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# Functional, biochemical and morphological studies on human bronchi after cryopreservation

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**1** Human isolated bronchi have been investigated as fresh tissue or after storage (7 and 30 days) at −196°C in foetal calf serum containing 1.8 M dimethyl sulphoxide.

**2** After cryopreservation, the maximal contractile response to acetylcholine (3 mM) was reduced (≈25%) but the difference did not reach significance statistically. Maximal responses to other spasmogens tested (histamine, [Nle$^{10}$]NKA(4-10), bradykinin, leukotriene $D_4$, U46619, and KCl) did not differ between unfrozen and frozen/thawed tissues. The sensitivity of cyropreserved tissues to the constrictor agents tested was similar to that of fresh tissues.

**3** The accumulation of inositol phosphates produced by acetylcholine in human bronchus *in vitro* was similar in fresh and cyrostored (30 days) tissues.

**4** Relaxant responses of acetylcholine (0.3 μM)-precontracted preparations to theophylline, isoprenaline, rolipram and sodium nitroprusside were unchanged after storage with the exception of the sensitivity to rolipram which was diminished in the 30-days cryostorage group.

**5** Light microscopic examination of sections taken from 30 days cryostored tissues indicates that the epithelium, submucosal tissue and smooth muscle were well preserved.

**6** These experiments suggest that cryopreservation of human bronchi results in maintenance of several morphological, functional (contraction/relaxation) and biochemical properties.

**Keywords:** Human isolated bronchus; cryopreservation; airway smooth muscle

## Introduction

Experiments with human tissues are expected to produce results of relevance for human pharmaclogy. However, a number of difficulties arise when conducting *in vitro* studies with human material. The pharmacological reactivity of the sample obtained may be altered due to anaesthesia, patient medication, tissue damage during or after surgery, or by the influence of physiological of pathological factors. In addition, important problems are the irregularity of supply and the short time of viability of the tissue. Therefore, it is convienient to have a reliable method of storing human material with the objective of maintaining functional and biochemical activities.

Different freezing methods and cryoprotective agents have been examined for the storage of isolated tissues for subsequent pharmacological experiments. Focusing on human tissues, Müller-Schweinitzer *et al.* (1986) showed that storage in liquid nitrogen (−196°C) of human saphenous veins immersed in foetal calf serum containing dimethyl sulphoxide (DMSO; 1.8 M) as a cryoprotective agent results in preservation of mechanical (contraction/relaxation) and biochemical (monoamine oxidase activity) properties. The response of human intrapulmonary arteries to contractile and relaxant agents is well-maintained after cryostorage at −196°C in foetal calf serum containing 1.8 M DMSO (Ellis & Müller-Schweinzter, 1991).

Most of the investigations on cryopreservation of mammalian tissue have been carried out in vascular smooth muscle (Müller-Schweinitzer, 1988), but other human tissues are also suitable for cryostorage. It has been established that different tissues differ in their susceptibility to the cryopreservation procedure. This makes necessary a validation of the technique of cryostorage for a particular tissue considered of interest to be studied. Although human isolated bronchus is widely used in pharmacological studies, little is known about the post-thaw functional recovery of cryostored airway smooth muscle. A recent study by Müller-Schweinitzer *et al.* (1993) showed that cryopreservation of human bronchi at −196°C maintains contractile and relaxant mechanisms. The present investigation was undertaken to find out whether cryostorage at −196°C in DMSO (1.8 M)-containing foetal calf serum is effective in the maintenance of pharmacomechancial, biochemical and morphological properties of human bronchus obtained from surgery.

## Methods

### *Tissue preparatiom and storage method*

Lung tissue was obtained from 57 (49 males and 8 females, 36 to 78 year old, mean age 62.0±1.6 years) patients who were undergoing surgery for lung carcinoma. None of the patients had a history of asthma. After the resection of one or more lung lobes, a piece of macroscopically normal tissue was cut free, submerged in physiological salt solution (for composition see drugs and solutions) at 4°C, and transported to the laboratory within 1 h after removal. Once in the laboratory, parts of the bronchus were dissected free from parenchymal lung tissue and preparations cut into rings (3-7 mm length × 2-3 mm internal diameter). In those experiments where bradykinin was the contractile agonist (see below), bronchial preparations of about 1 mm internal diameter were used (Molimard *et al.*, 1994). Preparations were distributed into three groups. One group consisted of 'fresh tissues' which were stored in physiological salt solution equilibrated with 5% $CO_2$ in $O_2$ at 4°C and used within 24 h of surgery. The other two groups comprised 'cryostored tissues' which were subjected to the same cryopreservation procedure but with two

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different times of storage, 7 and 30 days. In pharmacomechancial studies, preparations (2–8 rings) from a single patient were usually allocated at random into one or two of the three existing groups. In 10 patients (8 males and 2 females, 44 to 77 years old, mean age 58.0±3.1 years) sufficient material was obtained to enable equal distribution of preparations into each of the three groups. This permitted the responses to contractile and relaxant agonists to be studied in fresh, 7 days, and 30 days cryostored preparations which derived from the same patient.

