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COMPARISON OF EARLY FATES OF CADAVER RENAL ALLOGRAFTS FROM DIFFERENT METHODS OF HARVEST

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ABSTRACT

The early fate of cadaveric grafts harvested from four different methods depending on donor categories (A) in situ flushing with a simple catheter, B) in situ perfusion with a double balloon catheter, C) shipped kidneys from the United States, and D) from brain death and beating heart donors) were compared including histological studies of one-hour biopsied specimens. Early initiation of graft functions was naturally seen first in group D, which was followed by group B. In situ perfusion with a double balloon catheter is a very valuable method of harvest in those countries where death is declared after cessation of breathing and heart beat.

Key words: Cadaveric kidney transplant, In situ perfusion, Double balloon catheter, One hour biopsy, Cyclosporin A

INTRODUCTION

Prolonged warm ischemia is a major factor preventing immediate function of cadaver kidneys following transplantation. This period of time is most often a problem in cadaver kidneys removed after cardiac arrest of the donor.[1—4] In our joint transplant program at Aichi Cancer Center Hospital and Nagoya Second Red Cross Hospital, cadaver kidneys for transplantation were derived from four types of harvest as follows; (A) in situ hypothermic perfusion with a simple catheter after cardiac arrest of the donor; (B) in situ hypothermic perfusion with double balloon triplelumen catheter after cardiac arrest of the donor;[5] (C) shipped kidney from the United States;[6] and (D) heart beating donor.

The purpose of this study was to compare the early fate of kidneys harvested from the four different methods depending on donor categories by means of several criteria including findings of one-hour biopsied specimens.

MATERIALS AND METHODS

A total of 33 cadaveric kidneys were transplanted into 31 recipients. The first 3 cadaveric kidneys, which were harvested without any in situ perfusion, were excluded from this study.

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A pair of kidneys was shared by the two transplant hospitals of the Tokai Kidney Procurement Organization[7]; therefore, each kidney in this study came from a different donor. Only cases No. 4 and 5 are exceptional in that they received the graft from the same donor.

These thirty kidneys were divided into the following four groups according to the condition of the donor and/or the method of harvest.

A : In situ flushing with a simple catheter.

After donor's cardiac arrest or before impending cardiac arrest, a double-balloon-triple-14 or 16 French size) was inserted into the abdominal aorta through a femoral artery cutdown performed in ICU or in the patient's ward and the tip of the catheter, which has five side-holes, was positioned approximately at the level of the first lumbar spine. For the infussate we used cold (4°C) lactate Ringer's solution to which we added 3,000 units of heparin/l. This was infused with a Pressure infusor (Terumo, Tokyo, Japan). The donors were transferred to the operating room as quickly as possible.

B : In situ perfusion with a double balloon catheter.[5]

After donor's cardiac arrest or before impending cardiac arrest, a double-balloon-triple-lumen catheter* (No. 14, 16 or 18 French size) was inserted into the abdominal aorta through a femoral artery cutdown, and another simple catheter (No. 18 French size) for the venous

