CRYOPRESERVED HUMAN TISSUE IN PHARMACOLOGICAL RESEARCH

E. MÜLLER-SCHWEINITZER

Preclinical Research, Sandoz Pharma A.G., CH-4002 Basel, Switzerland

Dedicated to Prof. Dr. Dr. Ernst Mutschler *on the occasion of his 60th birthday*

Received in final form 25 July 1991

SUMMARY

Despite the relevance of human isolated tissue in human pharmacology, its use is still very much the exception rather than the rule. The major reason for this is that the supply of fresh human material is both irregular and unpredictable. This article considers the usefulness of cryopreservation to store human isolated tissues for subsequent pharmacological studies. Despite certain problems, such as some reduction in contractile force and endothelial function, the main biochemical properties and affinities of most agonists and antagonists have been shown to be well preserved. Hence, this technique offers clear potential for ensuring the supply of both vascular and non-vascular human material for pharmacological studies.

KEY WORDS: cryopreservation, isolated organs, pharmacology.

INTRODUCTION

Pharmacological studies on the mechanism(s) of action of compounds are generally performed on isolated tissues from various animals. From these studies, a large body of knowledge has been accumulated concerning species differences for various pharmacological mechanisms and data obtained from animal experiments, which in many cases have been shown to be relevant to human pharmacology. Naturally, though, if human tissue was made available, the results obtained from pharmacological experiments would be the most accurate and predictable for human pharmacology. Human tissue can be obtained from surgery or during autopsy. However, not all human tissue obtained from surgery or autopsy can be used, as it may be diseased when healthy tissue is required or it may be damaged during surgery, by ischaemic time periods during and after surgery, patient age, medication and anaesthesia. Therefore, besides obtaining tissue, the main problems are the diversity of the tissue types available and the irregularity of supply. Also, once removed from the patient, the tissue has a very short life span and experiments should commence as soon as possible, if not

1043–6618/92/020103–09/$03.00/0

immediately, but this is not always convenient. All these factors make studies with human tissues difficult to conduct. Hence the advantage of a simple and reliable method of storing human tissues in such a way as to preserve their functional attributes is readily apparent. Storage in such a way would also mean that tissue need not all be used within a few hours, but several days of experiments could be carried out from the same tissue divided into samples.

ENIGMA AND PRINCIPLES OF CRYOPRESERVATION

Freezing of living mammalian cells in physiological media without cryoprotective additives generally leads to the formation of membrane damaging ice crystals in intra- and/or extracellular spaces and only few if any cells survive [1, 2]. The field of cryopreservation started more than 40 years ago, when it was discovered that glycerol protects viable cells in suspension [3] and contractile proteins in muscle [4] from damage at subzero temperatures. Ten years later the cryoprotective activity of dimethyl sulphoxide (DMSO) was described [5] and it was adopted for the preservation of cells and tissues at low temperatures in aqueous solutions. Cryoprotective agents such as glycerol and DMSO are believed to protect mammalian cells from damage during freezing and thawing by reducing the amount of intracellular ice formed at subzero temperatures. This reduction in ice formation leads to an increase in the recovery rate of frozen stored cells after thawing [6]. Today, DMSO is a preferred cryoprotectant for the preservation of cells and tissues in various areas of research such as fertility and reproductive medicine, transplantation of blood cells, bone marrow, ligaments and tendons, corneal transplantation and aortic valve replacement and vascular reconstruction.

Functional studies on cryopreserved tissues were first performed with uteri and *Taenia coli* from guinea-pigs which had been frozen in physiological salt solutions containing 10–15% glycerol or DMSO. In those studies it was found that slow freezing in the presence of glycerol or DMSO increases markedly the recovery rate as assessed by the contractility of the tissues after thawing [7–14]. However, it was also noted that different cell types not only differ in their sensitivity to the cryoprotectant but may also be irreversibly damaged when exposed to it at 37°C. Aortae from cats constrict in response to 10% DMSO [15], while aortae from rabbits [16] and various canine veins [17] relax. Depending on the temperature and concentration used, exposure to DMSO may induce functional changes which persist even after repeated changes of the bathing medium [7, 8, 11, 17]. Furthermore, the presence of many different cell types within a tissue or organ implies that no one freezing/thawing procedure can satisfy them all [6]. Pharmacological studies, however, require the preservation of many different biochemical and functional properties of a tissue.

