatalyst); they were also counterstained with nuclear fast red. Cells with a red nucleus and cytoplasmic blue granules were interpreted as positively stained. Fresh tissue served as positive controls, and fresh tissue incubated without NBT or NADH served as negative controls. Sections were scored by a blinded observer on a range from 3 to 0, with scoring as follows: 3 (all red nuclei had blue staining); 2 (most of the red nuclei had blue staining); 1 (minority of the red nuclei had blue staining); 0 (no blue staining). Five random sections from each of the grafts were scored and the results subjected to a two-tailed analysis of variance.

Medial wall thickness measurements. Longitudinal sections of methylene blue–stained 1 μm-thick plastic sections of the graft were viewed with a light microscope (original magnification ×20), and the distance from the internal elastic lamina (IEL) to the external elastic lamina (EEL) was measured with a graticule with 100 μm subdivisions. Results from four grafts per group were tabulated, and the results underwent two-tailed analysis of variance.

Tissue culture. Four grafts were used in most of the groups. Explants of artery from vessels cold stored in vitro and reperfused vessels were cut open, placed in six-well, flat-bottom, tissue culture plates, and covered by a sterile coverslip, and complete tissue culture medium was added. The medium was changed every 48 hours. Visual inspection of plates was performed every 48 hours up to 14 to 15 days. Cells that had migrated out of the explant were examined by fluorescent immunohistochemical study for the presence of α-smooth muscle cell (SMC) actin (with monoclonal anti α-SMC actin [DAKO]) or EC vesicles (with monoclonal anti CD31, 1/40 dilution; [DAKO]).

RESULTS

Macroscopic appearance. The texture and appearance of cold-stored arteries was similar to fresh autologous arteries except for a translucent milky-white color and a somewhat sticky surface. The sticky nature of cold-stored grafts and compression during storage meant care was required to identify the lumen. The cold-stored grafts were no more difficult to suture than fresh autologous grafts. Leakages from the anastomoses occurred with a similar frequency to fresh autologous artery grafts. Pulsatile blood flow was visible within the graft after its insertion.

Histologic, TEM, and SEM observations. The histologic, TEM, and SEM morphologic observations are summarized in Table I and shown diagrammatically in Fig. 1. Fig. 2 shows a typical example of the histologic, SEM, and TEM appearance of mildly damaged cells from vessels cold stored in vitro, Fig. 3 shows the appearance of necrotic cells from vessels cold stored in vitro and Fig. 4 shows the structure of long-term cold-stored tissue after 24 hours reperfusion. Twenty-four hours of reperfusion did not change the extracellular framework of cold-stored arteries; however, infiltration of white blood cells (WBC) in reperfused vessels appeared to speed up the breakdown and removal of necrotic cells. Reperfusion appeared to increase necrotic cell numbers.

Immunohistochemistry. The results are summarized in Table II. In the normal artery the medial staining for α-SMC actin was uniform throughout the cytoplasm of SMCs, and the nucleus was recognized as a dark spot within the cytoplasm. No extracellular staining occurred. Autofluorescence of the IEL made EC staining difficult to recognize; however, there appeared to be an increase in the staining intensity luminally to the IEL. No fibroblasts stained.

In normal arteries all cells were positive for vimentin. Fibroblasts and EC stained more intensely than SMC, but the pattern of staining was similar to that of α-SMC actin.

NBT staining. The results are summarized in Table III. In vessels cold stored in vitro and reperfused vessels, it can be seen that NBT staining decreases as storage time increases, with the decrease being more rapid in reperfused vessels.

Tissue culture results. The results are summarized in Table IV. Cells that stained positively for α-SMC actin were regarded as SMC and cells that stained negative for α-SMC actin were assumed to be fibroblasts. No migrating cells stained with EC-specific antibody (CD31).

Medial wall thickness. The results are summarized in Table V. In the first 24 hours cold storage causes an increase in arterial wall thickness as a result of the combined effects of swelling of SMC and extracellular edema. Thereafter thickness decreases progressively, with the rate of shrinkage being much more rapid in reperfused vessels than in vessels cold stored in vitro. Both the decreased thickness with time and the differences between the groups are statistically significant.

