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Experimental preservation of sheep kidney for successful transplantation

Correlation of Results and Tetrazolium Test

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ALTHOUGH it is now possible to preserve isolated mammalian kidneys for 24 hours or longer, the functional potentiality of the organ so preserved is greatly reduced. At the present stage of human cadaver kidney transplantation, it is important to know the safe maximal period of ischemia which will permit adequate function of the organ to sustain life after transplantation. With evidence of the importance of histocompatibility in regard to the future fate of the transplanted cadaver kidney, it is urgent to define a safe period of ischemia which will allow such matching tests as are currently available.

This report presents the data of our experimental study of the effects of different periods of ischemia on the degree of irreversible renal damage and recovery of function after autotransplantation of kidneys in sheep.

Kidneys were preserved with hypothermia and hyperbaric oxygen and were evaluated after autotransplantation in the necks of the animals. Thus, the effects of preservation were studied without interference from complications induced by immunologic reactions. Lack of laboratory space prevented long-term evaluation of the results, but even at short term the differences in renal function indicated that one method was superior.

Materials and Methods

Thirty sheep kidneys were excised and were preserved as follows. Twenty-one sheep kidneys were preserved by a combination of hypothermia at 2 C and hyperbaric oxygen at a pressure of 30 psi (3 atm absolute) from 10

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Table 1.—*Composition of electrolyte solution used to flush the excised sheep kidneys*

Constituent	Amount per liter
Dextran	60.0 g
Dextrose	250.0 g
Mannitol	12.5 g
Heparin	40.0 mg
Sodium	147.0 meq
Potassium	5.0 meq
Calcium	5.0 meq
Magnesium	1.6 meq
Chloride	136.0 meq
Bicarbonate	20.0 meq
Phosphate	1.6 meq

sheep, and of 60 psi (6 atm absolute) from 11 sheep. Nine kidneys were preserved by hypothermia alone at 2 C.

With the animal under fluothane and oxygen anesthesia, a kidney was removed through an extraperitoneal lumbar incision. Appropriate lengths of the renal vessels and ureter were excised during the removal of the kidney. The renal artery and the vein were clamped simultaneously by separate clamps. After nephrectomy, the isolated kidney was flushed with a balanced electrolyte solution,[1] the composition of which is given in *Table 1*. The temperature of the solution was 4 C and it was not oxygenated.

The solution was flushed through the kidney under 1-m gravity pressure against a venous gradient of 4 cm of water. The kidney was then put inside the hyperbaric oxygen chamber* (*Fig. 1*). After the specified period of preservation, the hyperbaric chamber was decompressed and the kidney was autotransplanted into the neck of the sheep. The renal artery and vein were anastomosed end-to-end to the carotid artery and jugular vein, respectively, and a cutaneous ureterostomy was made in the neck. Contralateral nephrectomy was done at the time of transplantation only when the preserved kidney showed sustained urine output of from 1 to 2 ml per minute for 30 minutes. Animals were killed after 28 days when all was well, or earlier when there were signs of deterioration. Biopsy specimens were taken on the first and second postoperative days and then at weekly intervals. Tetrazolium viability tests were done on all preserved kidneys according to the method described.[2] A positive test, suggestive of viable tissue, was indicated by the development of a blue color within from 1 to 2 minutes.

* *Courtesy of Swenko Research & Development, Inc., Minneapolis, Minnesota.*

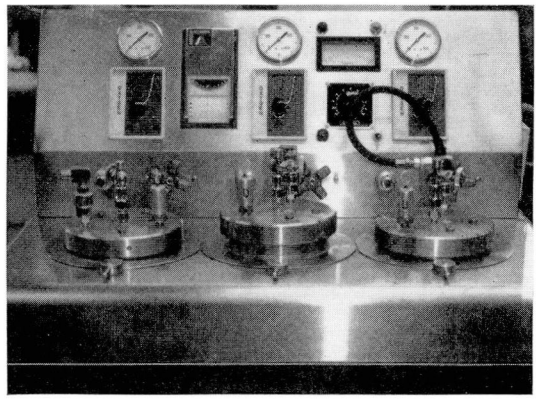

Fig. 1. Swenko Organ Preservation Unit composed of three hyperbaric oxygen chambers the pressures of which can be individually adjusted.

RESULTS

Table 2 shows the results of 21 experiments in which the hyperbaric oxygen chamber was cooled down to 2 C and oxygen under high pressure (OHP) at 30 psi was used in 10 cases, and at 60 psi in 11 cases. *Table 3* shows the results of preservation of nine kidneys by hypothermia alone.