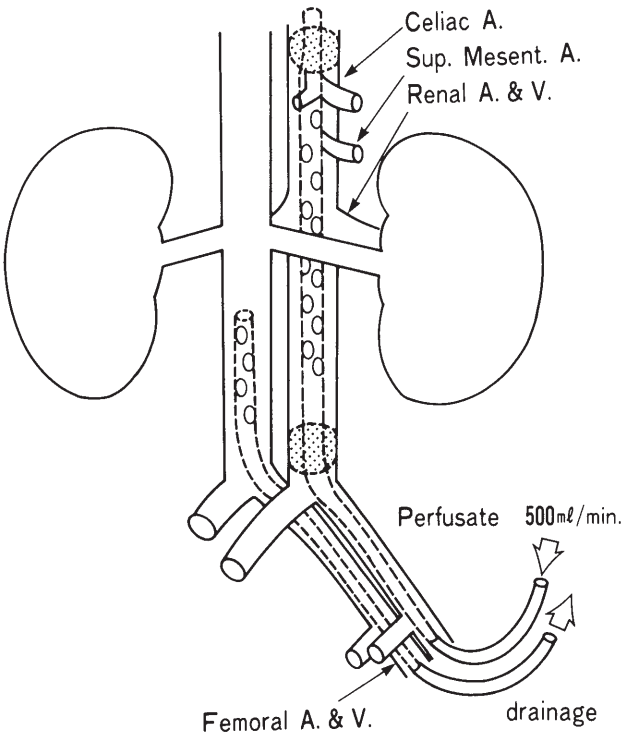

Fig. 1 In situ perfusion with a double balloon catheter

* : Create Medico Co. Tokyo, Japan

blood and infusate drainage was also inserted into the inferior vena cava through a femoral vein cutdown. A "pull-back technique" was used to position the balloons. Both catheters were connected with a specially designed machine, which has two roller pumps inside for both infusion and outflow. Cold lactate Ringer's solution, the same as in group A, was infused at the rate of 500 ml/min with continuous infusion (Figure 1). The donor was transferred to the operating room as quickly as possible.

C : Shipped kidneys from the United States.[6]

These kidneys were harvested in the United States with brain death and beating heart condition and sent to us in cold storage over Trans-Pacific.

D : From brain death and beating heart donors.

Recently, and these are exceptions, a few kidneys were harvested from brain death and beating heart donors.

After a pair of kidneys was harvested by evisceration techniques[8] they were ex vivo flushed with cold (4°C) modified Collins Solution (Euro-Collins).[9] Warm Ischemic Time (WIT) was considered the period between the donor's cardiac arrest and ex vivo flushing with this modified Collins Solution; and Cold Ischemic Time (CIT) was considered the period between the flushing with this solution and revascularization of the graft.

After a kidney was transplanted and just before closing the abdomen, a needle biopsy was taken from the graft and abdomen, a needle biopsy was taken from the graft and was submitted to histological examination including electron-microscopy.[10,11]

Immunosuppressive therapy for CD4-21 was a conventional combination consisting of Azathioprine, steroid and antilymphocyte globulin with additional graft irradiation at rejection episode. The one for CD22-33 was Cyclosporin A and steroid[12] also with the same therapy at rejection episode.

RESULTS

All clinical results were shown in Table 1. The period of systolic blood pressure less than 50 mmHg before cardiac arrest of donors was considerably long in some cases in groups A and B. The warm ischemic time (WIT), which includes in situ perfusion time, was also long in groups A and B. The cold ischemic time (CIT) in group C was naturally long, being 41 hours on the average.

Initiation of graft function in each group was resummarized in Figure 2. The number of post-transplant days with urine volume more than 1,000 ml/day was relatively high in group C although the number of cases was small.

The effects of the period of systolic blood pressure less than 50 mmHg and serum creatinine on the day of harvest to the initiation of graft functions in group B were shown in Figure 3. The significance of this is that as long as serum creatinine on the day of harvest is within the normal limit, the donor whose period of systolic blood pressure is less than 50 mmHg up to 3 hours is acceptable, and that the patient whose serum creatinine is more than 3 mg/dl is not recommended as a donor.

Light-microscopic findings of one-hour biopsy specimens are shown in Table 2 and electron-microscopic findings are shown in Tables 3 and 4.[10,12] These microscopic findings of one-hour biopsy specimens clearly show that the qualities of grafts well correlated with the