DISCUSSION

Cold-stored arteries function well as autografts, but it is not known how long it is before all cells are

Table I. Summary of morphological changes in rabbit femoral arteries stored at 4° C (part A) and after storage at 4° C and 24 hours reperfusion in the contralateral femoral artery (part B)

Cold storage time	*Intima*	*Media*	*Adventitia*
Part A			
Fresh artery	EC-most normal	SMC-most normal	Fibroblasts-most normal
	IEL-normal	EXF-normal	EXF-normal
24 hours	EC-some mild + most necrotic	SMC-some normal + some mild	Fibroblasts-most mild
	IEL-normal	EXF-normal	EXF-normal
1 week	EC-all necrotic	SMC-some mild + most necrotic	Fibroblasts-some mild + most necrotic
	IEL-normal	EXF-normal	EXF-normal
4 weeks	EC-all necrotic	SMC-all necrotic	Fibroblasts-all necrotic
	IEL-normal	EXF-normal	EXF-normal
10 weeks	EC-all necrotic	SMC-all necrotic	Fibroblasts-all necrotic
	IEL-normal	EXF-normal	EXF-normal
6 months	EC-all necrotic	SMC-all necrotic	Fibroblasts-all necrotic
	IEL-normal	EXF-normal	EXF-normal
Part B			
Fresh artery	EC-some normal + most necrotic	SMC-most normal + some mild	Fibroblasts-most normal
	IEL-normal	EXF-normal	EXF-normal
	WBC-mild	WBC-mild	WBC-mild
24 hours	EC-all necrotic	SMC-some normal + most mild	Fibroblasts-most mild
	IEL-normal	EXF-normal	EXF-normal
	WBC-mild	WBC-mild	WBC-mild
1 week	EC-all necrotic	SMC-some mild + most necrotic	Fibroblasts-some mild + most necrotic
	IEL-normal	EXF-normal	EXF-normal
	WBC-mild	WBC-mild	WBC-mild
4 weeks	EC-all necrotic	SMC-all necrotic	Fibroblasts-all necrotic
	IEL-normal	EXF-normal	EXF-normal
	WBC-mild	WBC-mild	WBC-mild
10 weeks	EC-all necrotic	SMC-all necrotic	Fibroblasts-all necrotic
	IEL-normal	EXF-normal	EXF-normal
	WBC-mild	WBC-mild	WBC-mild
6 months	EC-all necrotic	SMC-all necrotic	Fibroblasts-all necrotic
	IEL-normal	EXF-normal	EXF-normal
	WBC-mild	WBC-mild	WBC-mild

EXF, Extracellular framework. Grades of injury are *normal* (no cell damage, *mild* (dilated ER, golgi and mitochondria, intact plasmalemmas, marginated chromatin), and *necrotic* (severely dilated or ruptured endoplasmic reticulum, golgi apparatus, mitochondria, incomplete plasmalemmas and nuclear envelops, pycnotic or karyolytic nuclei). Grades of WBC infiltration are *none, mild, moderate,* and *severe*.

dead. On the basis of TEM, SEM, and tissue culture findings, this study shows that after 24 hours cold storage all endothelium is dead. After 1 week cold storage, histologic study and electron microscopy indicate that most fibroblasts and SMC are dead or severely damaged, but tissue culture shows that some cells are still alive. After 4 weeks cold storage all cells are dead, and most have broken down and disintegrated. These findings show that the end point for viability of arterial SMCs in cold-stored arteries is somewhere between 1 and 4 weeks.

Tissue culture is the most sensitive indicator of the presence of living cells. Morphologic methods cannot identify dead cells, but only the disintegration that follows death of a cell.[15] Electron microscopy can detect such changes at an earlier stage than can histologic study.

The findings of this study show that some elements of cells are resistant to cold storage and are still present in cells that by other criteria are clearly necrotic. Alteration in mitochondrial structure is one of the main indicators of ischemic necrosis of skeletal muscle,[16] and NBT staining for mitochondrial, membrane-bound enzymes is believed to be a sensitive marker of cell viability.[14] However, in this study intact mitochondria were seen by TEM in totally necrotic cells, and NBT staining was present in nonviable cells. This suggests that there is a small population of mitochondria that remain intact in necrotic, cold-stored cells. However, these cold-resistant mitochondria are removed when the tissue is reperfused, and consequently residual NBT staining disappears.