The cryostorage procedure was essentially as previously outlined by Ellis & Müller-Schweinizter (1991). The bronchial ring preparations were placed, within the first day after surgical removal, into 1.8 ml (working volume) liquid nitrogen storage ampoules (NuncCryoTubes) containing foetal calf serum as the vehicle for the cryoprotecting agent DMSO (1.8 M). Following an equilibration time of 60 min at 4°C, the ampoules were placed in a cryofreezing cylindrical container (11 × 14 cm; Nalgene) and slowly frozen at a mean cooling rate of about 1°C $min^{-1}$ in a freezer maintained at −80°C. After 4–12 h the ampoules were transferred into liquid nitrogen (−196°C) where they were stored for 7 days or 30 days until use. Before use, the tissues were exposed for 10–20 min to −80°C and then thawed within 2.5 min by placing the ampoules in a 37°C water bath. Thereafter, the bronchial rings were rinsed in a dish containing physiological salt solution at 37°C and used for pharmacomechancial, biochemical or morphological experiments.

### *Pharmacomechancial experiments*

The bronchial rings were suspended on tissue hooks in 5 ml organ baths containing physiological salt solution gassed with 5% $CO_2$ in $O_2$ at 37°C (pH 7.4). Each preparation was connected to a force displacement transducer (Statham UF-1 or Grass FTO3) and isometric tension changes recorded on a polygraph. The preparations were equilibrated for 90 min with changes in physiological salt solution every 15 min before adding drugs. A load of 2–2.5 g was maintained throughout the equilibrium period. Bronchial rings were initially contracted to maximal tension with acetylcholine 3 mM, and maximal relaxation was induced with theophylline 3 mM. The tissue was then allowed to equilibrate for a further 60 min period. The effects of contractile (acetylcholine, histamine, bradykinin, [$Nle^{10}$]-NKA(4-10), leukotriene $D_4$, U46619, and KCl) and relaxant (theophylline, isoprenaline, rolipram and sodium nitroprusside) agents were investigated by constructing cumulative concentration-response curves. Relaxation curves were obtained from a plateau contraction produced by acetylcholine (0.3 mM). Only one concentration-response curve was generated with each preparation. Experiments were terminated by the addition of acetylcholine 3 nM (experiments with spasmogens) or theophylline 3 mM (experiments with relaxants) to check for any time-related change in the maximal contractile or relaxant response of the preparation. In additional experiments, concentration-effect curves for acetylcholine or histamine were produced following 30 min incubation in the presence of physiological salt solution (control), atropine (0.1 μM; in acetylcholine experiments) or mepyramrine (0.1 μM; histamine experiments). Changes in force were measured from isometric recordings and expressed in g. The maximum response to a contractile or relaxant agent was expressed in absolute values (g) or normalized as a % of the response to acetylcholine (3 mM) of theophylline (3 mM). The molar concentration required to produce 50% of maximal effect ($EC_{50}$) was calculated by linear regression from the individual concentration-effect curve. These values were transformed into $pEC_{50}$ values i.e. the negative logarithm of the $EC_{50}$ value. In experiments using antagonists, the $K_B$ values were calculated using the equation: $K_B = [B]/(DR-1)$, where [B] is the concentration of the antagonist and DR (dose-ratio) is the ratio of $EC_{50}$ of agonist in the presence and absence of antagonist. The $K_B$ values were transformed into $pK_B$ values ($-\log K_B$ value).