Table 1 Clinical data of donors and recipients

BP < 50 : period between systolic blood pressure less than 50 mmHg and cardiac arrest
S.Cr. : serum creatinine of the donor on the day of harvest
WIT : warm ischemic time
CIT : cold ischemic time
* : average ± SD
@ : retransplant
Immuno Suppr. : immunosuppression
AZA : Azathioprine
CyA : Cyclosporin A
U.V. > 1 l/day : post transplant day of urine volume more than 1 l/day
S.Cr. < 2 mg/dl : post transplant day of serum creatinine of the recipient less than 2 mg/dl
Re-HD : restart of hemodialysis
X : non-function
† : dead
Rej. : rejection
#S.Cr. : functioning with this serum creatinine at the time of this writing

	CD	Age/sex	BP < 50	Donor S.Cr. (mg/dl)	WIT	CIT	Date of Trans-plant	Age/sex	Immuno Suppr.	Recipient U.V. > 1 l/day (day)	Recipient S.Cr. < 2 mg/dl (day)	Best S.Cr. (mg/dl)	Follow-up
	4	22 M	20′	2.8	47′	3°00′	8-30-'77	26 F	AZA	17	—	—	Re-HD 1.5 M
	5	22 M	20′	2.8	47′	3°36′	8-30-'77	24 F	AZA	0	2	1.5	Re-HD 8 D
(A)	6	60 F	40′	0.5	63′	12°15′	1-16-'78	35 M	AZA	2	—	3.0	Re-HD 1.0 M
	7	27 M	360′	0.9	73′	9°18′	4-29-'79	@38 M	AZA	—	—	—	X
	18	10 M	90′	2.6	99′	10°39′	8- 9-'82	23 M	AZA	13	61	1.3	#S.Cr. 1.3 mg/dl
			106.0±129.6*	1.9±1.0*	65.8±19.3*	7°45′±3°45′*				8.0±7.2*			
	8	53 F	120′	1.4	46′	5°44′	8-10-'80	28 F	AZA	13	29	0.9	†12 M (Hepatitis)
	9	19 M	120′	1.1	40′	4°57′	9- 4-'80	28 M	AZA	0	7	0.8	Re-HD 17 M (Rej.)
	10	18 M	60′	0.9	51′	4°19′	10-20-'80	46 M	AZA	7	13	0.9	Re-HD 4 M (Rej.)
	11	31 F	60′	1.0	49′	3°46′	12- 7-'80	34 M	AZA	1	13	1.0	#S.Cr. 0.9 mg/dl
	13	19 M	6′	1.3	70′	1°56′	10-22-'81	32 M	AZA	0	5	1.4	Re-HD 2 M (Rej.)
	14	19 M	6′	2.5	75′	2°26′	12-25-'81	31 M	AZA	7	13	1.2	Re-HD 1.5 M (Rej.)
	15	37 F	6′	0.6	42′	2°24′	2-11-'82	36 M	AZA	4	12	1.1	Re-HD 1.5 M (Rej.)
(B)	17	44 F	30′	2.8	74′	3°01′	6- 7-'82	26 M	AZA	15	21	1.1	Re-HD 11 M (Rej.)
	20	25 M	120′	3.6	62′	4°36′	9-15-'82	31 F	AZA	—	—	—	X
	21	18 M	6′	1.1	98′	3°57′	10-11-'82	28 F	AZA	6	9	1.8	Re-HD 1 M (Rej.)
	25	52 M	63′	1.0	93′	3°54′	2-13-'83	44 M	CyA	10	15	1.2	#S.Cr. 1.5 mg/dl
	26	56 F	90′	1.4	10′	7°35′	3-14-'83	@56 M	CyA	22	39	1.5	#S.Cr. 1.6 mg/dl
	31	59 F	60′	1.5	175′	10°15′	8-19-'83	47 M	CyA	11	—	3.2	#S.Cr. 3.2 mg/dl
	32	50 M	164′	1.1	116′	7°24′	9-13-'83	@32 F	CyA	1	21	1.9	#S.Cr. 2.7 mg/dl
	33	31 M	55′	0.3	60′	5°06′	9-19-'83	26 M	CyA	0	7	1.3	#S.Cr. 1.3 mg/dl
			64.4± 48.2*	1.4±0.8*	70.7±37.7*	4°45′±2°10′*				6.6±6.2*			
	12	9 M	0′	1.1	3′	29°38′	10-18-'81	56 M	AZA	1	4	1.4	Re-HD 10 D (Rej.)
	16	19 F	0′	1.3	12′	37°58′	4-10-'82	41 M	AZA	8	13	0.8	Re-HD 16 M (Rej.)
(C)	19	5 M	0′	0.4	1′	43°48′	9- 2-'82	29 M	AZA	27	57	1.4	Re-HD 11 M (Rej.)
	30	29 M	0′	2.1	3′	52°53′	8-11-'83	54 F	CyA	17	29	1.2	#S.Cr. 1.5 mg/dl
				1.2±0.6*	4.7± 4.3*	41°04′±8°29′*				13.0±9.6*			
	22	34 F	0′	0.8	1′	9°27′	11-30-'82	37 M	CyA	0	4	0.8	#S.Cr. 1.1 mg/dl
	23	11 M	0′	0.7	1′	1°38′	1- 7-'83	34 M	CyA	0	6	1.1	#S.Cr. 1.1 mg/dl
	24	68 M	3′	1.2	1′	10°19′	1-12-'83	38 F	CyA	4	9	1.5	#S.Cr. 1.5 mg/dl
(D)	27	64 M	1′	1.2	1′	5°59′	4-10-'83	30 M	CyA	10	—	2.1	#S.Cr. 2.8 mg/dl
	28	25 M	0′	1.0	1′	11°51′	5-14-'83	31 F	CyA	0	4	0.6	#S.Cr. 0.6 mg/dl
	29	40 M	9′	1.7	4′	3°33′	5-15-'83	31 F	CyA	6	11	0.8	#S.Cr. 0.8 mg/dl
			2.1± 3.2*	1.1±0.3*	1.5± 1.1*	7°07′±3°42′*				3.3±3.7*			