Various cryoprotective agents and freezing methods have been tested, taking the maximal contractile responses to noradrenaline of canine saphenous vein strips as parameter for the preservation of cell integrity during the freezing–thawing procedure. In those studies the best recovery was obtained when venous segments which had been immersed in fetal calf serum containing 1.8 M DMSO, were slowly frozen to −70 °C, stored in liquid nitrogen and thawed rapidly before being used

[18–20]. Other cryoprotective substances such as glycerol, *N*-methylacetamide, dimethylacetamide, *N*-methylformamide, dimethylformamide and polyethylene glycol 400 have also been tested on canine veins, but proved to be considerably less effective than DMSO [18, 20, 21].

BIOCHEMICAL AND MECHANICAL PROPERTIES

Comparative studies on the $^{45}Ca^{2+}$ uptake have shown that in canine saphenous veins which had been frozen in fetal calf serum containing 1.8 M DMSO, contractile responses and $^{45}Ca^{2+}$ uptake during both depolarization and α_2-adrenoceptor stimulation, are well preserved. There is a significant correlation between $^{45}Ca^{2+}$ uptake and contractile response during stimulation by KCl and guanfacine in the absence as well as in the presence of various calcium antagonists [22]. It has also been shown that after cryopreservation the *monoamine oxidase* activity in canine [19] and human veins [23] is well preserved. The same is true for the *endogenous prostaglandin synthesis*, which proved to be unchanged after cryopreservation of canine saphenous veins [19]. In addition, cryopreserved veins from dogs [24] and man [25, 26] have demonstrated intense prostacyclin I_2 (PGI_2) synthesis. Compared to that observed with fresh tissues, significantly higher levels of *tissue enzymes* such as lactic dehydrogenase (LDH), creatine phosphokinase (CPK), glutamic oxalotransaminase (GOT) and glutamic pyruvate transaminase (GPT) have been found in cryopreserved human veins [25]. In contrast, after the freezing of canine veins acid and alkaline phosphatase were significantly reduced but normal levels of LDH, GOT and GPT were obtained [17].

Assessment of the connective tissue function by quantifying the levels of [^{3}H]-proline incorporated into the tissue indicated that cryopreserved canine veins retain approximately 43.5% of values of fresh vein *collagen synthesis* [27]. Cryopreservation has been shown to preserve important functional elastic properties [27, 28], but the evidence suggests that under arterial haemodynamic conditions the compliance of cryopreserved canine veins is significantly reduced by about 50% [24]. Further evidence for this reduction was shown by the observation that helical strips from canine saphenous veins and basilar arteries which had been stored at −70 °C relaxed markedly less than those prepared from fresh vessels or from those which had been stored in liquid nitrogen [19]. Hence, not only the medium but also optimal storage temperatures seem to be important for the preservation of biomechanical properties of the tissues.

ENDOTHELIAL FUNCTION

The morphological integrity of endothelial cells after storage of various blood vessels, as assessed by light and scanning electron microscopy, has been questioned. Though some investigations by light and scanning electron microscopy have suggested that DMSO successfully protects viable venous and arterial allografts from cryoinjury during freezing [17, 29–31], other authors reported marked endothelial damage in cryopreserved blood vessels [32–35]. An

important factor, which might have contributed to these discrepancies, is the observation that endothelial injury during the preparation of blood vessels must be strictly avoided. Much effort has been made to develop techniques for the preservation of endothelial function *ex vivo*, especially in connection with vascular reconstructive surgery. Indeed, optimal preservation of viable endothelium may be achieved by the use of protein rich media, smooth muscle cell relaxants and optimal pH and temperature conditions [36–38].