The immunostaining for α-SMC actin in all cold-stored tissue shows that the antigens for these myofilaments are highly resistant to cold storage and 24 hours reperfusion. TEM confirmed that necrotic

Diagram 1 : Summary of the morphological changes to rabbit femoral arteries after cold storage and after 24 hours reperfusion.

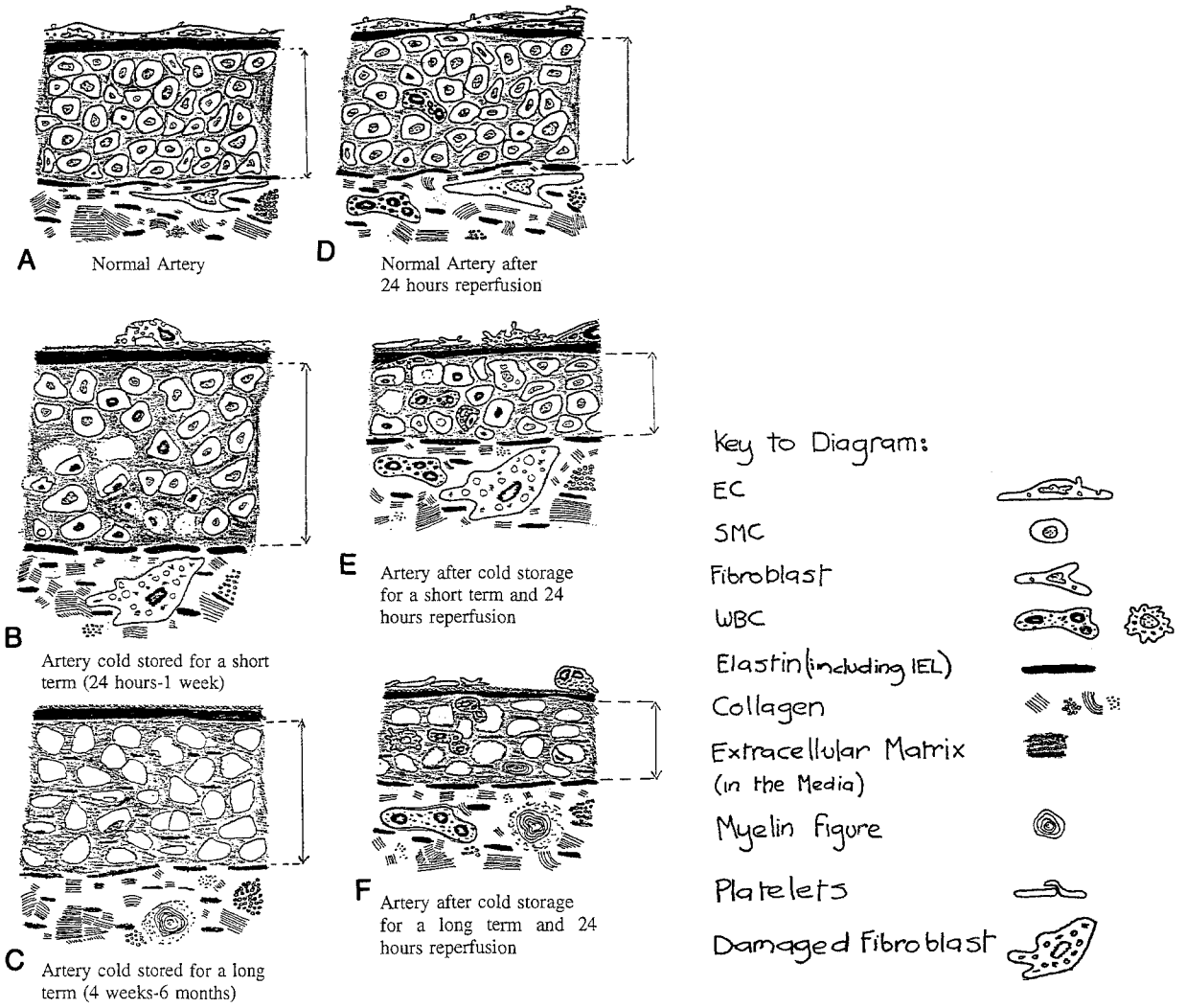

Fig. 1. Summary of morphologic changes to rabbit femoral arteries induced by storage at 4° C and by subsequent 24 hours reperfusion. Longitudinal view of rabbit femoral arteries. **A,** *Normal artery,* single layer of EC rest on IEL. Typically 15 to 20 layers of SMC occupy rabbit femoral artery media, although only six layers of SMC are drawn in this diagram. **B,** *Short-term cold storage,* In arteries cold-stored for short term (24 hours to 1 week), there is patchy cell necrosis and an increase in media thickness. EC are contracted or lost, and subendothelium is exposed. Pycnotic nuclei and mildly damaged cells (as depicted by fibroblast) are visible in media and adventitia. **C,** *Long-term cold storage,* After long period of cold storage (4 weeks to 6 months), all cells are necrotic, but extracellular framework remains intact. Media returns to normal thickness. Myelin figures (as seen replacing fibroblast) are occasionally present. **D,** *Normal artery reperfused for 24 hours,* Normal artery grafts are infiltrated with WBC, of which neutrophils and macrophages are mostly present. WBC are visible beneath endothelium, in media and in adventitia. **E,** *Short-term cold-stored artery reperfused for 24 hours,* WBC infiltration is similar to normal arteries. Platelets line exposed subendothelium. Thickness of media is significantly reduced. **F,** *Long-term cold-stored artery reperfused for 24 hours,* Media width is similar to short-term cold-stored arteries, and WBC infiltration is similar to short-term cold-stored and normal arteries. Extracellular framework is compressed but intact.