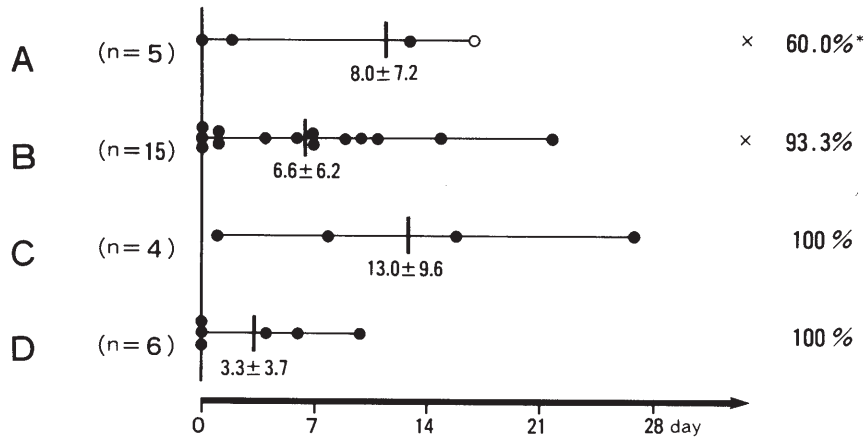

Fig. 2 Initiation of graft function among each group
Number of post-transplant days with urine volume more than 1000 ml/day
n : number of grafts
• : satisfactory function
o : unsatisfactory function
X : non-function
* : satisfactory initiation rate