### *Biochemical experiments*

Total inositol phosphate accumulation was determined as previously outlined (Naline *et al.*, 1994). Human bronchi obtained from 47 patients (41 males and 6 females, 36 to 78 years old, mean age 62.8±1.6 years) were used fresh (24 h storage at 4°C) or thawed after 30 days of cryostorage (-196°C) as indicated above. In short, small fragments (about 1 $mm^2$) of human bronchi weighing a total of about 10 g were washed in physiological salt solution and incubated in 25 ml physiological salt solution containing 50 μCi of *myo*-[$^3$H]-inositol for 4 h at 37°C. After this incubation, the tissue was washed twice with physiological salt solution. Aliquots of washed tissue (1.5–2 g) were placed in 2 ml final volume of physiological salt solution and incubated at 37°C for 30 min. Just before stimulation 20μl of LiCi was added (final concentration 10 mM); then the samples were stimulated with 20 μl physiological salt solution (control) or acetylcholine (1 μM, 10 μM, 0.1 mM or 1 mM, final concentration) for 5 min at 37°C. Stimulation was stopped, in an iced bath, by addition of 3ml chloroform/methanol/HCl 10N (100:200:4, v/v/v) and vigorous shaking; the tissues maintained at 4°C, were crushed with an Ultraturax. The samples were centrifuged (2000 *g*) for 10 min at 4°C. The aqueous phases were brought to pH 4 with 50 μl of ammonium formate 1.2 M and stored at −80°C until analysis. The separation of inositol phosphates was performed, according to Irvine *et al.* (1985), by an h.p.l.c ion-exchange system, and the radioactivity measured in a Flow-One on-line radioactivity flow detector (Packard, Meriden, U.S.A) as indicated in Naline *et al.* (1994).

### *Morphological study*

Fresh and frozen (30 days)/thawed tissues were fixed (24 h) in 10% formyl saline (0.1 M phosphate-buffered). Following tissue dehydration in serial alcohols and clearing with toluene, 5 mm transverse sections of bronchi were obtained from paraffin blocks. These sections were rehydrated and stained with haematoxylin and eosin. The integrity of the epithelium and other structures in these sections was assessed by light microscopy.

### *Drugs, solutions and statistical analysis of results*

Drug concentrations are expressed in terms of molar concentration of the active species. All drugs used were obtained from Sigma (St Louis, U.S.A) except for [$Nle^{10}$]NKA(4-10) which was obtained from Novabiochem (Laüfelfingen, Switzerland). Rolipram was supplied by Schering (Berlin, Germany). For the pharmacological studies, isoprenaline was dissolved in distilled water containing 0.57 mM ascorbic acid. Stock solutions of rolipram were prepared in 20% polyethylene glycol 300. All other drugs were dissolved in deionized water. Subsequent dilutions of drugs were made in the physiological salt solution. Drug vehicles at the concentrations employed did not alter the pharmacological reactivity of the preparation. *Myo*-[2-$^3$H]-inositol with PT6-271 (specific radioactivity, 10–20 Ci $mmol^{-1}$) was purchased from Amersham International (Amersham, Buckinghamshire, UK). The physiological salt solution had the following composition (mM): NaCl 119, KCl 5.4, $CaCl_2$ 2.5, $KH_2PO_4$ 0.6, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 11.1.

Values are given as mean±s.e. mean of *n* patients. The data were analysed first for normal distribution and then for statistical significance by parametric analysis of variance (ANOVA) and *post-hoc* Bonferroni test, with a *P*-value $<0.05$ taken as significant. Data were analysed by a computer using standard statistical packages.

## Results

### *Pharmacomechancial experiments*

Figure 1a shows that maximal contractions to acetylcholine (3 mM) in human bronchial rings obtained from the same patients for each of the groups were $1.30 \pm 0.16$ g in fresh tissues, $1.02 \pm 0.18$ g in 7-days cryostored tissues, and $0.94 \pm 0.22$ g in 30-days cryostored tissues ($n = 7$). These results suggest that after cryopreservation in a medium containing foetal calf serum as vehcile for DMSO 1.8 M, the maximal responses to acetylcholine tended to be reduced, but this reduction of responsiveness to acetylcholine in frozen/thawed tissues did not reach statistical significance ($P > 0.05$). Sensitivity ($pEC_{50}$) to acetylcholine did not differ between groups in these seven patients (Figure 1c).

When all patients studied were considered, the maximal responses to acetylcholine were also attenuated but this reduction did not reach statistical significance either (Table 1). Sensitivity to acetylcholine was similar in fresh and frozen/thawed tissues (Table1,Figure 2). A similar trend was observed for other contractile agonists (histamine, [Nle$^{10}$]NKA(4-10) and U46619), but reduction of responsiveness failed to reach statistical significance. (Table 1). Maximal responses to other spasmogens (bradykinin, leukotriene $D_4$ and KCl) did not differ between groups (Table 1). When contractile responses were normalized as percentages of the maximal response to acetylcholine (3 mM), the variability was reduced compared to the results in absolute values (g) and the differences found between groups for each spasmogen remained not significant (Figure 2). Sensitivity of the human bronchi to each of the constricting agents tested was not significantly different for fresh tissues compared to frozen/thawed tissues (Table 1, Figure 2). Treatment of human bronchial preparations with either atropine (0.1 μM) or mepyramine (0.1μM) antagonized the contractions produced by acetylcholine or histamine respectively, and produced a parallel rightward displacement of the log concentration-response curves of the corresponding agonist. $pK_B$ values for atropine against acetylcholine were $8.76 \pm 0.19$ ($n = 7$) in fresh tissues, $8.65 \pm 0.23$ ($n = 6$) in 7-days frozen tissues, and $9.01 \pm 0.14$ ($n = 6$) in 30-days frozen tissues. $pK_B$ vlaues for mepyramine against histamine were $8.25 \pm 0.28$ in fresh tissues, $7.94 \pm 0.36$ in 7-days frozen tissues, and $8.13 \pm 0.19$ in

