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Effects of pH on the Cold Preservation of the Isolated Rat Heart

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Abstract

Storage solutions of varying pH have been used for the simple cold preservation of the heart for transplantation. However, few studies have focused on the optimal pH for a storage solution. In the present study, we investigated the effects of storage solutions with 5 different pHs (6.60, 7.00, 7.40, 7.80, and 8.20 at 4°C) on cardiac function, the leakage of cytosolic enzymes, and the myocardial metabolites content. We used the isolated perfused working rat heart model and a 6-hour preservation period in the different storage solutions. We found that cardiac function was best preserved at a pH of 7.00 or 7.80. In the hearts preserved at a pH of 7.00 or 7.80, the leakage of creatine kinase and lactate dehydrogenase was significantly less than at a pH of 7.40 ($p<0.01$). ATP was maintained at a significantly higher concentration in the pH 7.00 solution as compared with the other solutions ($p<0.01$). No significant differences were observed in the creatine phosphate and lactate levels among the five different pH groups. The results showed that the cardiac function and other parameters of cold-preserved hearts were relatively well maintained at both a pH of 7.00 and 7.80, suggesting the possible existence of a biphasic optimum pH for cold preservation.

Introduction

In both experimental and clinical heart transplantation, cold preservation of the heart has been used to reduce energy requirements and to preserve organ viability. The most common method is simple cold storage in intracellular type solutions, such as Collins' solution[6], after an initial rapid arresting of cardiac contraction with high-potassium solution[11]. In experimental studies, some investigators have successfully transplanted hearts that have been preserved in this way for up to 26 hours[20,21,22]. However, cold preservation does not totally prevent the effects of anoxia and ischemia[7,9], and there is a gradual attenuation of cardiac function as the preservation period increases. The ideal storage solution still remains the subject of considerable debate. There have been numerous experimental studies dealing with myocardial protection during simple immersion preservation of the heart, but few have focused on the pH of the storage solution.

Accordingly, we examined the effects of the pH of the storage solution on poststorage cardiac function, the release of cytosolic enzymes, and the myocardial metabolites content.

Key words: Cold preservation, Rat heart, pH, Cardiac function
索引語：低温保存，ラット心臓，pH，心機能
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Materials and Methods

Solutions: The compositions of the solutions used in this experiment are listed in Table 1. The pH of the storage solution (Collins' M solution, Midorijuji Co., Osaka, Japan) was adjusted with HCl, and tris (hydroxymethyl) aminomethane to 6.60, 7.00, 7.40, 7.80, or 8.20 at 4°C.

Cold preservation: Male Sprague-Dawley rats (weight, 250–350 g) were anesthesized with pentobarbital (60 mg/kg, i.p.), and then heparin (1,000 units) was injected intraperitoneally. The pericardium was incised and the inferior vena cava was transected just below the right atrium. The heart was immediately arrested by the retrograde infusion of Young's solution (5 ml, 4°C) through a cannula which had been inserted into the abdominal aorta. It was then perfused with 20 ml of St. Thomas cardioplegic solution at 4°C, and finally flushed with 20 ml of cooled Collins' solution at one of the experimental pH levels. Each heart was then rapidly excised and stored in one of the 5 types of Collins' solution (pH 6.60, 7.00, 7.40, 7.80, and 8.20) for 6 hours at 4°C to assess the effects of varying the pH of the storage solution.

Working heart preparation: After 6 hours of cold preservation, the hearts were attached to the working heart apparatus. Langendorff perfusion was instituted at 20°C, as described by KOHDA and COLLEAGUES[10], and the heart was gradually warmed to 37°C by 20 min of Langendorff perfusion. The working mode of perfusion was then instituted, and the cardiac function was recorded for 40 min. The perfusion apparatus used was similar to those of NEELY et al.[14] and MOCHIZUKI et al.[12]. In brief, in the Langendorff mode, the heart was perfused through the cannulated aorta at a constant pressure of 80 cm H_2O. In the working mode, the perfusate entered the cannulated left atrium and passed into the left ventricle, from which it was spontaneously ejected through the aortic cannula against an afterload pressure of 75 cm H_2O. Aortic pressure was monitored via the side arm of the aortic cannula, and a catheter was inserted via the left atrium into the left ventricle to measure left ventricular (LV) systolic pressure, LV dp/dt, and LV end-diastolic pressure. These hemodynamic parameters were monitored using a polygraph and were recorded with a thermal pen recorder. Aortic and coronary flow rates were measured by timed collection, and cardiac output was calculated as

Table 1. Composition of the solutions.