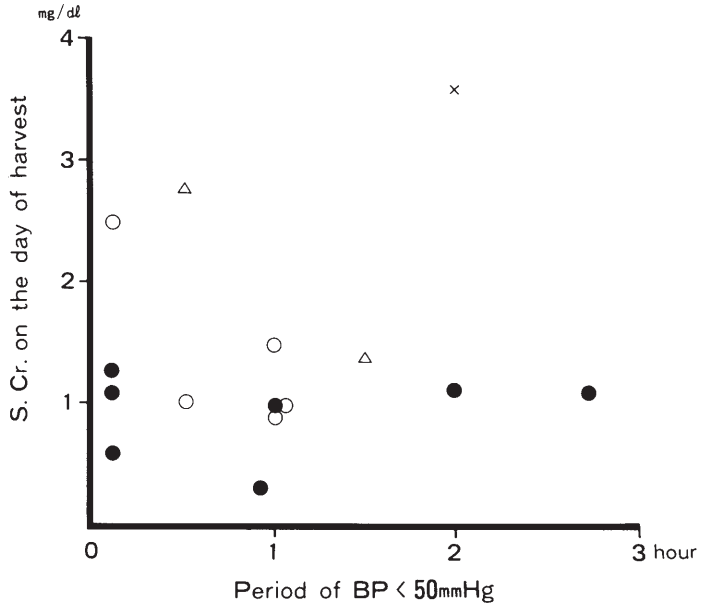

Fig. 3 Effects of the period of systolic blood pressure less than 50 mmHg and serum creatinine of the donors on the day of harvest to the initiation of graft functions in Group B (n = 15)
Number of post-transplant days with urine volume more than 1000 ml/day
• : 0 — 6 days
o : 7 — 13 days
Δ : more than 14 days
X : non-function

Table 2 Light microscopic findings of one-hour biopsy specimen
0 : none, D : diffuse, F : focal, 1 : mild, 2 : moderate, 3 : remarkable

		Thrombi	Tubules Vacuoles	Tubules Flat cytoplasma	Tubules Detachment	Edema	Arteriole Intimal thickening
(A)	CD-18	F2	D1	D2	F3	0	0
(B)	CD-13	0	D2	D1	F1	0	0
	14	0	D2	F1	F1	0	0
	15	0	D2	D2	F1	0	0
	17	0	D1	D2	F1	D1	1
	20	D3	F1	F2	0	0	0
	21	0	F1	F1	F1	0	0
	25	0	F1	D1	F1	0	0
	26	0	D1	D2	F1	0	2
	31	F3	D1	D2	F2	0	2
	32	0	D1	F1	F1	0	0
	33	0	D2	D2	D1	0	—
(C)	CD-12	0	F1	F1	0	0	0
	16	0	F2	D2	F1	0	0
	19	0	F1	D1	F2	0	0
	30	0	F1	0	F1	0	0
(D)	CD-22	0	D1	D1	F1	0	0
	23	0	F1	F1	0	0	0
	24	0	D1	F1	F1	D2	0
	27	0	D2	D3	D2	D1	2
	28	0	F1	F1	0	0	1
	29	0	D2	D1	F2	0	1

Table 3 Electron microscopic findings of one-hour biopsy specimen (glomeruli)
0 : none, 1 : segmental/mild, 2 : moderate, 3 : remarkable

		Disappearance of Fenestrae	Thrombi	Foot Process Fusion	Belb Formation	Cell Debris in urinary space
(A)	CD-18	1	2	1	1	1
(B)	CD-13	1	0	1	0	0
	17	2	0	1	0	1
	20	1	3	1	1	2
	21	0	0	2	0	1
	25	0	0	1	0	2
	26	0	0	1	1	1
	31	1	0	1	1	1
	32	0	0	1	0	0
	33	0	0	1	0	0
(C)	CD-12	1	0	1	1	2
	16	0	0	1	1	1
	30	0	0	1	1	0
(D)	CD-22	0	0	0	0	1
	23	0	0	0	2	1
	24	0	0	1	1	1
	27	0	0	0	1	0
	28	0	0	0	0	1
	29	0	0	0	1	1

Table 4 Electron microscopic findings of one-hour biopsy specimen (tubules)
0 : none, 1 : segmental/mild, 2 : moderate, 3 : remarkable