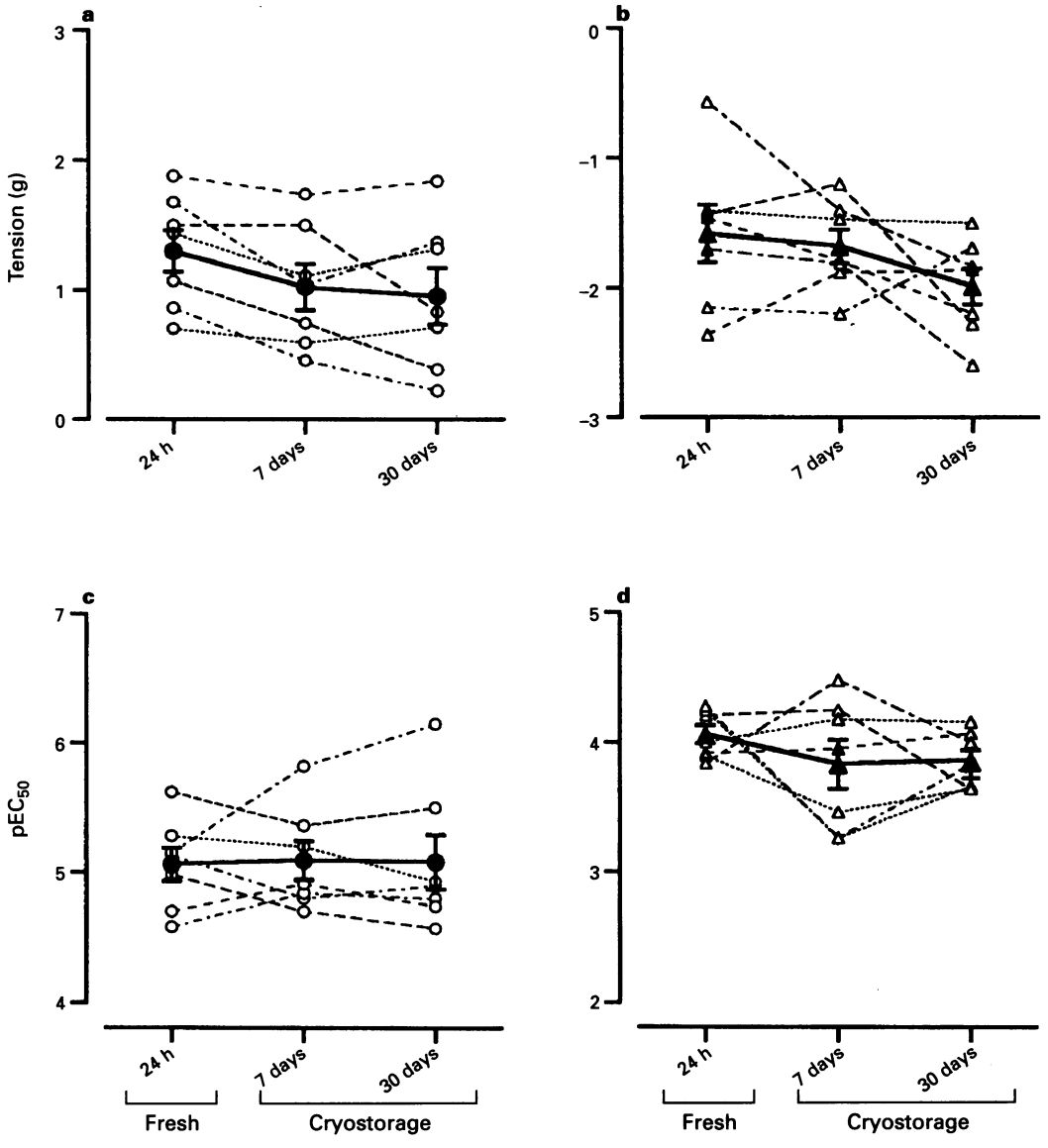


**Figure 1** Responsiveness (assessed by maximal responses to agonist, 3 mM (a, b)) and sensitivity estimated as $pEC_{50}$ values (c, d) of human isolated bronchus to acetylcholine (●,○; a, c) and theophylline (▲, △; b, d). The responses were obtained in fresh tissues (24 h storage at 4°C) and after 7 or 30 days of cryostorage (foetal calf serum and 1.8 M DMSO). The responses in each of the three groups were elicited by bronchial rings isolated from the same patients ($n = 7$). Individual (○, △) and mean ± s.e.mean (●, ○) values are shown.