Thromboresistance is directly related to an adequate production of fibrinolytic substances by endothelial cells. Therefore, the quantitative determination of *fibrinolytic activity* offers an additional tool to estimate endothelial integrity. Comparative experiments have shown that the fibrinolytic activity of cryopreserved veins from dog [33, 35, 39] and man [25] parallels largely the degree of endothelial injury as assessed by scanning electron microscopy.

Pharmacological evidence for the release of *endothelium-derived relaxing factor(s)* (*EDRF*) which mediate the relaxant responses of some hormones and neurotransmitters in various vascular tissues [40, 41] represents another means for estimating the function of vascular endothelium. Recently it has been shown that the endothelium-mediated relaxant response to acetylcholine of rabbit central ear artery and its main side branch is well preserved after storage of the arteries for several days at −70°C while suspended in newborn calf serum containing 1.8 M DMSO [42]. Similar results have been reported for the EDRF-mediated responses to acetylcholine, thrombin and calcium ionophore of canine coronary arteries following cryostorage at −75°C during immersion in Krebs–Henseleit solution containing 20% FCS and 1.8 M DMSO [43]. The same applies for the endothelium-dependent relaxant responses to both 5-HT and substance P of porcine circumflex coronary arteries which had been suspended in fetal calf serum containing 1.8 M DMSO while being stored in liquid nitrogen [44]. By contrast, experiments with rings from frozen/thawed rat aorta were less successful in this respect, since the acetylcholine-induced relaxation, an effect supposed to be strictly dependent on the endothelium in this preparation [40], was markedly attenuated in frozen/thawed tissues [18]. Whilst this discrepancy might be due to species differences, it may also reflect different susceptibility of endothelial cells from arteries of different sizes to the freezing/thawing process [42].

ADRENERGIC NEUROTRANSMISSION

Sympathetic neurotransmission represents a further functionally important mechanism of isolated blood vessels. The cocaine-induced enhancement of contractile responses to noradrenaline of cryopreserved canine saphenous veins suggested that these mechanisms were well preserved [19]. More direct evidence for an unimpaired neuronal uptake mechanism was obtained by measurements of basal and stimulation-induced tritium overflow from canine saphenous vein strips after preincubation with [^{3}H]noradrenaline. Though the basal outflow from frozen/thawed vein strips was slightly higher than that observed with strips from fresh veins, the absolute tritium overflow during sustained electrical stimulation was similar in both tissue groups [20]. Similar conclusions have been drawn from

experiments with rabbit ear arteries which had been immersed in newborn calf serum containing 1.8 M DMSO and stored at −70 °C. After thawing the contractile responses to electric field stimulation of these arteries were similar to those measured with fresh tissues [42].

SMOOTH MUSCLE FUNCTION

Functional changes following cryopreservation have been estimated by measuring the *contractility* of cryopreserved veins and arteries from various species in response to numerous agonists. In these comparative studies quantification was derived by determination of two parameters, namely the *efficacy* (maximal contractile effect) and the *affinity* (the concentration producing 50% of maximum response, i.e., the EC_{50} or pD_2 value) of the agonist. Table I summarizes the

Table I
Maximal contractile responses of cryopreserved blood vessels expressed as percentage of that produced by unfrozen tissues

