Heart Preservation during 24 Hours for Transplantation

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Abstract

***(J. Extra-Corpor. Technol.* 19[3] p. 305–311 Fall 1987, 11 ref.) To promote the development of heart transplantation by developing a complete technique of prolonged preservation of the graft in an independent, miniaturized and computerized container, a surgical technique is described, which allows continuous protection of the myocardium during removal, preservation and grafting.**

The use of cardioplegia allows diastole arrest, an essential point for good preservation. The preservation system consists of a refrigerated and thermostatic container. The heart is perfused by a micropump and the perfusate is oxygenated and bubble-free due to a special filter. Captors are placed in the perfusion, thermic catheter, K+, pH, lactate, electrodes and the results are analyzed by a microcomputer and transmitted to a feedback control system which adjusts the parameters. This device can be portable. With this type of device, good results were obtained as evidenced by:

- **ckmb release was low**
- **the histological lesions are rare and reversible**
- **after transport, the heart maintains electrical and mechanical activity.**

Introduction

Presently cardiac transplantation is still fraught with numerous impediments. One of the main obstacles is a lack of donor hearts in the face of an increasing demand. This is due to several factors, but the present delay of three or four hours of ichemia necessary for transport represents a very important snag.

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That is the reason for the realization of a preservation system (transportable, reliable) allowing improvements in graft quality which will permit an extended graft harvest area, allow better evaluation on immunological factors and the realization of transplantation, outside an emergency situation, that is so important.

Materials and Methods

1) Experimental Animal

They are female sheep of 3 or 4 years old. Their choice has been determined upon the four following criteria:

- an advanced selection level allowing us to get a homogeneous batch
- lack of blood group
- docility, allowing observation
- weight of 80 to 100 lbs., approximately human weight

2) Preparation and Anesthesia

- The animal fasted for 24 hours before an operation
- One hour before the operation it is premedicated with Vetranquyl (Aceparamazine) 1 cc/20 lbs.
- anesthesia is induced with Nesdonal and maintained with Halothane
- the animal is laid backside on the table and a sounding line, blood pressure, central venous pressure and electrocardiogram are monitored

A 6 animal batch had the advantage of being prepared by perfusion for 3 hours:

- glucose : 0.5 gr/P/hour
- insulin : 1 U/P/hour
- potassium : 0.2 P/hour
- Solumedrol (Méthylprednisolone): 500 mg
- Verapamil : 5 mg

3) Surgical Method[3,8,9]

We are using a prototype plastic aortic clamp made by Medicorp Society of Nancy (France). The clamp is moulded in one whole piece and its elasticity allows a soft aortic clampage without causing endothelial injury even after a long period (Figure 1).

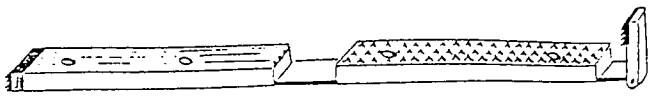

Figure 1

The inferior branch has longitudinal striations which allow it to slip easily around the aorta. The superior clasp is closed in such a way as to catch the adventitia, therefore preventing its sliding. The closing system is automatic and allows an appropriate tightening (Figure 2). In order to facilitate its positioning, the inferior branch is introduced into a silastic tubing (Figure 3), so that it slips easily behind the aorta without catching the adventitia.

Figure 2

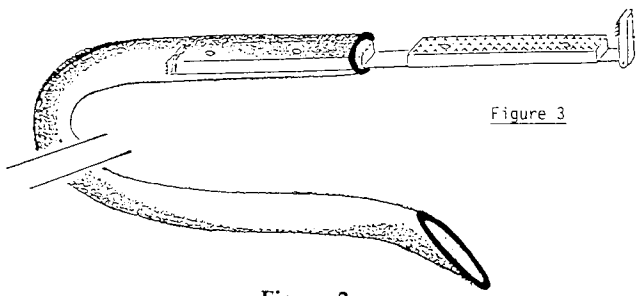

Figure 3

The second instrument of this method is a plastic catheter fitted with a metallic guide. It is placed at the aortic bulb and fixed to the adventitia with 2 rings located at its base (Figure 4).