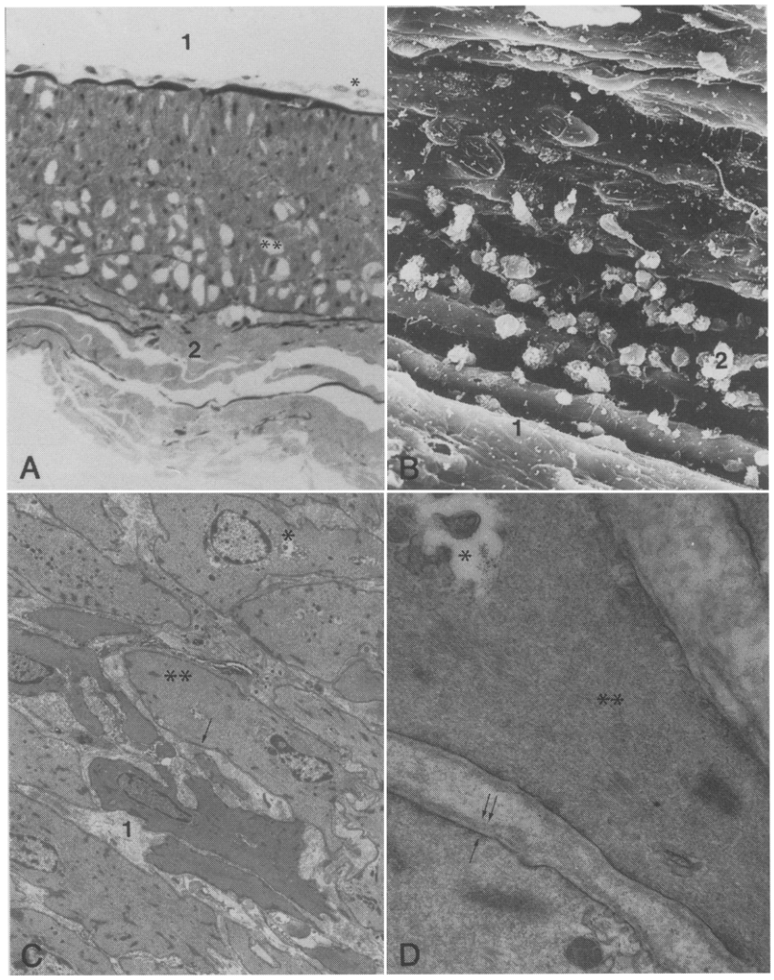

Fig. 2. Mildly damaged cold-stored rabbit femoral artery. **A,** Longitudinal section of mid graft of 1-week cold-stored artery. Note karyolytic ECs (*) and spaces (**) in media as result of necrosis of SMCs. *1* indicates lumen; *2* indicates adventitia. (Methylene blue stain; original magnification ×440.) **B,** SEM of mid graft of 24-hour cold-stored artery. Note general necrosis of most ECs. *1* indicates normal ECs; *2* indicates severely damaged ECs. (Original magnification ×2000.) **C,** TEM of media in mid graft of 24-hour cold-stored artery. Typical mildly damaged SMCs. Note mildly dilated synthetic organelles (*) and intact plasmalemma *(arrow)* and myofilaments (**). *1* indicates extracellular framework. (Uranyl acetate and lead citrate staining; original magnification ×4500.) **D,** TEM of media in mid graft of 24-hour cold-stored artery. Typical mildly damaged SMCs. Note mildly dilated synthetic organelles (*) and intact plasmalemma *(arrow)*, basal lamina *(double arrows)*, and myofilaments (**). (Uranyl acetate and lead citrate staining; original magnification ×40,000.)

cells often retained their myofilaments. However, these myofilaments may disappear after a period of cold storage that is greater than 6 months or a period of reperfusion longer than 24 hours.

Vimentin is more susceptible to cold storage than α-SMC actin, but both are unaffected by a short period of reperfusion. Vimentin staining is present in arteries cold stored for 4 weeks but is absent after 10 weeks cold storage. The functions of vimentin are not fully understood, but it has been suggested that loss of other components of the cytoskeletal network, that is, loss of vinculin or microtubule staining, are early signs of irreversible ischemic injury.[17,18] The findings show that staining for NBT, α-SMC actin or vimentin are not useful measures of cell viability in arterial SMCs.

It is well established that when ischemic tissue is reperfused further cell injury may occur.[19] Such ischemia-reperfusion injury would explain the significantly fewer undamaged cells seen by electron