**Table 1** Maximal responses ($E_{max}$) and sensitivity ($pEC_{50}$) of human isolated bronchus to acetylcholine (ACh), histamine, [Nle$^{10}$]NKA(4–10), bradykinin, leukotriene $D_4$ U46619, KCl, theophylline, isoprenaline, rolipram, and sodium nitroprusside (SNP)

| | *Fresh tissues* | | | *7 days storage* | | | *30 days storage* | | |
|---|---|---|---|---|---|---|---|---|---|
| | *n* | $E_{max}$ (g) | $pEC_{50}$ | *n* | $E_{max}$ (g) | $pEC_{50}$ | *n* | $E_{max}$ (g) | $pEC_{50}$ |
| ACh | 15 | 1.83±0.31 | 5.16±0.09 | 11 | 1.11±0.17 | 5.32±0.17 | 11 | 1.14±0.19 | 5.11±0.18 |
| Histamine | 14 | 1.66±0.23 | 5.86±0.12 | 11 | 0.92±0.19 | 5.80±0.12 | 12 | 1.35±0.21 | 5.96±0.18 |
| [Nle$^{10}$]NKA(4–10) | 6 | 1.27±0.41 | 6.69±0.18 | 6 | 0.70±0.26 | 6.98±0.17 | 6 | 0.76±0.23 | 6.98±0.18 |
| Bradykinin | 6 | 0.57±0.11 | 6.93±0.27 | 6 | 0.48±0.08 | 7.22±0.44 | 6 | 0.62±0.17 | 6.79±0.33 |
| Leukotriene $D_4$ | 4 | 0.72±0.26 | 8.58±0.30 | 4 | 0.79±0.38 | 8.37±0.49 | 6 | 0.67±0.18 | 8.70±0.17 |
| U46619 | 7 | 1.65±0.14 | 7.41±0.05 | 7 | 1.06±0.19 | 7.53±0.08 | 7 | 1.46±0.23 | 7.50±0.08 |
| KCl | 6 | 0.64±0.33 | 2.27±0.12 | 6 | 0.45±0.20 | 2.47±0.14 | 6 | 0.71±0.30 | 2.34±0.16 |
| Theophylline | 8 | −1.42±0.25 | 3.99±0.09 | 7 | −1.68±0.13 | 3.83±0.19 | 8 | −1.87±0.17 | 3.87±0.07 |
| Isoprenaline | 7 | −1.43±0.24 | 6.84±0.07 | 6 | −1.28±0.22 | 6.88±0.13 | 7 | −1.42±0.18 | 6.55±0.15 |
| Rolipram | 10 | −1.18±0.22 | 6.19±0.39 | 11 | −1.72±0.40 | 5.33±0.46 | 8 | −1.05±0.34 | 4.63±0.28* |
| SNP | 6 | −1.30±0.46 | 6.04±0.17 | 6 | −1.26±0.51 | 6.26±0.18 | 4 | −0.95±0.35 | 6.18±0.23 |

The responses were obtained in fresh (24 h storage at 4°C) and cryostored (7 or 30 days) tissues. Data are mean±s.e.mean of *n* patients. *$P<0.05$ compared to fresh tissues.

30-days frozen tissues ($n=6$ for each group). No significant differences were found for these $pK_B$ values between frozen and unfrozen tissues.

Responses to relaxants were obtained in preparations which had been contracted to a plateau by a submaximal concentration (approximately $EC_{20}$) of acetylcholine (0.3 mM). Figure 1b,d shows that the responsiveness and sensitivity to theophylline (3 mM) of human bronchial rings derived from the same patients did not differ between groups. These results suggested that cryopreservation did not alter relaxation. When all patients studied were considered, the efficacy and potency of a number of relaxants (i.e. theophylline, isoprenaline, sodium nitroprusside, and rolipram) were not significantly different in fresh compared to frozen/thawed tissues with the exception of the sensitivity to rolipram in 30-days frozen tissues (Table 1, Figure 3). The mean log concentration-response curve to isoprenaline obtained in 30-days cryostored tissues was shifted to the right compared to that obtained in fresh tissues (Figure 3) but this displacement did not reach significance when assessed at the $EC_{50}$ level (Table 1). The baseline values of tension before commencing the concentration-response curves to each of the spasmogens or the relaxants tested did not significantly differ between groups (data not shown).