Figure 4

The superior vena cava is dissected and a suture is placed to do its ulterior ligature. The inferior vena cava is placed on a lace, then the aorta is dissected until the brancheo-cephalic arterial trunk and a silastic lace is put at the aortic root (Figure 5).

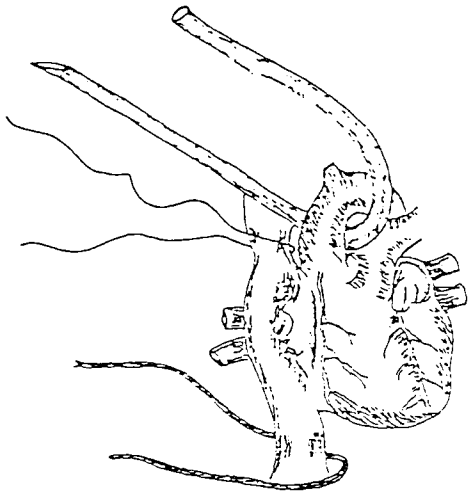

Figure 5

After heparin (intravenous injection of 200 units/lb.), the cardioplegia catheter is placed at the aortic bulb level and the posterior branch of the clamp is introduced into the silastic lace (Figure 6). The superior vena cava is ligated and divided, the inferior vena is divided, and the aortic clamp is closed (Figure 7). After aortic occlusion the cardioplegia perfusion begins (Figure 8).

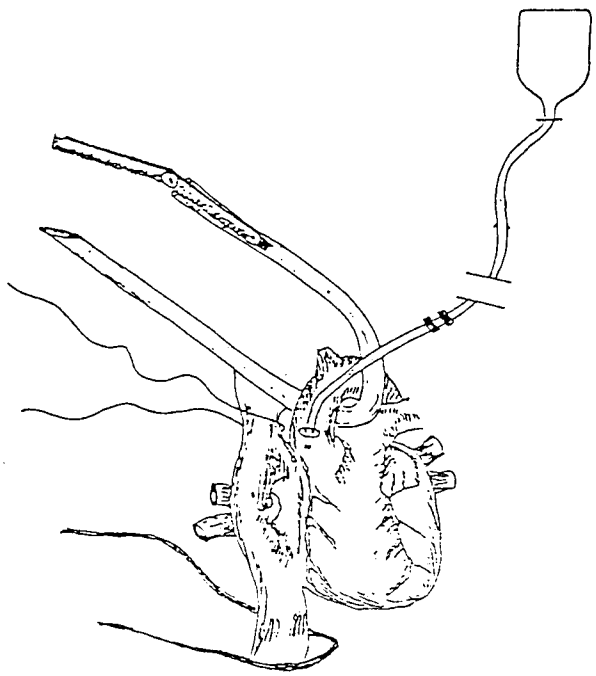

Figure 6

During the cardioplegia perfusion, the heart is excised and the atria are prepared for transplantation (Figure 9). The heart is carefully washed in two basins filled with iced physiological solution (Figure 10).