		Brush Border Injury	Vacuoles	Swelling	Mitchondria Dislocation of Cristae	Ballooning
(A)	CD-18	1	2	1	1	1
(B)	CD-13	0	1	0	0	0
	17	1	1	1	1	1
	20	0	3	1	1	1
	21	0	2	2	2	2
	25	0	1	1	1	1
	26	1	2	1	1	1
	31	0	2	2	1	1
	32	0	1	0	0	0
	33	0	1	0	0	0
(C)	CD-12	0	1	0	0	0
	16	0	1	1	0	0
	19	0	1	1	1	0
	30	?	1	1	1	0
(D)	CD-22	0	1	1	1	0
	23	0	1	0	0	0
	24	?	2	2	2	1
	28	0	1	0	1	0
	29	0	1	0	1	0

donor's condition before harvest. Remarkable exhibitions of thrombi, either diffuse or focal, are definitely deleterious to the immediate or long-term graft functions and were never seen in either group C or D. Exhibitions of vacuoles, flat cytoplasma and detachment of tubules also have deleterious effects on the immediate or long-term graft functions (Figure 4 and 5).

All patients including two cases of retransplants who received immunosuppression with Cyclosporin A have functioning grafts at the time of this writing. Their serum creatinine levels tend to be slightly higher compared with the Azathioprine group although some of them are apparently due to the damaged qualities of the graft.

DISCUSSION

The declaration of death after cessation of breathing and heart beat and the short supply of cadaver organs, both of which are strongly based on socio-religious background, have been major obstacles in the advance of organ transplantation in Japan. Despite these circumstances, several efforts have been made for the improvement of cadaver kidney transplants. Introduction of in situ perfusion before nephrectomy has been one of the major

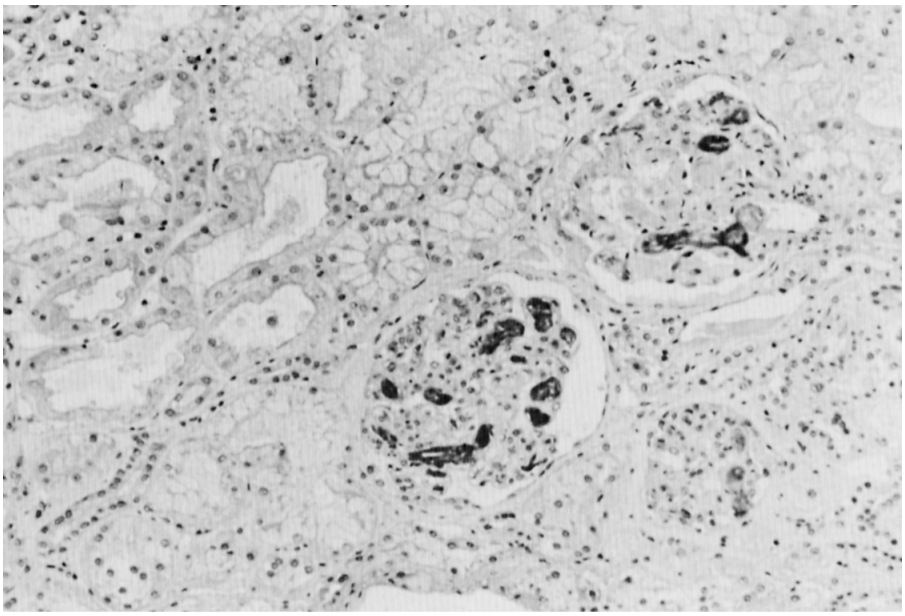

Fig. 4 One-hour biopsied specimen of patient No. 20.
Hematoxylin-eosin : × 100
Many microthrombi are seen in the glomeruli and the capillary lumens are not well open. Mild vacuolization and flattening of cytoplasma are sporadically seen in the epitherial cells of tubules.