### *Biochemical experiments*

Incubation of fresh tissues with acetylcholine (1 μM–1 mM) resulted in a concentration-dependent increase of total [$^3$H]-inositol phosphates above basal levels, with a maximum for

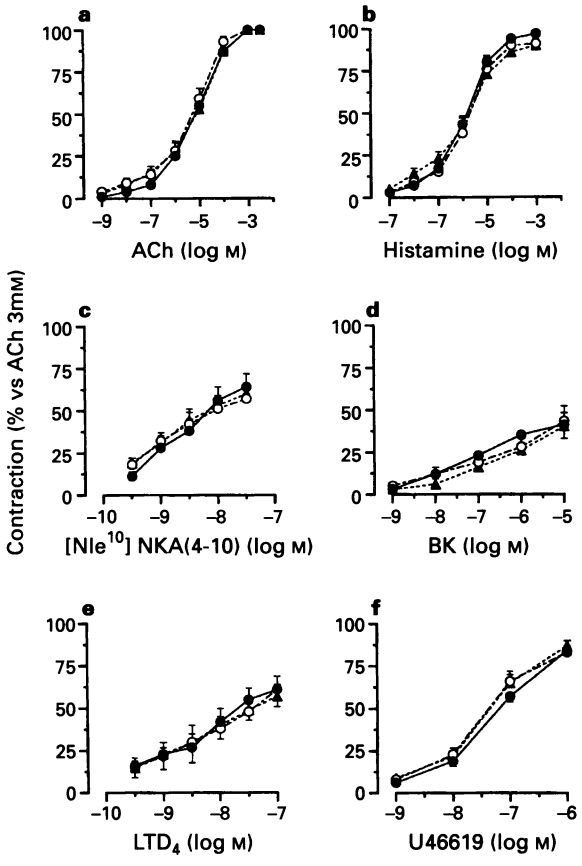


**Figure 2** Cumulative concentration-response curves obtained in fresh (●) and cryostored (7 days, ○; or 30 days, ▲) human bronchial rings for the contractile responses to (a) acetylcholine, (b) histamine, (c) [Nle$^{10}$]NKA(4-10), (d) bradykinin, (e) leukotriene $D_4$ and (f) U46619. Ordinates represent contractile responses expressed as percentages of the contraction to acetylcholine (3 mM). Abscissae represent agonist concentration as log M. Points are mean±s.e. mean of the number of experiments shown in Table 1.

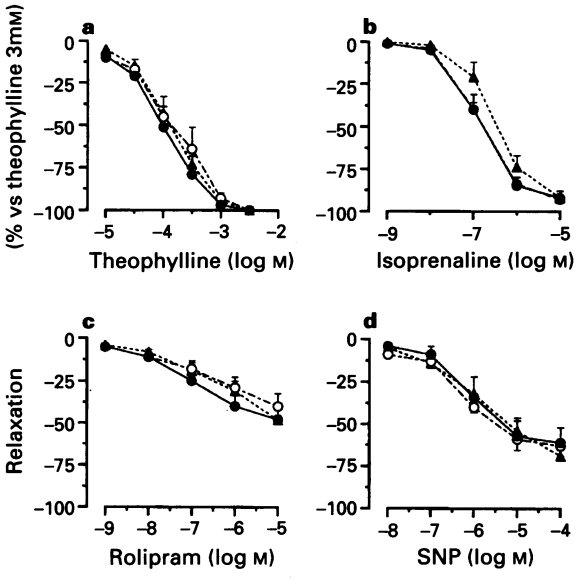


**Figure 3** Cumulutive concentration-response curves obtained in fresh (●) and cryostored (7 days, ○; or 30 days, ▲) human bronchial rings for the relaxant responses to (a) theophylline, (b) isoprenaline, (c) rolipram, and (d) sodium nitroprusside. Ordinates represent relaxant responses expressed as percentages of the relaxation to theophylline (3 mM). Abscissae represent agonist concentration as log M. Points are mean±s.e. mean of the number of experiments shown in Table 1.