All kidneys that were preserved for from six to eight hours put out urine immediately at rates of from 1 to 2 ml or more per minute, allowing contralateral nephrectomy at the time of transplantation in the neck. Histologically, the kidneys showed mild degrees of tubular necrosis, which completely regressed.

Kidneys preserved for from 9 to 12 hours had immediate urine outputs of from 1 to 2 ml per minute, after which oliguria or anuria developed within 24 hours. Within one week, diuresis occurred and contralateral nephrectomy was performed. Histologically, the kidneys showed moderate to severe degrees of tubular necrosis, which completely regressed.

Kidneys preserved for more than 12 hours were anuric, after which urine output was less than 600 ml per day. After contralateral nephrectomy, progressive azotemia developed. There was histologic evidence of severe tubular necrosis that showed only focal regeneration of the tubules. Focal glomerular necrosis was present in all kidneys.

Table 3 shows the results of nine experiments in which the kidneys were preserved solely by hypothermia at 2 C. Kidneys preserved for from six to eight hours showed no significant difference in function from that of those preserved by a combination of hypothermia and hyperbaric oxygen. The latter was of definite advantage when preservation longer than eight hours was required.

Table 2.—*Data on excised sheep kidneys preserved at 2 C under high oxygen pressure*

Period of preservation, hours	Kidneys preserved under 30 psi		Kidneys preserved under 60 psi	
	Number	Remarks	Number	Remarks
6 to 8	3	Immediate diuresis after transplantation and contralateral nephrectomy. Normal blood urea nitrogen content and almost complete regeneration of tubules in 3 to 4 weeks. Tetrazolium test positive.	None.	
9 to 12	3	Oliguria and then diuresis. Contralateral nephrectomy in 1 to 2 weeks after transplantation. One had normal and two had increased blood urea nitrogen and serum creatinine content. Majority of tubules regenerated, but there were some tubular atrophy and interstitial cellular infiltrates. Normal glomeruli. Tetrazolium test positive.	5	Initial oliguria and then diuresis. Contralateral nephrectomy after 1 week. Normal blood urea nitrogen and creatinine content after 3 weeks. Majority of tubules regenerated after 3 weeks. Normal glomeruli, blood vessels, and anastomosis. Tetrazolium test positive.
13 to 24	4	No diuresis. Azotemia after contralateral nephrectomy. No evidence of regeneration after 3 weeks in majority of tubules. Focal glomerular necrosis, and kidney soft and pale. Blood vessels and anastomosis all patent. Tetrazolium test negative.	6	No diuresis. Azotemia after contralateral nephrectomy. No evidence of regeneration of majority of tubules. Focal glomerular necrosis and some glomeruli had basement membrane thickening and proliferation. Blood vessels and anastomosis all patent. Kidney soft and pale. Tetrazolium test negative.

DISCUSSION

Preservation of kidneys for 24 hours has been reported by Manax, Largiader, and Lillehei,[3] and by Humphrey and associates.[4] Ischemia of organs at normal body temperature if prolonged leads to irreversible changes. De Duve's[5] concept of intracellular cytoplasmic particles, the lysosomes, has been revived by Janoff[6] in the field of tissue hypoxia to

Table 3.—*Data on excised sheep kidneys preserved solely by hypothermia at 2 C*

Period of preservation, hours	Kidneys preserved, number		Remarks	Histologic data
	Total	Functioning		
6 to 8	3	3	Normal blood urea nitrogen and serum creatinine content 3 weeks after contralateral nephrectomy.	Normal after 3 weeks.
9 to 12	3	1	Oliguria; increased blood urea nitrogen and serum creatinine content.	No regeneration of tubules after 3 weeks. Interstitial edema, cellular infiltrates and some necrotic glomeruli.
12 to 24	3	0	—	Severe tubular and cortical necrosis.

explain the mechanism of tissue damage. Lysosomes contain a variety of hydrolytic enzymes that are most active in the acid pH range and are therefore activated by the tissue acidosis during hypoxia. Release of the enzymes leads to propagation of injury from cell to cell. Hypothermia and hyperbaric oxygen may help to preserve organs by means of the protective action on the intracellular lysosomes. Tubular necrosis develops despite the use of hypothermia and hyperbaric oxygen, and the severity of tubular necrosis is proportional to the duration of ischemia. After 12 hours of preservation, more than 50 percent of the renal tubules suffer irreversible damage (*Fig. 2*).

