Cryoprotective Effect of Dimethylsulfoxide, Dextran, and Magnesium on Guinea Pig Uteri (34418)

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A cryoprotectant is a chemical that will allow cells to survive freezing. Farrant (1) reported that dimethylsulfoxide (DMSO), in concentrations high enough to prevent freezing, will allow guinea pig uteri to surivve at —50°. Lin and Carroll (6) reported that magnesium was ineffective in protecting guinea pig uteri frozen to —196°. But both of these chemicals, as well as dextran, will protect cardiac muscle if frozen at temperatures no lower than —10° (3), but only DMSO affords cryoprotection to cardiac muscle at lower temperatures (4). We felt that it would be profitable to compare the cryoprotective effectiveness of DMSO, magnesium, and dextran on guinea pig uteri at temperatures in the range of —10 to —20°.

Materials and Methods. The uterus was excised from virgin mature guinea pigs (approximately 20 weeks old) which had been anesthetized with ether. The uterus was immediately placed in Locke–Ringer's solution (composition: NaCl, 153.9 m*M*; KCl, 5.6 m*M*; $CaCl_2$, 2.2 m*M*; $NaHCO_3$, 16.8 m*M;* dextrose, 5.5 m*M*; and distilled water to make 1 liter) which was oxygenated with a gas mixture of 95% oxygen and 5% carbon dioxide and kept at 30°, pH 7.4. After severing the uterine fundus, each uterine horn was tied to a glass rod. The free end of each horn was tied to a transducer and the horns were immersed in individual organ baths containing Locke–Ringer's solution kept at 37°. One g of tension was applied to each horn and contractions were recorded by a direct writing oscillograph.

Following a 20-min equilibration period, some of the uteri were frozen and other uteri were frozen following administration of one of three cryoprotectants (DMSO, $MgCl_2$ or dextran: mol wt 70,000). Subzero slow cooling (<1°/min) was accomplished by exposing the uteri to a —20° environment (acetone chilled with dry ice). Direct myometrium contact with the acetone was avoided by sealing an uterus in a 10-ml plastic centrifuge tube. Cooling was monitored with a copper–constantan thermocouple with a time constant of 50 msec. The amplified signal from the thermocouple was recorded by a direct ink-writing servorecorder. Fourteen uteri at —14° were thawed after 20-min exposure to the cold bath, 6 at —16° after 30-min exposure. Temperature profile is shown in Fig. 1.

Uteri treated with cryoprotectants were all frozen to —16°. When cryoprotectants were used, they were administered in the organ bath prior to freezing. Unless otherwise noted, the solvent for the cryoprotectants was Locke–Ringer's solution. Six uteri were treated with 1% dextran, 8 with 3%, 10 with 6%, 8 with 10%, 8 with 12% and 10 with 20%. Six uteri were treated with 66 m*M* $MgCl_2$ and 6 were treated with 66 m*M* $MgCl_2$ plus 100 m*M* dextrose. In addition to 6 uteri treated with 2*M* DMSO, a group of 8 horns was treated with gradually increasing concentration of DMSO (2, 4, and 5*M*) at various temperatures (0,—20°, respectively) and ultimately kept at —50° for 20 min. This treatment, after Farrant (1), prevented the ice formation in these preparations. One group of 8 horns without the benefit of a cryoprotectant was similarly cooled to —50°.

After rapid thawing, the horns were returned to Locke–Ringer's solution in the organ bath as prior to freezing. Contractile activity was again recorded. Uteri spontane-

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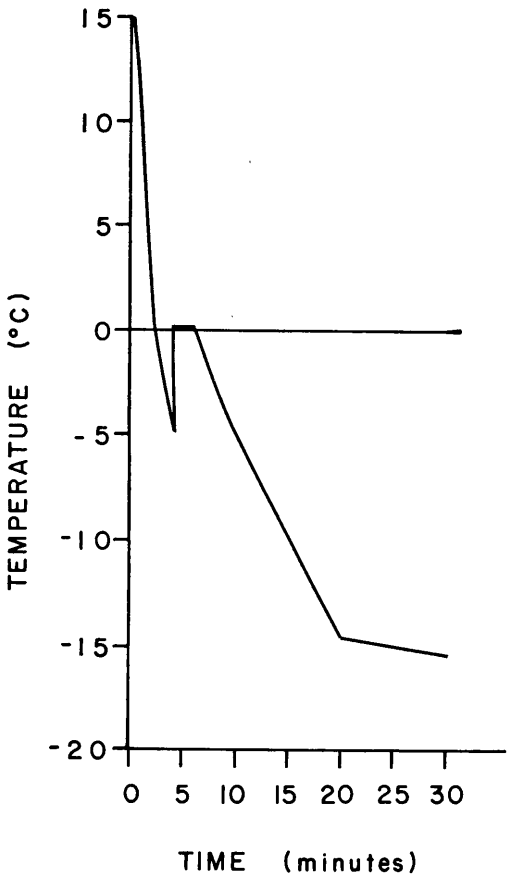

FIG. 1. Typical temperature profile of guinea pig uteri placed in a —20° environment. Temperature was sensed by a copper–constantan thermocouple placed inside the organ.

ously resuming contractile activity within 20 min of thawing were considered to be "survivors" of freezing. After a 20-min period of observation, the horns were treated with a 0.02 mg/100 ml solution of acetylcholine.

Results. With our technique, uteri kept in the —20° cold bath reach —14° after 20-min exposure and —16° after 30 min. These characteristic temperature profiles (Fig. 1) for the uteri are obtained regardless of the cryoprotectant or the dose of the cryoprotectant. A clearly defined isotherm was routinely detected in the uterine preparations.