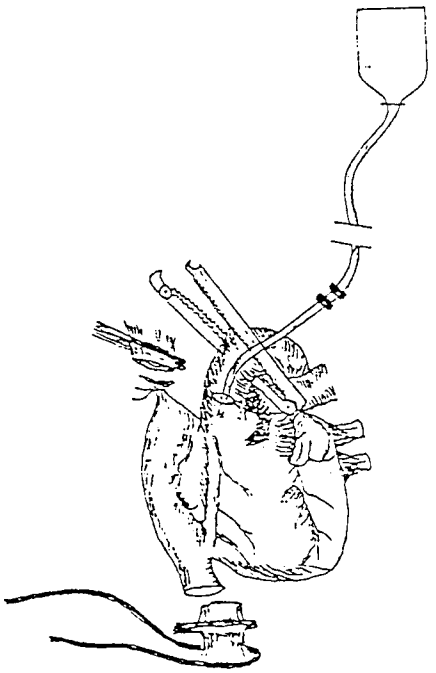

Figure 7

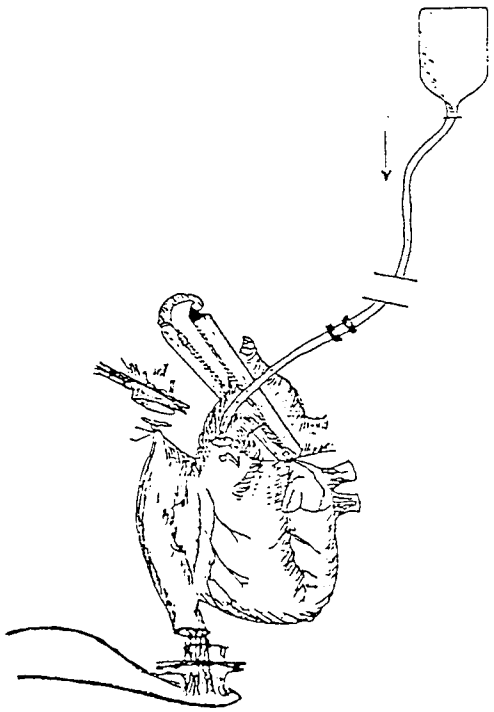

Figure 8

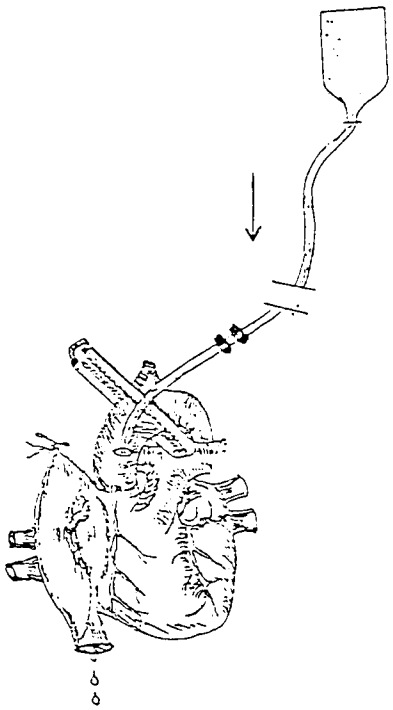

Figure 9

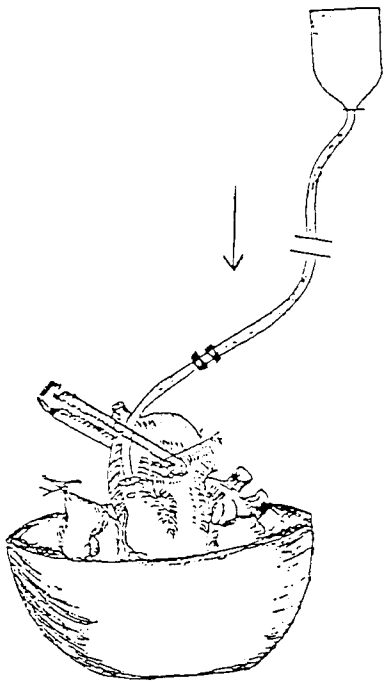

Figure 10

The heart is placed into the thermostatically controlled transportation, and the cardioplegia is then stopped (Figure 11).

When it arrives, the heart is removed from its container and cardioplegia is prepared and perfuses the graft prior to the transplant; the catheter is disconnected during the declampage (Figure 12).

