Successful cryopreservation of auricle fragments from rat hearts at − 196 °C

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Summary. The influence of electrolyte composition and glucose concentration of a cryoprotective medium on the survival of auricle fragments from adult rat hearts after storage at − 196 °C was investigated. Using a K^+-, Mg^{++}-, Ca^{++}-rich solution with increased glucose concentration, a high rate of surviving fragments was found after cryopreservation.

Long-term preservation of isolated heart muscle cells at − 196 °C was found to be feasible[1-3]. Certain successes were also obtained in cryopreservation of fragments of heart muscle (frog[4], mouse[5]) and of small embryonic hearts (chicken[6], mouse[7]). Maintenance of viability of whole hearts of neonatal and adult mammals was not successful under these conditions.

A cryoprotective medium (solution for cryopreservation) is necessary for storage of biological materials in frozen state. It usually consists of a basal medium; the cryoprotectant proper and special additives by which cryoprotective effectiveness is further enhanced. In most investigations on cryopreservation, only the type and concentration of cryoprotectants was varied (and included in the theoretical considerations). Only a few experimental studies were performed on the composition of the basal medium for low-temperature preservation[8].

The studies reported here were undertaken to explore the influence of alterations in electrolyte composition and glucose concentration of a Krebs-Henseleit solution used as basal medium for experiments on the survival of auricle fragments from adult rat hearts after cryopreservation at − 196 °C.

Material and methods. Fragments of both auricles of adult male albino rats, average weight 250 g, were used as test objects. The experimental arrangement is shown in figure 1. After preparation, the heart fragments (1-2 mm^3) were immediately placed in glass vials containing 1 ml of chilled cryoprotective medium A, B or C (table 1). After an incubation period of 30 min required for the distribution of cryoprotectant dimethylsulfoxide (3.1 M in the cryoprotective medium) within the tissue, the fragments were frozen by storage of the vials in a freezing chamber (− 25 °C) for 20 min (cooling rate 1.2 °C/min) and plunging the vials thereafter into liquid nitrogen. After storage at the different stages of cryopreservation (stage 1: 30 min storage at 4 °C, stage 2: 20 min storage at − 25 °C, stage 3: 24 h storage at − 196 °C) the vials containing the specimens were im-

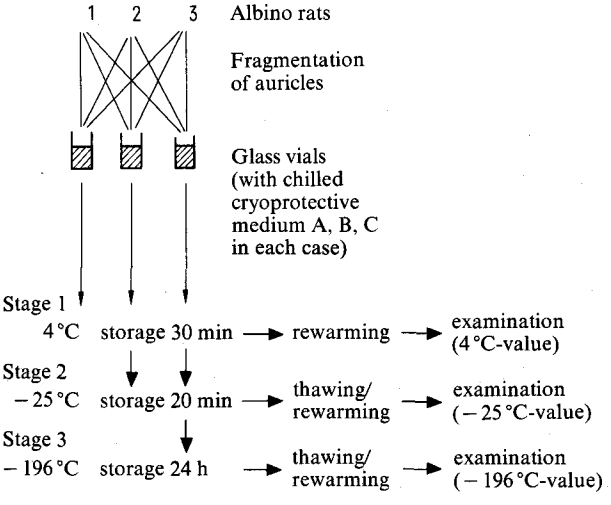

Fig. 1. Experimental arrangement. Constant parameters: 1. Concentration of DMSO (3.1 M), 2. Freezing procedure (3-step method), 3. Examination (testing of the contractility, i.e. percentages of fragments contracting 80 min after rewarming).

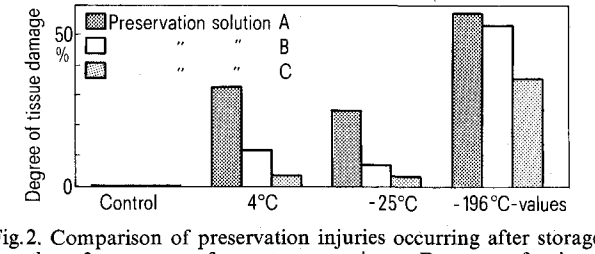

Fig. 2. Comparison of preservation injuries occurring after storage at the 3 stages of cryopreservation. Degree of tissue damage = difference between percentages of contracting fragments after storage at the different stages. Dates used for comparison see table 2.