Blood vessel (species)	*Cryomedium +DMSO (M)*	*Temp.*	*Agonist and maximum effect*		*Ref.*
Basilar artery (man)	FCS+1.8 M	−190 °C	5-HT	40%	18
Pulmonary artery (man)	FCS+1.8 M	−190 °C	U 46619	76%	35
Saphenous vein (man)	FCS+1.8 M	−190 °C	5-HT	100%	23
Basilar artery (dog)	FCS+1.8 M	−70 °C	5-HT	69%	19
Coronary artery (dog)	*KHS+1.8 M	−75 °C	$PGF_{2\alpha}$	55%	43
Jugular vein (dog)	KHS+2.1 M	−100 °C	Adrenaline	75%	46
Jugular vein (dog)	KHS+2.1 M	−140 °C	Adrenaline	48%	17
Jugular vein (dog)	KHS+2.8 M	−140 °C	Adrenaline	38%	17
Jugular vein (dog)	KHS+3.5 M	−140 °C	Adrenaline	15%	17
Saphenous vein (dog)	FCS+1.8 M	−190 °C	Noradrenaline	59%	19
Saphenous vein (dog)	FCS+1.5 M	−190 °C	Noradrenaline	57%	19
Saphenous vein (dog)	FCS+1.8 M	−70 °C	Noradrenaline	42%	19
Saphenous vein (dog)	FCS+2.6 M	−190 °C	Noradrenaline	19%	18
Saphenous vein (dog)	FCS+1.8 M	−190 °C	Guanfacine	42%	22
Saphenous vein (dog)	FCS+1.8 M	−190 °C	5-HT	32%	19
Saphenous vein (dog)	FCS+1.8 M	−70 °C	5-HT	28%	19
Saphenous vein (dog)	FCS+1.8 M	−190 °C	Tryptamine	55%	19
Saphenous vein (dog)	FCS+1.8 M	−70 °C	Tryptamine	43%	19
Saphenous vein (dog)	FCS+1.8 M	−190 °C	KCl	36%	22
Coronary artery (pig)	FCS+1.8 M	−190 °C	$PGF_{2\alpha}$	36%	44
Coronary artery (pig)	FCS+1.8 M	−190 °C	5-HT	20%	44
Ear artery (rabbit)	FCS+1.8 M	−70 °C	Noradrenaline	60%	42
Ear artery (rabbit)	FCS+1.8 M	−70 °C	Histamine	61%	42
Aorta (rat)	FCS+1.8 M	−190 °C	Noradrenaline	35%	18

FCS, fetal calf serum; KHS, Krebs–Henseleit solution; *with 20% FCS.

parameters calculated on various blood vessels which had been stored at −70°C to −190°C while being suspended in media containing DMSO as a cryoprotectant. Best recovery was obtained with vessels immersed in media containing 1.8–2.1 M DMSO.

In addition, *endothelium-independent relaxations* in response to agonists such as isoprenaline, forskolin, 3-isobutyl-1-methylxanthine, nitroprusside, atriopeptin III, PGI_2, aminophylline, papaverine and various potassium channel activators are also well preserved. After cryopreservation in DMSO containing media, endothelium-independent relaxant responses of rabbit ear arteries [42], canine [43] and porcine coronary arteries [44] and also in human pulmonary arteries [45] have been shown to be well maintained.

In most blood vessels investigated up to now, the maximum contractile force was found to be diminished when compared to the contractile force produced by unfrozen tissues. However, there was always a very good correlation when the *affinities* for different agonists on frozen/thawed and unfrozen vessels were compared [18–20, 22, 42–45]. Thus a highly significant correlation of pD_2 values for various tryptamine derivatives on fresh and frozen/thawed canine basilar arteries [19] and human saphenous veins [23] has been demonstrated. Futhermore, the affinity parameters for all antagonists investigated up to now (pA_2 and pD'_2 values) proved to be unchanged after cryopreservation. The same is true for antagonists tested against noradrenaline and 5-hydroxytryptamine on human saphenous veins [23] and for calcium channel blocker tested against KCl and guanfacine on canine saphenous veins [22].

CRYOSTORAGE OF HUMAN TISSUE

Human cells and tissues are quite well preserved when immersed in a medium containing an appropriate concentration of a cryoprotectant such as glycerol or DMSO and stored at subzero temperatures. Under optimal conditions both contractile force and affinities for various agonists and antagonists on segments from cryopreserved human saphenous and femoral veins are unchanged if compared to data obtained with fresh tissues [18, 23]. The same applies for human bronchi obtained from tumour surgery. After cryopreservation the spontaneous tone developed by these tissues is as high as that observed with fresh bronchi [18]. Moreover, it has been demonstrated that after cryopreservation even the ciliary function in human airway tissues [47, 48] is well retained.