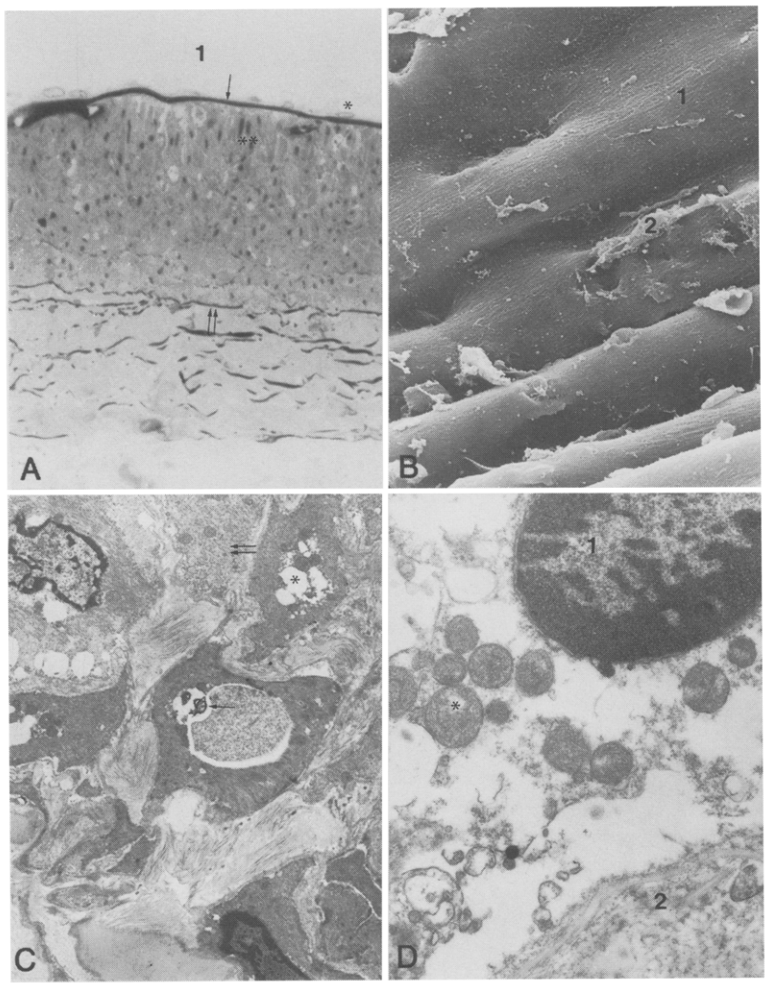

Fig. 3. Severely damaged cold-stored rabbit femoral artery. **A,** Longitudinal section of mid graft of 6-month cold-stored artery. Note total karyolytic ECs (*) and pycnotic SMCs (**). *1* indicates lumen; *arrow* indicates IEL; *double arrows* indicate EEL. (Methylene blue staining; original magnification ×460.) **B,** SEM of mid graft of 1-week cold-stored artery. Note loss of most ECs and exposed IEL. *1* indicates exposed IEL; *2* indicates severely damaged ECs. (Original magnification ×2500.) **C,** TEM of media in mid graft of 1-week cold-stored artery. Typical severely damaged SMCs. Note grossly dilated synthetic organelles (*), ruptured nucleus with myelin figure *(arrow)*, and loose myofilaments *(double arrows)*. (Uranyl acetate and lead citrate staining; original magnification ×5400.) **D,** TEM of EC in mid graft of 1-week cold-stored artery. Unusual severely damaged cell. Note that despite lack of plasmalemma, there is necrotic nucleus (*1*), mitochondria with intact outer membranes (*) and intact extracellular framework (*2*). (Uranyl acetate and lead citrate staining; original magnification ×23,500.)

microscopy in 1-week cold-stored vessels reperfused for 24 hours than in nonreperfused vessels stored in the cold for the same period. It is not clear whether a longer period of reperfusion would have led to the death of the residual viable cells.

The rapid reduction in medial thickness seen after 24 hours reperfusion of cold-stored arteries is due to the rapid removal of necrotic SMC by the inflammatory cells that infiltrate the wall of cold-stored vessels in the early stages of reperfusion (Fig. 1). It is clear that cold storage, with or without 24 hours reperfusion, leaves the extracellular framework, including the IEL and EEL, intact and little altered. The findings of Gray et al.,[12] with the same model, show that the extracellular framework is still intact after 3 to 12 weeks reperfusion. Until 6 weeks reperfusion SMC necrotic debris is still present in the media, which has been invaded by fibroblasts, and the luminal surface is lined with endothelial-covered neointima.