Table 1. Composition of the basal media. The solutions were oxygenated with 95% O_2:5% CO_2 and thereafter the pH adjusted to 7.4 at room temperature

	Solution A (mmoles/l)	Solution B (mmoles/l)	Solution C (mmoles/l)
NaCl	121.1	58.9	58.9
KCl	5.4	15.8	15.8
$CaCl_2$	2.5	4.5	4.5
KH_2PO_4	1.2	1.2	1.2
$NaHCO_3$	25.5	25.5	25.5
$MgSO_4$	1.2	30.0	30.0
Glucose	5.5	5.5	166.0
Osmolality (mosmol/l)	310	290	450
pH (at 25 °C)	7.4	7.4	7.4

Table 2. Influence of various preservation solutions on the contractility of the heart auricle fragments during the 3 stages of cryopreservation

Storage	Contractility (percentages of contracting fragments)		
Stage/Time	Solution A (%)	Solution B (%)	Solution C (%)
Control (without storage)	100	100	100
Stage 1 (4 °C, 30 min)	67	88*	97**
Stage 2 (− 25 °C, 20 min)	50	82*	94**
Stage 3 (− 196 °C, 24 h)	21	38*	60**

*Statistically significant in comparison to solution A ($p < 0.05$); **Statistically significant in comparison to solution B ($p < 0.05$). Composition of solutions employed see Table 1.

mersed into a water-bath at 37 °C for thawing and rewarming. The fragments were washed with Krebs-Henseleit solution at 37 °C and the contractility examined. The contractility, i.e. percentages of contracting fragments 80 min after rewarming, was used as a criterion for viability. Contraction of the fragments was triggered by electric stimulation, using rectangle pulse, 12 V, 2 msec, in special chambers perfused with oxygenated Krebs-Henseleit solution and addition of 3 µg/l adrenaline.

Results and discussion. In the present experiments, we have observed the effects of changes in composition of the cryoprotective medium on contractility of heart auricle fragments following storage at −196 °C. Earlier studies from this laboratory have shown that variations in electrolyte composition of preservation solutions can account for the differences in contractility of auricle fragments occurring after short-term storage at temperatures between −15 °C and −50 °C[9,10]. Now we could observe that by increasing the potassium ion, magnesium ion and calcium ion concentrations in the preservation solution B at a constant level of DMSO, about 40% of frozen and thawed heart fragments could survive a storage in liquid nitrogen (table 2). With an additional increase of the glucose level in the preservation solution to 166 mM, the number of heart fragments able to contract after a storage of 24 h at −196 °C increased to 60% (table 2). The average differences were statistically significant and therefore show the cryoprotective effectiveness of the changed electrolyte and glucose composition.

Corresponding to our experience and to that of Sumida[5] we had chosen a relatively high concentration of DMSO. Under these conditions, considerable injuries by osmotic and toxic influences of the cryoprotectant should be taken into consideration, even with hypothermia[9,11]. Figure 2 shows a comparison of the cryoprotective effectiveness of preservation solution A, B and C. It is evident that K^+-, Mg^{++}- and Ca^{++}-rich preservation solutions act during the first stages of cryopreservation, whereas the degree of tissue damage occurring between −25 °C and −196 °C can be reduced by addition of glucose in preservation solution C. The level of ATP in ischemic heart tissue has been associated with contractility restoration[12,13]. The use of cardioplegic intracellular type solution under hypothermic conditions results in a reduction of metabolic requirements of heart tissue and in a better maintenance of energy-rich phosphates[14,15]. A comparison of our results in the various stages of storage shows the advantageous effect of depolarizing solution B. The improved contractility by increased glucose concentration in solution C suggests that glucose in connection with DMSO has a protective influence on the cell membrane and/or results in additional dehydration of heart tissue during freezing (smaller degree of damage at stages 2 and 3 in figure 2). Therefore the influence of glucose could be explained as an additional or competitive effect related to the cryoprotective effect of DMSO.

Our experiments show that, not only isolated heart muscle cells, but also heart auricle fragments of adult rats, can be frozen and thawed with a high degree of survival of contracting fragments. The composition of the basal medium in the cryoprotective medium used for cryoprotection plays an important role in the cryopreservation of biological materials.

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Increased blood pressure in the SHR is not related to a deficit in renomedullary PGE_2[1]

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Summary. Synthesis of prostaglandin E_2 by renal medulla from SHR and WKY rats was compared during early postnatal development. Although arterial blood pressure was significantly higher in SHR as early as 6 weeks of age, no difference in renal medullary prostaglandin synthesis was observed.

Current research on hypertension has been directed, in part, toward the hypothesis that deficiencies of renal vasodilators could play a role in the etiology of essential hypertension[2,3]. Attention has focused on prostaglandin E_2 (PGE_2) because this potent vasodilator[4,5] is synthesized in large amounts by the renal medulla[6]. Therefore, a natural hypothesis was that a deficiency in renal PGE_2-production and/or release contributed to the etiology of hypertension[7]. Indeed, several groups have reported decreased release of PGE_2 from kidneys of hypertensive rats[8-10].

Unfortunately, most animal models of hypertension require either surgical or pharmacological manipulation of the kidneys, making extrapolation of the data to the etiology of essential hypertension in man difficult. The development of the spontaneously hypertensive rat (SHR) made it possible to quantify renal vasodilators in a hypertensive rat model that did not require prior manipulation of the kidney.

If a deficiency of PGE_2 is involved in the etiology of hypertension, the deficiency should appear before the development of hypertension. Alternatively, if a deficit in