Though after cryopreservation the contractile force of human arteries such as basilar, cerebral and pulmonary arteries is generally reduced by 25–50%, the affinities for the majority of pharmacologically active drugs proved to be unchanged [18, 45]. In addition, the responses of cryopreserved human pulmonary arteries to endothelium-independent vasodilators have been shown to be comparable to those observed with unfrozen samples [45]. A correlation of the pD_2 values that have been determined on fresh and frozen/thawed human blood vessels is shown in Fig. 1. All these vessels had been frozen slowly to −70°C and subsequently stored in liquid nitrogen while being immersed in fetal calf serum containing 1.8 M DMSO. Before being used, the tissues had been thawed within a

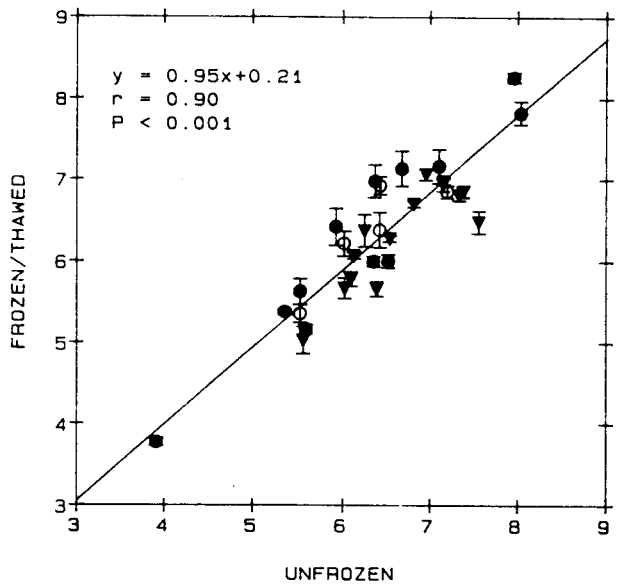

Fig. 1. Correlation between drug potencies as assessed by the pD_2 values, for various agonists determined on unfrozen (abscissa scale) and frozen/thawed (ordinate scale) human saphenous veins (▼), human basilar arteries (○) and human pulmonary arteries (●). Data were compared by linear regression analysis. The correlation coefficient and regression for the correlation are indicated.

few minutes by placing the ampoules into a water bath at 37°C. As can be seen from Fig. 1, there exists a highly significant correlation between the pD_2 values determined on blood vessels from both groups.

These findings indicate the usefulness of the cryopreservation technique for ensuring the supply of both vascular and non-vascular human material for pharmacological studies. In view of the often uncertain and limited predictability of data obtained in animal experiments, the use of cryopreserved human tissue for *in vitro* experiments should be considered.

REFERENCES

1. Litvan GG. Mechanism of cryoinjury in biological systems. *Cryobiology* 1972; **9:** 182–9.
2. Mazur P. Slow freezing injury in mammalian cells. In: *The freezing of mammalian embryos*. Amsterdam: Elsevier, North Holland, 1977: 19–42.
3. Polge C, Smith AU, Parkes AS. Revival of spermatozoa after vitrification and dehydration at low temperatures. *Nature* (*Lond*) 1949; **164:** 666.
4. Szent-Györgyi A. Free-energy relations and contraction of actomyosin. *Biol Bull* 1949; **96:** 140–61.
5. Lovelock JE, Bishop MWH. Prevention of freezing damage to living cells by dimethyl sulphoxide. *Nature* 1959; **183:** 1394–5.
6. Pegg DE. Principles of tissue preservation. In: Morris PJ, Tilney NL, eds. *Progress in transplantation*. Edinburgh-London-Melbourne-New York: Churchill Livingstone, 1985: 69–105.