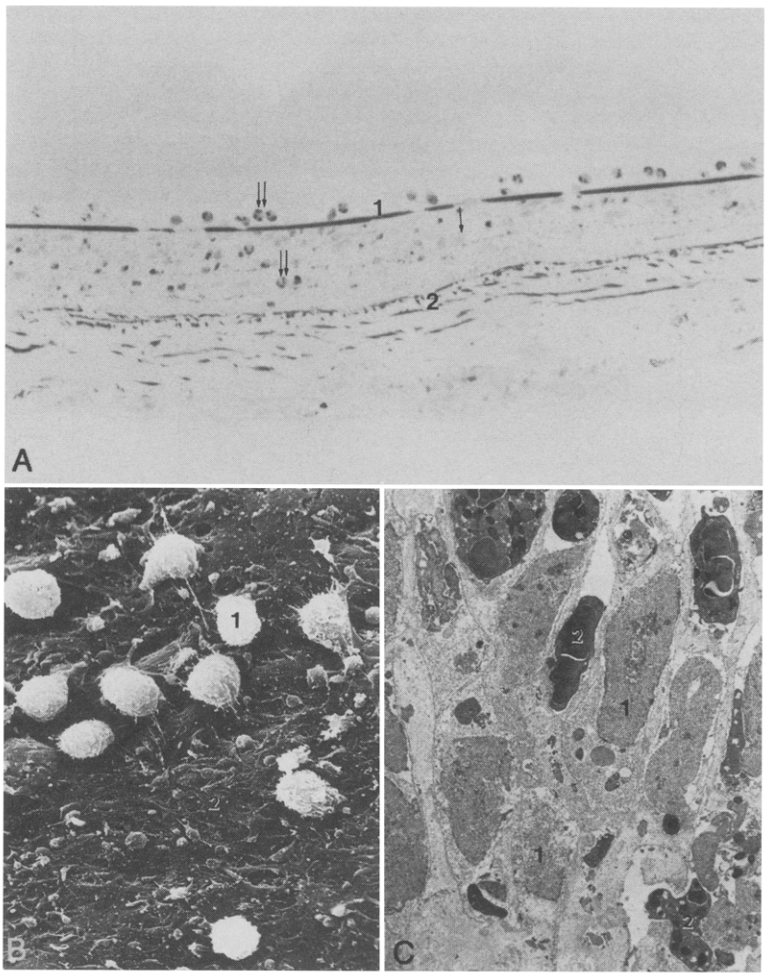

Fig. 4. Severely damaged cold-stored rabbit femoral artery after 24 hours reperfusion in contralateral femoral artery. **A,** Longitudinal section of mid graft of 4-week cold-stored artery. Note total loss of endothelium and karyolytic SMCs *(arrow)*. Neutrophils *(double arrows)* lined luminal surface and infiltrate into media. *1* indicates IEL; *2* indicates EEL. (Methylene blue staining; original magnification ×380.) **B,** SEM of mid graft of 6-month cold-stored artery. Note total loss of endothelium. Exposed IEL is covered by platelets and WBCs. *1* indicates WBCs; *2* indicates platelets. (Original magnification ×4100.) **C,** TEM of media in mid graft of 1-week cold-stored artery. Typical severely damaged SMCs (*1*) with WBC infiltration (*2*) and an intact extracellular framework. (Uranyl acetate and lead citrate staining; original magnification ×4200.)

Contamination of stored grafts was not a problem. With more than 120 grafts collected by use of sterile procedure, only one 6-month graft had dried out and become contaminated with fungal and bacterial flora. It is known that cold-stored skin grafts are highly susceptible to infection.[20] However, unlike in skin, contamination is not a concern because these arterial grafts are harvested from a sterile environment by use of a sterile technique.

These findings expand and are in general agreement with earlier published studies on human saphenous veins stored at 4° C in normal saline solution,[21] rabbit external jugular veins stored in heparinized normal saline solution at 37° C,[22] rabbit aorta stored in Krebs solution at 4° C,[23] and rat femoral veins stored at 4° C in lactated Ringer's solution.[24]