Hypothermia lowers the rate of oxygen consumption by the tissues. At 2 C, oxygen consumption of the tubular cells is less than 5 percent of normal, and the tubular transport of water, sodium, and potassium ceases.[7] Intracellular pH of the tubular epithelium is dependent on the anaerobic metabolism during the ischemic period, and tends to decrease with the passage of time and may actually set in motion the lytic action of the lysosmal enzymes that cause tissue damage.[5]

Hyperbaric oxygen, by increasing the rate of diffusion of oxygen through the tissues and at an oxygen pressure of 60 psi may diffuse to the renal medulla[8] and may just meet the minimal requirement of tissues at 2 C. Our observation that hypothermia alone permits storage of viable kidneys for eight hours is in agreement with the data of others.[9, 10] Ischemia of kidneys preserved by a combination of hypothermia with OHP longer than 12 hours led to increasing severity of irreversible damage. At the

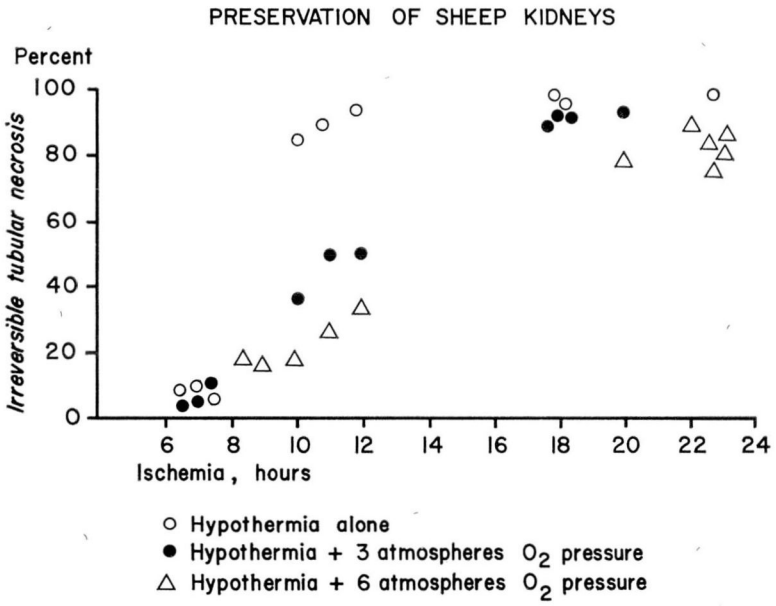

Fig. 2. Correlation of duration of ischemia with the degree of irreversible tubular damage. Note that hypothermia alone did not permit storage of the kidney longer than eight hours if the kidney were to remain functional.

present time, it seems that the damage due to ischemia of preservation is progressive, and, in a majority of kidneys, long-range normal function was not obtained.[11] Addition of intermittent pulsatile slow perfusion of the kidney while stored at 2 C under 30 psi of oxygen pressure, for 24 hours, did not improve the function of the preserved kidney.[12]

At this stage of cadaver kidney transplantation, the future fate of the renal allograft is to a great extent not known because of the possible host-versus-graft immunologic reaction. It is therefore important to shorten the ischemic period as much as possible, so as to permit complete recovery of the kidney from the ischemic damage, and also to allow such histocompatibility testing as lymphocyte cytotoxicity.[13] Both of the above time requirements were met by preserving excised kidneys in temperatures ranging from 0 to 2 C under high oxygen pressure. As the kidney suffers from irreversible damage at normal body temperature, it should be removed from the cadaver donor within one hour,[14] and the kidney cooled immediately by perfusion with refrigerated isotonic balanced electrolyte solution containing albumin or dextran and heparin.

Electron microscopy of kidneys cooled to 4 C for more than 12 hours showed swelling, pallor, and increased density of matrical granules of mitochondria in the proximal tubular epithelium, which could be correlated to the functional failures after autotransplantation.[15]

SUMMARY

Twenty-one sheep kidneys were preserved by hypothermia (2 C) and hyperbaric oxygen, and nine kidneys by hypothermia alone. Autotransplantation into the neck of the animal was performed in each case to evaluate the function of the preserved kidney. Preservation up to eight hours by either method yielded normal function of the kidney after autotransplantation and immediate contralateral nephrectomy. Hypothermia alone permitted storage of kidneys for eight hours at the longest, after which time hyperbaric oxygen had a definitely beneficial effect up to 12 hours. Kidneys stored longer than 12 hours did not produce adequate function to sustain life. The results of the tetrazolium viability tests correlated with the functional integrity of the stored kidneys and their period of preservation.

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