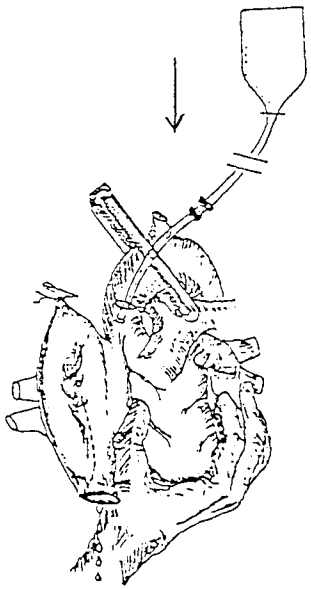

Figure 11

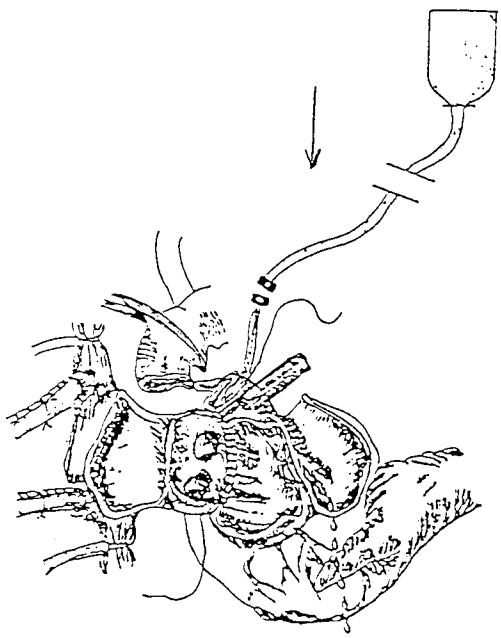

Figure 12

4) The Preservation System.[10]

This system should be light, transportable, reliable and fitted with alarms. It should maintain a constant temperature of 4°C. In addition this system should oxygenate the preservation solution, and should be equipped with sounding leads for essential parameters.

As shown in Figure 13, the system consists of:

1—Carbogen cylinder
2—Gas filter
3—Bubbling oxygenation
4—Computer screen for display of temperature, pH, sodium, potassium, heart weight
5—Transformer + Rheostat
6—Peristaltic pump
7—Pump motor
8—Bubble filter
9—Perfusion column of 10 centimeters of water
10—Aortic clamp support
11—Temperature probe
12—pH probe

Cardioplegia and Perfusion Solution[5,11]

Constitution of cardioplegia solution

	G/liter	mM
NaCl	6.00	102.0
$NaHCO_3$	0.38	4.0
Kcl	0.75	10.0
$MgSO_4$, H_2O	3.5	14.0
$CaCl_2$, H_2O	0.15	1.1
Procaine Hcl	0.27	1.0
Insulin	20 U/l	—
Dextrose	50	27
Verapamil	1.5 mg	—
Osmolality	320 mosmol/liter	
pH at 4°C	7.4	

Constitution of the storage solution and perfusate

	Gr/liter	mM
NaCl	6.76	115.7
$NaHCO_3$	2.10	23.0
$KHPO_3$	1.12	8.9
$CaCl_2 + H_2O$	0.16	1.1
$MgSO_4$ H_2O	3.48	14.4
Glucose	2.00	11.1
Sucrose	2.5	7.6
Glycerol	12.6	136.0
Taurine	0.5	4.0
Procaine Hcl	0.27	1.1
Chlorpromazine	0.005	—
Phenoxybenzamine	0.01	—
Osmolality	385 Mosmol/liter	
pH at 8°C	7.2–7.4	

Results

The first group of animals was used to perfect the surgical technique, to test the preservation system and to standardize sounding-leads. Consequently, precise measurements were done on a second batch of animals. Each heart had electrical and mechanic activity.

1) Biophysical Results[6]

Measurements were made on preservation solutions and showed constant concentrations during the 24 hours for:

$Na+$, $Cl-$, $Ca++$, Glucose, creatinine
PO_2, PCO_2

The amount of CKMB, pH and the weight varied according to the cardioplegia type. With the standard cardioplegia, the following was shown:

		Animal Number					
	Time	**7**	**8**	**9**	**10**	**11**	**12**
pH	0–8 hours	+ 0.1%	− 2.8%	− 2.8%	− 0.16%	− 9.5%	− 2.2%
pH	8–16 hours	− 1.6%	− 0	− 0.3%	− 0.3%	− 1.3%	− 0.4%
pH	16–24 hours	− 0.79%	− 1.3%	− 0.8%	− 0.32%	− 7%	− 2.4%
pH	0–24 hours	− 2.5%	− 4.1%	− 3.8%	− 8.1%	− 4.8%	− 5%
Poids	0–24 hours	+75%	+53%	+56%	+66%	+58%	+53%
CK Mb	0–8 hours	+ 0.3%	+ 0.63%	—	+ 2	+ 2.3%	+12.7%
CK Mb	8–16 hours	+ 0.5%	+ 1.14%	+ 0.2%	+ 1.09%	+ 0.37%	+ 0.45%
CK Mb	16–24 hours	+ 5.8%	+14%	+ 0.78%	+ 0.59%	+ 0.34%	+ 3.87%
CK Mb	0–24 hours	+15.7%	+12.3%	+ 3.4%	+ 5.3%	+ 5.1%	+ 9.6%