	Young's solution	St. Thomas' solution	Collins' M solution	modified Krebs-Henseleit solution
Na	145	120	10	118
K	75	16	117	4.7
Ca	0	1.2	0	2.5
Mg	200	16	3	1.2
Cl	145	143	15	127
PO_4	0	0	102	1.2
SO_4	200	0	3	1.2
HCO_3	0	10	10	25
Na_2EDTA	0	0	0	0.5
citric acid	75	0	0	0
Glucose	0	0	139	11
pH	6.5 at 4°C	7.8 at 4°C	7.4 at 4°C	7.4 at 37°C

EDTA: Ethylenediaminetetraacetic acid.

the sum of these flow rates. The perfusate used was a modified Krebs-Henseleit solution (Table 1) which was bubbled with 95% O_2 and 5% CO_2. Perfusate temperature was maintained at 37°C, except for the early period of Langendorff perfusion, as mentioned above.

Creatine kinase and lactate dehydrogenase assay: The leakage of creatine kinase and lactate dehydrogenase into the coronary effluent during the 20 minutes of Langendorff perfusion was determined by an automatic analyzer (736-40, Hitachi, Tokyo, Japan). Total creatine kinase and lactate dehydrogenase leakage were expressed as international units (IU) per heart.

Myocardial metabolites analysis: For analysis of myocardial metabolites, the hearts preserved for 6 hours were rapidly frozen with a Wallenberger clamp precooled in luquid nitrogen. Frozen samples were then homogenized and the extracts were used to determine the concentrations of ATP, creatine phosphate, and lactate. ATP was determined by the luciferine-luciferase method, while creatine phosphate and lactate were measured by enzymatic methods[2]. Values of these metabolites were expressed as micromoles per wet tissue weight.

Statistical analysis: Results are expressed as the mean±the standard error of the mean. Data were evaluated by analysis of variance and then the unpaired Student's t-test. Differences were considered statistically significant if the p value was less than 0.05.

Animal care: All animals received care in compliance 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" prepared by the National Academy of Sciences and published by National Institutes of Health (NIH Publication No. 85-23, revised 1985).

Results

Cardiac function

The functioning of the cold-preserved hearts was measured 40 min after the institution of perfusion using a working heart model. After 6 hours of cold preservation, the heart rate was the same among the 5 different pH groups (Table 2). However, hearts preserved at a pH of 7.00 or 7.80 showed significantly better recovery of aortic flow rate, cardiac output, and LV max dP/dt than those preserved at a pH of 7.40. With the hearts preserved in the pH 7.00 solution, the aortic pressure, LV systolic pressure, and LV end-diastolic pressure were also well maintained as compared with those in the pH 7.40 series. The coronary flow in the pH 7.80 group was significantly greater than

Table 2. Functioning of isolated rat hearts after cold storage for 6 hours at 4°C.

pH at 4°C	CF	AoF	CO	AoP	LVP	LVEDP	LV max dp/dt	LV max −dp/dt	HR
6.60 (n=8)	12.2±1.1	20.2±4.7	32.4±5.0	77.7±3.6*	87.7±4.2	7.1±1.6	2.7±0.1*	1.6±0.1	248±22
7.00 (n=8)	12.4±2.5	32.8±3.6*	45.0±2.8*	81.1±8.4*	98.3±8.1*	4.7±1.6*	3.1±0.2*	2.5±0.5*	250±19
7.40 (n=14)	11.9±1.4	18.6±5.9	30.7±5.6	72.1±5.6	87.1±8.6	9.1±3.7	2.2±0.3	1.9±0.3	258±37
7.80 (n=8)	13.7±0.8*	25.3±5.6**	39.1±6.4*	73.3±3.9	91.8±14.0	8.3±1.9	2.7±0.2*	2.2±0.5	236±22
8.20 (n=8)	11.4±2.2	18.0±7.0	29.8±6.6	67.6±11.0	79.2±20.0	13.1±3.4**	2.2±0.4	1.8±0.7	246±36

CF: Coronary flow rate (ml/min), AoF: Aortic flow rate (ml/min), CO: Cardiac output (ml/min), AoP: Aortic pressure (mmHg), LVP: Left ventricular pressure (mmHg), LVEDP: Left ventricular end-diastolic pressure (mmHg), LV max dP/dt, −dP/dt: Left ventricular maximum dP/dt, −dP/dt (10^3 mmHg/s), HR: Heart rate (beats/min). Each value is the mean±S.D.