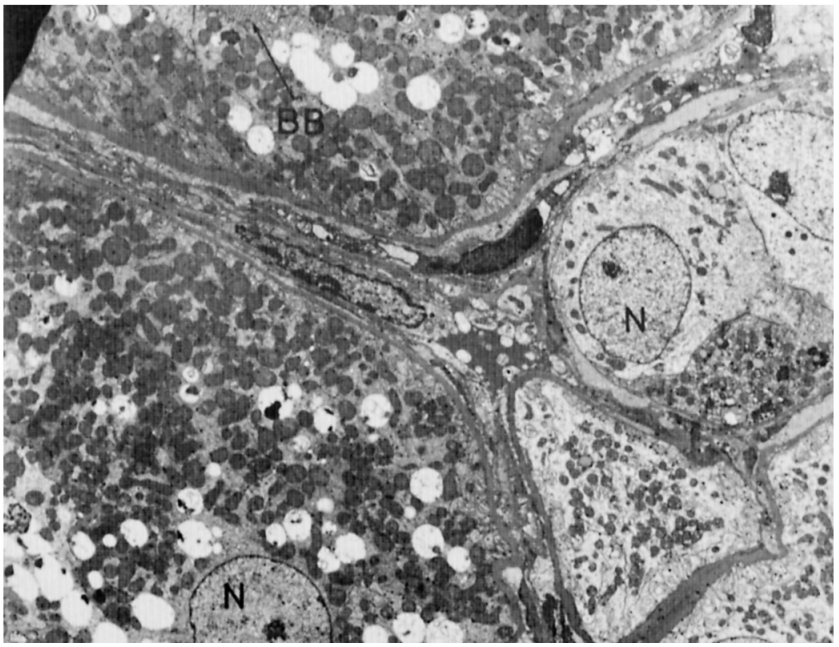

Fig. 5 One-hour biopsied specimen of patient No. 22 (Tubules).
Immersion fixation : × 4000
Mild vacuolization is seen in the epitherial cells of tubules, which are otherwise well preserved with normal-shaped mitochondria.
N : nucleus
BB: brush border

achievements. In situ perfusion with double-balloon catheter has shown especially satisfactory results as long as the donor has good kidney function.

Our in situ perfusion studies in canines showed that modified Collins' solution which has an intracellular-like electrolyte composition is apparently more deleterious than lactate Ringer's solution at a two-hour perfusion. We used modified Collins' solution only for ex vivo flushing.

Human cadaver kidney preservation is currently achieved by two methods, cold storage and machine perfusion. The great advantage of the former method compared to the latter is its simplicity and low cost. However, while it was demonstrated that pulsatile preservation for periods of 36-67 hours did not adversely effect the success rate of transplantation,[13] it was generally thought that the safe period of storage by cold preservation was markedly shorter, and many transplant centers were reluctant to accept kidneys stored by this method for more than 24 hours.[14]

Halasz[15] and Collins[16] reported excellent function of canine kidneys preserved by cold storage for 48 hours provided there was no warm ischemia time. In the human, extension of cold storage time up to 30 hours was reported by Kreis[17] and up to 44 hours, by Barry.[18] Squifflet comfirmed, in a large population, the absence of adverse effects of cold storage up to 51 hours on the incidence of acute tubular necrosis (ATN) and on the quality of graft function.[9]

Histological examination of one-hour biopsy specimens provides adequate information about the qualities of grafts in connection to (1) the donor's hemodynamic status at the time of organ harvesting, (2) preservation conditions, as well as total ischemic time, and (3) blood flow taking place through the graft when vascular clamps are released. It also serves as a standard control, when biopsy examination of the graft becomes necessary later on, although electron microscopic study provides additional information. Light-microscopic findings also seem to provide clinically adequate information.

Many Japanese transplant centers are very much obliged to the U.S.-shipped kidney supply; however, medical cost reimbursement plans in Japan do not allow for the expenses incurred in obtaining such kidneys. Currently, Japanese patients who receive such kidneys must bear the expenses personally.[6] The effects of long-term cold ischemic time over a long term survival of the graft also remains to be investigated.