The result with the standard cardioplegia and 20 mg Verapamil addition is as shown:

		Animal Number					
	Time	**1**	**2**	**3**	**4**	**5**	**6**
pH	0–8 hours	+ 0.9%	− 0.9%	− 2.1%	− 2.6%	− 2.5%	− 3.1%
pH	8–16 hours	− 0.3%	− 1.8%	− 0.4%	− 1.4%	− 0.3%	− 0.9%
pH	16–24 hours	− 0.2%	− 0.1%	− 3.5%	− 0.6%	0%	− 0.5%
pH	0–24 hours	− 1.4%	− 2.8%	− 6%	− 4.6%	− 2.2%	− 4.5%
Poids	0–24 hours	+66%	+44%	+74%	+68%	+59%	+41%
CK Mb	0–8 hours	+ 1.68%	+ 2.1%	0%	+ 5.67%	+ 3.6%	+ 6.89%
CK Mb	8–16 hours	+ 2.125%	+ 0.93%	+ 0.8%	+ 0.08%	+ 1.41%	+ 0.13%
CK Mb	16–24 hours	+ 1.4%	+ 0.17%	+ 3.15%	+15%	+ 0.62%	+ 0.176%
CK Mb	0–24 hours	+ 6.5%	+ 7.75%	+ 6.5%	+15%	+17%	+ 9.53%

2) Histological Results[7]

With light microscopy, the myosytes were clear after 24 hours preservation time; the loss of color shows the disappearance of glycogen (Figure 14). We cannot differentiate between cells of prepared hearts (glucose and insulin perfusion for 3 hours before transplantation) and the nonprepared hearts.

With electron microscopy (Figure 15), at the top of the photograph, you can see mitochondria which look normal. The glycogen is abundant between mitochondria appearing under dark granules. At the base of the photograph is a myosyte after 24 hours preservation. Mitochondria are very clearly expanded without any membrane break. In the cytosol, between the mitochondria glycogen granules are rare.

The myofibriles are squeezed by mitochondrial dilatation. These injuries are reversible.