7. Farrant J. Pharmacological actions and toxicity of dimethyl sulfoxide and other compounds which protect smooth muscle during freezing and thawing. *J Pharm Pharmacol* 1964; **16:** 472–83.
8. Farrant J. Calcium-dependent contraction of smooth muscle after freezing in the presence of dimethyl sulphoxide. *J Physiol (Lond)* 1964; **170:** 33P–4P.
9. Farrant J. Mechanism of cell damage during freezing and thawing and its prevention. *Nature* 1965; **205:** 1284–7.
10. Farrant J, Walter CA, Armstrong JA. Preservation of structure and function of an organized tissue after freezing and thawing. *Proc Roy Soc B (Lond)* 1967; **168:** 293–310.
11. Elford BC. Functional recovery of smooth muscle after exposure to dimethyl sulfoxide and low temperatures. *Cryobiology* 1970; **7:** 148–53.
12. Elford BC, Walter CA. Preservation of structure and function of smooth muscle cooled to 79 °C in unfrozen aqueous media. *Nature (New Biol)* 1972; **236:** 58–60.
13. Elford BC, Walter CA. Effects of electrolyte composition and pH on the structure and function of smooth muscle cooled to −79°C in unfrozen media. *Cryobiology* 1972; **9:** 82–100.
14. Taylor MJ, Pegg DE. The effect of ice formation on the function of smooth muscle tissue stored at −21 or −60°C. *Cryobiology* 1983; **20:** 36–40.
15. Spilker B. Pharmacological studies on dimethyl sulfoxide. *Arch Int Pharmacodyn* 1972; **200:** 153–67.
16. Jackson CV, Karow AM, Carrier GO. Influence of dimethyl sulfoxide (Me_2SO) on vascular smooth muscle. *Arch Int Pharmacodyn* 1979; **237:** 4–15.
17. Weber TR, Dent DL, Lindenauer SM, *et al.* Viable vein graft preservation. *J Surg Res* 1975; **18:** 247–55.
18. Müller-Schweinitzer E. Cryopreservation: a useful technique for storing tissues for pharmacological investigations. *TIPS* 1988; **9:** 221–3.
19. Müller-Schweinitzer E, Tapparelli C. Pharmacological studies on frozen stored canine saphenous veins and basilar arteries. *Naunyn Schmiedeberg's Arch Pharmacol* 1986; **332:** 74–8.
20. Müller-Schweinitzer E, Tapparelli C. Cryopreservation of isolated blood vessels for pharmacological studies: experiments on canine and human veins. In: Nobin A, Owman C, Arneklo-Nobin B, eds. *Neuronal messengers in vascular function.* Amsterdam: Elsevier Science Publishers (Biomedical Division), 1987: 105–10.
21. Müller-Schweinitzer E. Cryopreservation of isolated blood vessels. *Folio Haematol, Leipzig* 1988; **115:** 405–10.
22. Ebeigbe AB, Müller-Schweinitzer E, Vogel A. Effects of calcium channel blockade in canine saphenous veins after storage at −190 °C. *Brit J Pharmacol* 1988; **94:** 381–8.
23. Müller-Schweinitzer E, Tapparelli C, Victorzon M. Functional studies on human veins after storage at −190°C. *Brit J Pharmacol* 1986; **88:** 685–7.
24. Showalter D, Durham S, Sheppeck R, *et al.* Cryopreserved venous homografts as vascular conduits in canine carotid arteries. *Surgery (St Louis)* 1989; **106:** 652–9.
25. Louagie YA, Legrand-Monsieur A, Lavenne-Pardonge E, *et al.* Viability of long-term cryopreserved human saphenous veins. *J Cardiovasc Surg* 1990; **31:** 92–100.
26. Passani SL, Angelini GD, Breckenridge IM, Newby AC. Endothelial function can be preserved during cryo-storage of human saphenous vein. *Eur J Cardiothorac Surg* 1988; **2:** 233–6.
27. Brockbank KGM, Donovan TJ, Ruby ST, Carpenter JF, Hagen P-O, Woodley MA. Functional analysis of cryopreserved veins. Preliminary report. *J Vasc Surg* 1990; **11:** 94–102.
28. L'Italien GJ, Maloney RD, Abbott WM. The preservation of the mechanical properties of venous allografts by freezing. *J Surg Res* 1979; **27:** 239–43.
29. Weber TR, Lindenauer SM, Dent TL, Allen E, Salles CA, Weatherbee L. Long-term patency of vein grafts preserved in liquid nitrogen in dimethylsulfoxide. *Ann Surg* 1976; **184:** 709–12.
30. Boren CH, Roon AJ, Moore WS. Maintenance of viable arterial allografts by cryopreservation. *Surgery* 1978; **83:** 382–91.