*: $P<0.01$ compared to pH 7.4 solution. **: $P<0.05$ compared to pH 7.4 solution.

in the pH 7.40 group. Hearts preserved at a pH of 8.20 showed a significantly greater LV end-diastolic pressure than did those preserved at a pH of 7.40. Thus, cardiac function appeared to be preserved in the following order of pH levels: 7.00＞7.80＞6.60≧7.40≧8.20.

Creatine kinase leakage

The total creatine kinase leakage during 20 minutes of reperfusion was 14.3±7.3 IU/heart at a pH of 6.60, 12.0±5.1 at a pH of 7.00, 20.4±5.3 at a pH of 7.40, 12.9±3.4 at a pH of 7.80, and 18.7±6.5 at a pH of 8.20 (Fig. 1). Thus, the release of creatine kinase from the hearts preserved at a pH of 7.00 or 7.80 was significantly less than that from hearts preserved at a pH of 7.40 ($p<0.01$).

Lactate dehydrogenase leakage

The total lactate dehydrogenase leakage during 20 minutes of reperfusion was 14.5±8.2 IU/heart at a pH of 6.60, 12.8±4.2 at a pH of 7.00, 19.4±4.4 at a pH of 7.40, 12.9±2.0 at a pH of 7.80, and 18.7±6.9 at a pH of 8.20 (Fig. 2). In the pH 7.00 and 7.80 groups, the release of lactate dehydrogenase was significantly less than in the pH 7.40 series ($p<0.01$).

Myocardial metabolism

Creatine phosphate: At the end of the 6-hour preservation period, no significant differences was observed in the content of creatine phosphate among the five groups (Table 3).

ATP: In the hearts preserved at a pH of 7.00, the ATP level was significantly higher than in the hearts at other pH levels. In the pH 8.20 group, the ATP content was approximately a third of that at a pH of 7.00.

Lactate: Hearts preserved in at a pH of 7.00 or 7.80 tended to accumulate less lactate than hearts preserved at the other levels of pH, although there was no statistically significant difference bet-

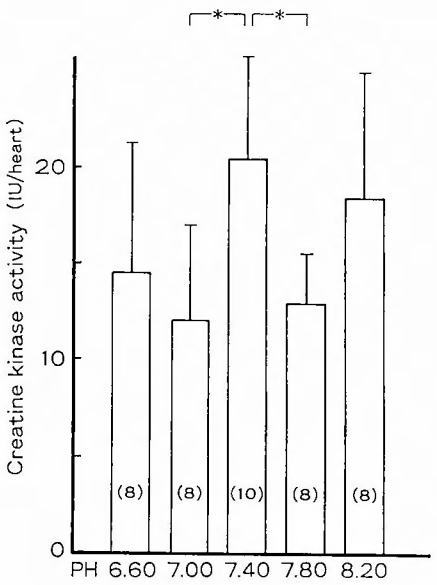

Fig. 1. Total amount of creatine kinase activity (IU/heart) leaked during a 20-min reperfusion period. Values are expressed as the mean±SD. ( ): number of experiments. *: $P<0.05$.

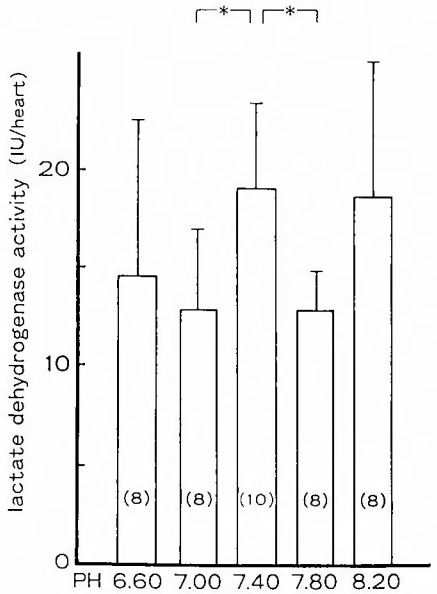

Fig. 2. Total amount of lactate dehydrogenase activity (IU/heart) leaked during a 20-min reperfusion period. Values are expressed as the mean±SD. ( ): number of experiments. *: $P<0.05$

Table 3. Myocardial creatine phosphate (CrP), ATP, and lactate contents at the end of cold storage for 6 hours at 4°C (μmol/gm tissue weight)

pH of storage solution	CrP	ATP	Lactate
6.60	0.60±0.23	2.43±1.10	16.9±4.6
7.00	0.59±0.24	4.90±1.25*	11.6±7.9
7.40	0.54±0.22	3.20±1.39	19.5±8.1
7.80	0.56±0.19	2.80±0.80	13.3±10.0
8.20	0.61±0.28	1.81±0.72	20.1±8.3

Each value is the mean±S.D. N=9 in all of the groups.
*: significantly different ($p<0.05$) compared with the other groups.

ween the five groups (Table 3).