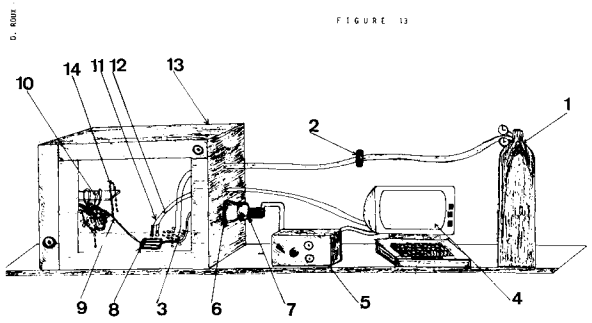

Figure 13

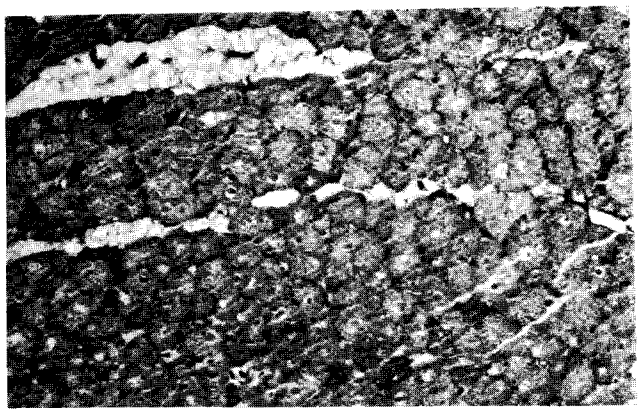

Figure 14

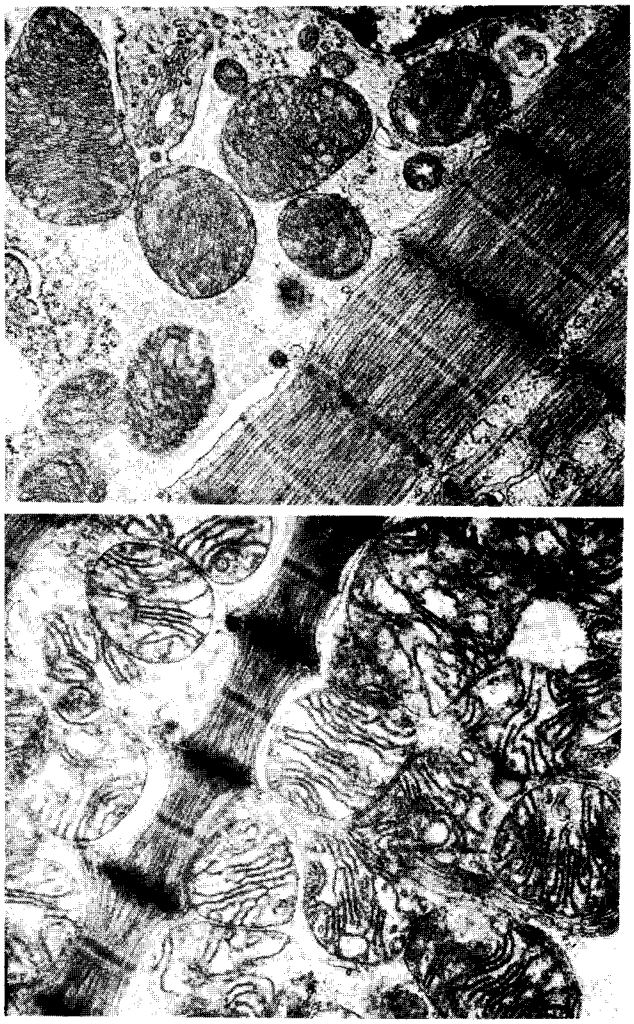

Figure 15

Discussion

Several points deserve comments.

Surgical Method: This method is very important because it perfectly preserves the myosyte energy substrate. The heart is under hypothermic perfusion and oxygenated from clampage for the excision, to declampage after transplantation. Coronary perfusion is important to eliminate the blood cells because red cells can obstruct the preservation system filter; and platelets are eliminated (possible thromboxane release).

Preservation Solution Criteria: Asepsis; neutral pH; oncotic and osmotic pressures equivalent to blood for preventing edema.[1] Some of the components are not stable (for instance, procaine and insulin).

For this reason we use a stable solution to which we add the nonstable elements just before use. The nutrient elements are useless because they are not consumed during preservation. On the other hand, the membrane stabilizing elements prevent reperfusion arrythmias.[2]

Histological Photograph: Under light microscopy we can see that intracellular glycogen has disappeared in spite of glucose and insulin present inside the perfusion solution. This is because insulin is not active at a low temperature. Therefore, the hearts have to be prepared with a glucose and insulin solution in order to increase cellular stores of glycogen. Under electron microscopy, we notice mitochondria swelling which compresses the myofibriles.

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Questions from the Audience

Question: Aaron Hill, Falls Church, VA: Very nice presentation. Could you tell me something about the perfusate that was in the solution, of the composition of that perfusate?
Response: Yes. There are two types of solution. One is for the cardioplegia and this is a crystalloid solution with potassium. Also, because it seemed to us quite important to have a cardiac arrest in diastole, we flush an injection of a calcium blocker.
Question: Do you have calcium blocker in the perfusate as well, in the transport unit? Do you have a calcium channel blocker like deltiazam, cardiazem or something like that? Or the European name for the drug? Veraplasmen? Do you use that in the perfusate as well?
Response: Yes.
Question: What about the time frame? How long do you think you can prolong the heart?
Response: I see because of its work during 24 hours. Why not in the next step try it any time? Why not?
Question: You should be able to prolong it beyond 24 hours?
Response: Yes.