Reperfusion of cold-stored grafts causes localized inflammation, reduces histochemical staining, and leads to a reduction in wall thickness as a result of the lytic action of inflammatory cells on the necrotic cells within the vascular wall but has only a minimal effect on the residual connective tissue framework of the cold-stored artery. However, 24 hours reperfusion

Table II. Semiquantitative score of immunofluorescence staining for αSMC actin and vimentin antigens in rabbit femoral arteries cold stored *in vitro* (group A) and reperfused vessels (group B)

	Antigen Storage time			
	α SMA actin		*Vimentin*	
	Group A	*Group B*	*Group A*	*Group B*
0 hour	+ +	+	+ +	±
24 hours	+	+	+	±
1 week	+	+	±	±
4 weeks	+	±	±	±
10 weeks	+	±	−	−
6 months	+	±	−	−

Scoring range was: + + maximal staining; + weak overall staining; ± patchy staining with cells present that did not stain; − no staining.

Table III. Semiquantitative score (mean ± standard deviation) of NBT staining in the media of rabbit femoral arteries cold stored *in vitro* (group A) and reperfused vessels (group B)

	NBT	
Storage time	*Group A 5 observations/graft (n = 3)*	*Group B 5 observations/graft (n = 3)*
Normal	2.53 ± 0.19	1.53 ± 0.09
24 hours	2.8 ± 0.16	1.33 ± 0.09
1 week	1.87 ± 0.34	0.8 ± 0.28
4 weeks	1.53 ± 0.34	0.4 ± 0.16
10 weeks	1.33 ± 0.41	0.0 ± 0.0
6 months	0.0 ± 0.0	0.0 ± 0.0

Scoring range was *3* all red nuclei had blue staining; *2* most red nuclei had blue staining; *1* minority of red nuclei had blue staining; *0* no blue staining.
A two-tailed analysis of variance for unpaired data showed an overall difference for NBT staining between group A and group B ($p < 0.01$); and an overall difference for NBT staining over time ($p < 0.01$).