Two of the 14 uteri cooled to —14° (without the benefit of a cryoprotectant), when thawed, contracted spontaneously and also when treated with acetylcholine. None of the 6 uteri cooled to —16° contracted when thawed.

Survival rates increased, however, when the uteri were treated with dextran. Twenty-four of 50 uteri (Table I) treated with varying concentrations of dextran (1, 3, 6, 10, 12, and 20% solutions; w/v) contracted spontaneously after being cooled to —14° but none contracted after being cooled to —16°. A 10% solution of dextran proved the most effective in protecting uteri from freeze-injury. Seven of 8 preparations treated with 10% dextran contracted spontaneously after thawing and 5 of these gave an appropriate tonic response to acetylcholine.

Magnesium chloride (66 or 120 m*M*) in Locke–Ringer's solution did not afford any cryoprotection to the uteri. The hypertonicity of the magnesium–Locke–Ringer's solution may have been injurious to the uteri. Two of 6 uteri contracted rhythmically after freezing at —14° in a solution of 66 m*M* $MgCl_2$ plus 100 m*M* dextrose, but neither of them contracted when treated with acetylcholine.

All 6 uteri treated with 2 *M* DMSO actually froze after cooling to —14°. Four of these organs, when thawed, contracted spontaneously and all 6 of them contracted in response to acetylcholine stimulation.

Four of 8 uteri which had been treated with 5 *M* DMSO contracted spontaneously when returned to the organ bath after being cooled to —50°. Three of these uteri gave a

TABLE I. Contractile Ability of Thawed Uteri after Freezing to —14°.[a]

Dextran (%; w/v)	Spontaneous activity	Activity after acetylcholine, 0.02 mg/100 ml
0	2/14	2/14
1	2/6	0/6
3	4/8	1/8
6	4/10	6/10
10	7/8	5/8
12	1/8	1/8
20	6/10	1/10

[a] All dextran mixtures were prepared in Locke solution. Experimental results are expressed as number of uteri that responded/number of uteri in the group.

normal response to stimulation with acetylcholine. All 8 horns which were not treated with a cryoprotectant and cooled to —50° failed to contract when thawed.

The uterine response to acetylcholine after thawing was inconsistent. Some uteri contracted spontaneously after thawing but did not give an appropriate contractile response to acetylcholine and some uteri would contract only after acetylcholine stimulation. This observation is in agreement with other reported results on smooth muscle (5) and is in contrast to the observation that the drug ouabain is a sensitive pharmacological indicator of freeze-induced injury in mammalian cardiac muscle (3).

Discussion. Even though DMSO, dextran, and magnesium are effective cryoprotectants for some cells, they are not equally effective for all cells (2). Whereas only DMSO provides partial cryoprotection for rat hearts frozen to —15° (3), we have shown that magnesium will not protect uterine smooth muscle frozen to —14°. If neither myocardium or myometrium is frozen further to —17°, DMSO is the only effective agent. Lin and Carroll (6) previously observed that magnesium is ineffective as a cryoprotectant for guinea pig uterine tissue frozen to —196°.

DMSO is a cryoprotectant that can cross cell membranes. Farrant (1) reported in 1967 that if guinea pig uteri were kept from freezing by high concentrations of DMSO, they could survive cooling to —50°. When he used lesser DMSO concentrations which could not prevent freezing at such low temperatures, the thawed uteri developed a contracture. Although our survival ratio upon using 5 *M* DMSO is not as good as Farrant's, we have confirmed his observation. In addition, we have demonstrated that uterine smooth muscle can survive actual freezing to at least —16° provided DMSO or dextran is used as a cryoprotectant.

This is the first report that dextran can afford some cryoprotection to uterine smooth muscle frozen at relatively high temperatures. The optimum dextran concentration seems to be a 10% solution (w/v). Karow and Carrier (3) and Karow (4) previously reported that dextran affords cryoprotection to cardiac muscle if the freezing temperature is no lower than —10°. In both tissues this high molecular weight compound probably enters the extravascular compartment, yet it is doubtful if the substance can penetrate the cellular compartment.

The various proposed mechanisms of cryoprotection, supported by many different authors, was reviewed recently (2). Elucidation of the action of such chemically diverse cryoprotectants has been difficult. Biochemical disturbances can result merely from hypothermia (above 0°) or from thermal shock (rapid cooling to suprazero temperatures). Freezing sequesters water in the form of ice and thereby causes tissue injury from the nonspecific effects of hypertonic solutions or from the specific effects of high concentrations of individual solutes. These chemical effects of concentration are more injurious at relatively high subzero temperatures where reaction rates are faster than at lower temperatures. In addition, cryoprotectants have an intrinsic toxicity which is augmented not only by concentration, but also by duration of tissue exposure. The observation that DMSO or dextran, but not magnesium, will protect the contractile process in smooth muscle at —10 to —15° while DMSO but neither dextran nor magnesium are effective in cardiac muscle has not yet aided in clarifying the factors involved in cryoinjury and cryoprotection.

Summary. Fourteen percent of guinea pig uteri survived freezing to a core temperature of —14° but none to —16°. Dose-response studies utilizing DMSO, dextran, and $MgCl_2$ as cryoprotectants were conducted on guinea pig uteri frozen to —16°. Uteri treated with $MgCl_2$ did not survive freezing. Uteri treated with dextran in concentrations as high as 20% survived freezing to —14° but not to —16°. The optimal dextran concentration, 10%, allowed a 62% survival at —14°. Uteri treated with 2 *M* DMSO survived freezing to —16°; and when treated with 5 *M* DMSO, survived cooling to —50°.

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