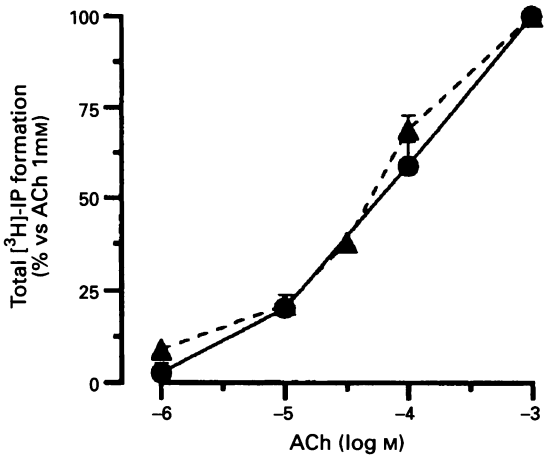


**Figure 4** Concentration-response curves obtained in fresh (●) and cryostored (30 days, ▲) human bronchial rings for the total [$^3$H]-inositol phosphate production in response to acetylcholine. To normalize responses from different experiments, data are expressed as a percentage of the response to ACh 1 mM which was measured in every experiment. Each point represents the mean ± s.e. mean from 19 (fresh) and 15 (cryostored) separate experiments.

acetylcholine (1 mM) of 185 ± 20% ($n = 19$) and an $EC_{50} = 4.20$. The corresponding values obtained in bronchi subjected to 30 days of cryostorage were 147 ± 11% ($n = 15$) and 4.30, which did not differ significantly from values obtained in fresh tissues (Figure 4).

### *Morphological study*

Light microscopic examination of sections taken from 30 days cryostored tissues revealed that the epithelium was well preserved, and the submucosal tissue or the underlying smooth muscle was not damaged (Figure 5). The morphological characteristics of cryopreserved tissues did not differ from those observed for fresh tissues under light microscopic examination ($n = 6$).

## Discussion

The present work shows that post-thaw mechanical responses of cryostored human bronchi to a variety of contractile and relaxant agents are well preserved. The results from pharmacomechancial experiments previously reported by Müller-Schweinizter *et al.* (1993) for the same preparation were essentially confirmed in the present study and extended to other spasmogens and relaxants as well as to the biochemical and morphological properties of the cryostored tissues.

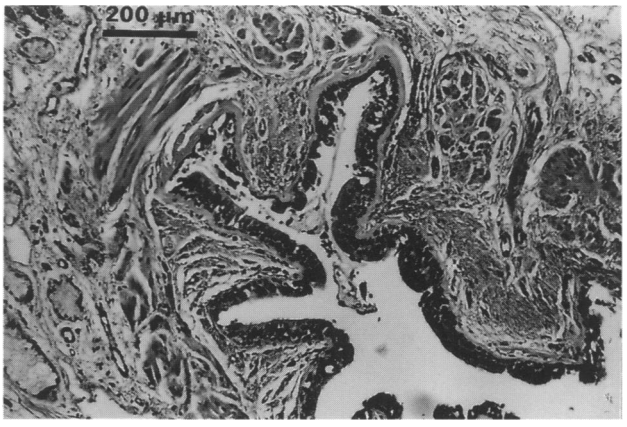


**Figure 5** Preparations of human bronchus after 30 days of cryostorage. Transverse section stained with haematoxylin-eosin. The bronchial lumen is lined by a columnar ciliated epithelium. Note the regular morphology of smooth muscle cells associated in bundles. Calibration bar = 200 µm.

We decided to use 1.8 M DMSO in foetal calf serum to protect human bronchial tissue from cryoinjury during freezing (Müller-Schweinizter & Tapparelli, 1986). Under these experimental conditions, histological examination of frozen/thawed tissues by light microscopy did not reveal any alteration or damage in the epilthelium, submucosa or smooth muscle. This finding is consistent with other reports (Morris *et al.*, 1973; Wulffraat *et al.*, 1985; Deschamps *et al.*, 1989) which demonstrated that cryopreservation of human and canine airways in DMSO-containing media preserves epithelium integrity, mucus production and ciliary activity.

De Jongste *et al.* (1985, 1986) have reported that the variability of responses between patients was significantly larger than that between strips within patients. According to their findings, the sensitivity (assessed as $pEC_{50}$) could be measured accurately and reproducibly and showed a relatively small within-patients variation. In contrast, responsiveness (assessed as maximal response) showed a considerable within-patients variation (De Jongste *et al.*, 1985, 1986). In the present study, we have found that despite the apparent preservation of smooth muscle in the cryostored human bronchial preparations, maximal contraction to acetylcholine (3 mM) appeared to be partly reduced when compared to fresh tissues from the same patients (see Figure 1). However, the differences in responsiveness failed to reach statistical significance. Essentially the same conclusion was reached for acetylcholine (3 mM)-induced contraction when the results for all patients were considered (see Table 1). Müller-Schweinizter *et al.* (1993), likewise, reported that with bronchial rings from different patients for each group (fresh versus 3–4 weeks frozen tissues) maximal contraction to carbachol (0.1 mM) was reduced in cryopreserved tissues, yet the difference was not significant. However, Müller-Schweinizter *et al.* (1993) found a reduced sensitivity to carbachol in frozen tissues whereas we found no difference in the sensitivity to acetylcholine, irrespective of whether the analysis was made within-patients (see Figure 1) or between patients (see Table 1). In addition, we found that the potency of atropine as a muscarinic antagonist remained unaltered in cryostored tissues.