Discussion

The best preservation of cardiac function was obtained at a pH of 7.00 followed by a pH of 7.80. Leakage of two cytosolic enzymes, creatine kinase (CK) and lactate dehydrogenase (LDH) was also significantly lower in the hearts stored of these pH levels. These results suggested that slightly acidic or alkaline deviation of the storage solution from the pH of 7.40 is appropriate for cold preservation.

There are many reports relating to pH and myocardial damage. Some investigators have found that slight acidosis is cytoprotective for hearts subjected to ischemic or hypoxic conditions[4,8,17]. For cardioplegic solution, relatively good preservation has been reported using both acid and alkaline solutions[3,16]. These findings have argued with our present results that there may be some benefit in a slight pH deviation from 7.40 for cardiac preservation.

Regarding the intramyocardial ATP content after 6 hours of preservation, a significatly higher level was observed after storage at a pH of 7.00. Since the ATP content under ischemic conditions is known to show a positive correlation with functional recovery[19], the relatively good recovery of cardiac function seen in hearts stored at a pH of 7.00 may have been related to maintenance of the ATP content. Since the lactic acid level was not significantly different among the groups, the higher ATP level in hearts stored at a pH of 7.00 should not be due to production by anaerobic glycolysis, but rather due to a reduction of ATP degradation. An increase of the extracellular hydrogen ion concentration is reported to inhibit the inflow of membrane-bound Ca^{2+} into cells[5], to decrease hydrolysis of ATP by ATPase, and/or to restore cardiac function[13]. Bercot et al. have demonstrated that acidic solution is advantageous for maintaining the ATP level[1] in the case of heart preservation.

Rahn et al. have urged attention to two specific points so as to maintain optimum metabolic function at low temperatures[18]. First, the pH of the intracellular fluid should remain around the neutral point of water, and second the liquid environment should remain relatively alkaline. Therefore, to maintain optimum metabolic function at a low temperature, it is necessary to increase the intracellular pH depending on the changes in the neutral point, and when the temperature is lowered from 37°C to 3°C, the pH of the extracellular fluid should be increased from 7.4 to 7.8. This may in part account for the relatively good recovery of cardiac function and the decreased leakage of cytosolic enzymes seen in the hearts stored at a pH of 7.80.

The heart rate after 6 hours of cold preservation was the same among our five different pH

groups (Table 2). Recently, we have demonstrated that pacemaking activity is little affected by cold storage for-up to 24 hours although the contractile activity of the atrial and ventricular myocardium is markedly reduced[15]. This suggests that the sinus node may be resistant to factors such as a long period of cold storage and pH deviation.

In summary, the present results suggested that slight deviation of the pH of a storage solution from 7.40 to 7.80 or 7.00 improved the cold preservation of the rat heart, although the precise mechanism of this effect remains to be determined.

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和文抄録

心保存における至適 pH

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現在，心臓移植手術における donor 心の保存は，単純浸漬保存法が主流であり，その際に用いられる保存液の組成については多くの研究がなされている．しかしながら，低温保存における保存液の至適 pH に関する研究は充分ではなく，まだ結論が得られていないのが現状である．

そこで今回，保存液（コリンズ液）の pH を6.6，7.0，7.4，7.8，8.2の5群に分けて，ラット心臓を6時間単純浸漬保存し，その後各群を working heart model で再灌流して，心機能，CPK，LDH 漏出量および保存直後の心筋内代謝産物含量を測定し，至適 pH についての検討を加えた．

その結果，pH 7．0 と pH 7.8 保存群で良好な心機能が得られた．また，CPK，LDH 漏出量は pH 7.0 と pH 7.8 で pH 7.4 と比較して有意に低値であった．保存直後の ATP 含量については，他群と比して pH 7.0 保存群で有意に高値を示した．クレアチンリン酸および乳酸値については5群間で有意差を認めなかった．

以上，本研究により保存液の pH として7.4 から酸性側およびアルカリ性側に偏位した pH 7.0 および pH 7.8 で良好な保存が得られ，至適 pH は2峰性であることが示唆された．