31. Balderman SC, Montes M, Schwartz K, Hart T, Bhayana JN. Preparation of venous allografts. A comparison of techniques. *Ann Surg (Philadelphia)* 1984; **200:** 117–30.
32. Calhoun AD, Baur GM, Porter JM, Houghton DH, Templeton JW. Fresh and cryopreserved venous allografts in genetically characterized dogs. *J Surg Res* 1977; **22:** 687–96.
33. Malone JM, Moore WS, Kischer CW, Keown K, Conine R. Venous cryopreservation: endothelial fibrinolytic activity and histology. *J Surg Res* 1980; **29:** 209–22.
34. Gottlob R, Stockinger L, Gestring GF. Conservation of veins with preservation of viable endothelium. *J Cardiovasc Surg* 1982; **23:** 109–16.
35. Sachs SM, Ricotta JJ, Scott DE, De Weese JA. Endothelial integrity after venous cryopreservation. *J Surg Res* 1982; **32:** 218–27.
36. Stanley JC, Sottiurai V, Fry RE, Fry WJ. Comparative evaluation of vein graft preparation media: electron and light microscopic studies. *J Surg Res* 1975; **18:** 235–46.
37. Haudenschild CC, Gould KE Quist WC, Logerfo FW. Protection of endothelium in vessel segments excised for grafting. *Circulation* 1981; **46:** II/102–II/107.
38. Brockbank KGM, Bank HL, Schmehl M. Ischemia and saphenous vein endothelial integrity. *Transplant Proc* 1989; **21:** 1384–8.
39. Street DL, Russ GA, Ricotta JJ. Platelet avidity of cryopreserved veins: thrombogenicity of cryopreserved veins. *J Surg Res* 1988; **45:** 363–9.
40. Furchgott RF, Zawadzki JV. The obligatory role of the endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; **288:** 373–6.
41. Furchgott RF. The requirement for endothelial cells in the relaxation of arteries by acetylcholine and some other vasodilators. *TIPS* 1981; **2:** 173–5.
42. Thompson L, Duckworth J, Bevan J. Cryopreservation of innervation, endothelial and vascular smooth muscle function of a rabbit muscular and resistance artery. *Blood Vessels* 1989; **26:** 157–64.
43. Ku DD, Willis WL, Caulfield JB. Retention of endothelium-dependent vasodilatory responses in canine coronary arteries following cryopreservation. *Cryobiology* 1990; **27:** 511-20.
44. Schoeffter P, Müller-Schweinitzer E. The preservation of functional activity of smooth muscle and endothelium in pig coronary arteries after storage at −190°C. *J Pharm Pharmacol* 1990; **42:** 646–51.
45. Ellis P, Müller-Schweinitzer E. Maintainance of functional activity of human pulmonary arteries after cryopreservation. *Br J Pharmacol* 1991; **103:** 1377–80.
46. Dent TL, Weber TR, Lindenauer SM, *et al.* Cryopreservation of vein grafts. *Surg Forum (Philadelphia)* 1974; **25:** 241–3.
47. Morris GJ, Buckland R, Rubenstein D, Farrant J. Preservation of human tracheal organ cultures at −196°C. *J Med Microbiol* 1973; **6:** 123–6.
48. Wulffraat NM, Veerman AJP, Stamhuis IH. Frequency and coordination of ciliary beat after cryopreservation of respiratory epithelium. *Cryobiology* 1985; **22:** 105–10.