Breakdown of membrane polyphosphoinositides and formation of inositol phosphates has been proposed as the transduction mechanism involved in the pharmacomechancial coupling of muscarinic receptor stimulation and airway smooth muscle contraction (Baron *et al.*, 1984). This mechanism has been demonstrated in human bronchial smooth muscle (Meurs *et al.*, 1989). A correlation exists between the ability of muscarinic agonists to elicit smooth muscle contraction and accumulation of inositol phosphates (Meurs *et al.*, 1988). The accumulation of inositol phosphates caused by acetylcholine in fresh human bronchi was similar in magnitude to that obtained in frozen/thawed tissues (this study). This finding suggests that any reduced contraction to acetylcholine observed in cryopreserved human bronchus is not attributable to alterations of the phosphoinositide turnover.

Other spasmogens (histamine, [Nle$^{10}$]-NKA(4-10), bradykinin, $LTD_4$, U46619, and KCl), which operate through different mechanisms, were tested in the present study and produced maximal contractions of frozen/thawed tissues that were not significantly different from those obtained in unfrozen tissues. The sensitivity of cryostored human bronchus to the different spasmogens tested was similar to that observed in fresh tissues. Müller-Schweinizter *et al.* (1993) found that maximal response to histamine (0.1 mM) in cryopreserved human bronchus although apparently reduced was not significantly different from that elicited in fresh tissues. In contrast, the sensitivity to histamine was significantly reduced in frozen/thawed tissues (Müller-Schweinizter *et al.*, 1993). Spasmogens other than acetylcholine and histamine were not

studied by Müller-Schweinizter *et al.* (1993). We also found reductions in the maximal responses to a number of spasmogens but statistical significance was not reached (Table 1). However, both the shape of the concentration-response curves (see Figure 2) and the $pEC_{50}$ values (see Table 1) were not different in fresh versus cryopreserved tissues. The antihistamine efficacy of mepyramine was not altered in cryopreserved tissues (this study). These results suggest that the contractility of cryopreserved human bronchus is not impaired. When spasmogenic responses were normalized as a percentage of the maximal contraction to acetylcholine (3 mM), it was found that the variability was reduced compared to the results expressed in absolute values (g), and the differences between groups were not significant. This indicates that normalization of spasmogenic response may be interesting in studies of pharmacological reactivity of cryostored human bronchi.

Relaxant responses of cryopreserved tissues to theophylline and isoprenaline were not significantly different in fresh and cryopreserved tissues. The sensitivity of the preparations to theophylline did not differ between groups but sensitivity of 30-days cryostored tissues to isoprenaline was diminished although the maximal effect was similar in all groups. These results are in contrast with those of Müller-Schweinizter *et al.* (1993) who found a decrease in the maximal relaxation and potency of papaverine and isoprenaline in cryopreserved human bronchus. This finding was ascribed to a reduced elasticity of human bronchus after cryopreservation (Müller-Schweinizter *et al.*, 1993). In the present study, maximal relaxation to rolipram and sodium nitroprusside was similar in fresh and frozen/thawed tissues, although sensitivity to rolipram was reduced in cryopreserved preparations. Therefore, our results show that maximal relaxation to a variety of relaxant agents was not impaired in the human bronchus after cryostorage. We have essentially followed the protocol for cryostorage outlined by Ellis & Müller-Schweinizter (1991) for human pulmonary arteries. However, in their bronchial study, Müller-Schweinizter *et al.* (1993) stored human material at 4°C until transport to the laboratory within three days after removal whilst in the present study we transported the pieces within the first 24 h. Another difference is that Müller-Schweinizter *et al.* (1993) used 1 g as the resting tension of the ring preparations. We equilibrated ring preparations of similar diameter under a resting tension of 2–2.5 g which was found optimal in assessing drug-induced relaxation (Naline *et al.*, 1989; Cortijo *et al.*, 1992; 1993; Sarriá *et al.*, 1994).

In conclusion, we provide experimental evidence that cryopreservation of human bronchi in foetal calf serum containing 1.8 M DMSO effectively maintains numerous morphological, functional (contractile and relaxant mechanisms) and biochemical properties. These results confirm and extend those of Müller-Schweinizter *et al.* (1993) and give further support to the use of this technique for storing human airway smooth muscle for subsequent pharmacological experiments.

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