Table IV. Summary of tissue culture results of rabbit femoral arteries cold stored in *in vitro* (group A) and reperfused vessels (group B)

		24 hr		*1 wk*		*2 wks*	*4 wks*	*12 wks*
Storage time and group	*Fresh artery*	*A*	*B*	*A*	*B*	*A*	*A*	*A*
Positive growth/No. of explants	4/4	4/4	4/4	4/4	4/4	0/2	0/4	0/1
Time cells first appeared (days)	5-6	5-6	3-4	8-13	3-4	No growth	No growth	No growth
No. cells in culture after 14 days	Confluent	Confluent	Confluent	>50	Confluent	−	−	−
Actin-positive cells present	Yes	Yes	Yes	Yes	Yes	−	−	−
Actin-negative cells present	Yes	Yes	Yes	Yes	Yes	−	−	−
CD31-positive cells present	No	No	No	No	No	−	−	−

shortens the time lag before cell migration and proliferation in explant cultures (Table III). This is unlikely to be due to blood-borne inflammatory factors but may be due to the release of factors from WBCs present in the reperfused arteries or to alterations in the extracellular framework as a result of reperfusion. It may be that specific extracellular matrix glycosaminoglycans such as heparan sulphate, which have an inhibitory effect on cell proliferation,[25,26] are reduced or nullified during reperfusion or that reperfusion activates a factor such as basic fibroblast growth factor, which is known to be present in the extracellular matrix[27,28] and which is a mitogen for SMC.[29,30]

Table V. Semiquantitative score (mean ± standard deviation) of media thickness measurements of the media of rabbit femoral arteries cold stored *in vitro* (group A) and reperfused vessels (group B)

	Mean thickness μm 10 observations/graft (n = 4)	
Storage time	*Group A*	*Group B*
Normal	81.5 ± 6.7	93.9 ± 4.0
24 hours	127.6 ± 7.3	103.9 ± 34.1
1 week	116.4 ± 10.5	44.8 ± 9.3
4 weeks	89.6 ± 7.8	47.5 ± 19.7
10 weeks	85.8 ± 2.5	48.6 ± 6.0
6 months	79.4 ± 9.4	41.1 ± 3.9

A two-tailed analysis of variance for unpaired data showed an overall difference for medial thickness between group A and group B ($p < 0.01$); and an overall difference for medial thickness over cold storage time ($p < 0.01$).

What are the likely clinical applications of cold-stored arteries? Because the extracellular framework remains intact after long periods of cold storage and after up to 12 weeks reperfusion,[12] cold-stored arteries provide a reliable but noncontractile vascular conduit. In rabbits 100% of 4-week cold-stored grafts remained patent 3 to 6 weeks after reinsertion, and 60% remained patent after 12 weeks reinsertion.[12] In clinical situations when a patient has lengths of artery left over after an operation, the vessels are normally discarded; however, it may be feasible to cold store the vessels should vascular grafts be required at a later date. There are only a few suitable arterial donor sites, which limits the clinical value of cold-stored arterial autografts. However, if arteries lose sufficient antigenicity during cold storage to be acceptable as allografts, they would provide a valuable prosthesis for vascular grafting and could form the basis for the establishment of a "vascular bank."

Recognition of cellular antigens in vascular EC has been shown to be the major stimulus to rejection of vascular allografts.[31,32] Hence, the total absence of ECs and the necrosis and breakdown of all medial SMC in arteries stored for 4 weeks at 4° C might reduce the allogenic reaction in such grafts to very low levels. Allaire et al.[33] has shown that vascular allografts made acellular by chemical treatment can achieve high patency rates and do not provoke immunologic reactions. Four-week cold-stored allografts are not strictly acellular because cellular debris is still present in the graft. Much of this debris is removed in the early stages of reperfusion, but Gray et al[12]. found that patches of debris are still present after 3 and 6 weeks reperfusion but not after 12 weeks. If allografts that have been cold stored until all cells are reduced to structureless debris do not excite a rejection response and behave in a similar manner to the chemically treated grafts used by Allaire et al.,[33] then cold-stored arterial allografts would be a valuable vascular prosthesis. Findings in a preliminary study of cold-stored arterial allografts in rabbits has been very encouraging, and further studies are currently in progress.[34]

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