The number of kidney donations has been slowly but steadily increasing. The rigid attitude to a declaration of death cessation of breathing and heartbeat will hopefully change in the near future.

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REFERENCES

1) Schweizer, R.T., Bartus, S.A., Sutphin, B.A., Improved methods of in situ hypothermic perfusion of cadaver

kidneys. *Transplant. Proc.*, **9**, 1587—1589, 1977.
2) Scheweizer, R.T., Sutphin, B.A., Bartus, S.A., In situ cadaver kidney perfusion. Experimental and clinical studies. *Transplantation,* **32**, 482—484, 1981.
3) Anderson, C.B., Sicard, G.A., Etheredge, E.E., Delayed primary renal function and cadaver renal allograft results. *Surg. Gynecol. Obstet.*, **149**, 697—702, 1979.
4) Luciani, J., Frantz, P., Thibault, P. *et al.* Early anuria prevention in human kidney transplantation. *Transplantation,* **28**, 308—312, 1979.
5) Garcia-Rinaldi, R., Lefrak, E.A., Defore, W.W. *et al.* In situ preservation of cadaver kidneys for transplantation: Laboratory observations and clinical application. *Ann. Surg.*, **182**, 576—584, 1975.
6) Iwaki, Y., Terasaki, P.I., Kinukawa, T. *et al.* Trans-pacific kidney shipment. *Transplantation,* **35**, 506—508, 1983
7) Takagi, H., Uchida, K., Ohshima, S. *et al.* Prospective HLA-DR matching in cadaveric renal transplantation. *Transplant. Japon.* in press.
8) Merkel, F.K., Jonasson, O., Bergan, J.J., Procurement of cadaver donor organs: Evisceration technique. *Transplant. Proc.*, **4**, 585—589, 1972.
9) Squifflet, J.P., Pirson, Y., Gianello, P. *et al.* Safe preservation of human renal cadaver transplants by Euro-Collins solution up to 50 hours. *Transplant. Proc.*, **13**, 693—696, 1981.
10) Reimer, K.A., Ganote, C.E., Jennings, R.B., Alterations in renal cortex following ischemic injury. III. Ultrastructure of proximal tubules after ischemia or autolysis. *Lab. Invest.*, **26**, 347—363, 1972.
11) Coffey, A.K., Andrews, P.M., Ultrastructure of kidney preservation: Varying the amount of an effective osmotic agent in isotonic and hypertonic preservation solutions. *Transplantation,* **35**, 136—143, 1983.
12) Hakala, T.R., Starzl, T.E., Rosenthal, J.T. *et al.* Cadaveric renal transplantation with Cyclosporin-A and steroids. *Transplant. Proc.*, **15**, 465—470, 1983.
13) Seim, S.K., Maisel, K.E., Merkel, F.K., The effect of length of preservation on renal transplant survival. *Surg. Gynecol. & Obstet.*, **145**, 709—713, 1977.
14) Chatterjee S., In manual of renal transplantation. Springer, New York, pp76—79, 1979.
15) Halasz, N.A., Collins, G.M., Forty-eight-hour kidney preservation. A comparison of flushing and ice storage with perfusion. *Arch. Surg.*, **111**, 175—177, 1976.
16) Collins, G.M., Halasz, N.A., Forty-eight-hour ice storage of kidneys: Importance of cation content. *Surgery.* **79**, 432—435, 1976.
17) Kreis, H., Noël, L.H., Moreau, J.F., *et al.*, Bilogic, pathologic, and radiologic studies of cadaver kidneys preserved in Collins' solution. *Transplant. Proc.*, **9**, 1611—1616, 1977.
18) Barry, J.M., Farnsworth, M.A., Bennet, W.M., Human kidney preservation by flushing with intracellular solution and cold storage. *Arch. Surg.* **113**, 830—832, 1978.