Henry Ford Hospital Medical Journal

Volume 18 | Number 2 Article 10

6-1970

In Vitro Kidney Preservation Techniques

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Henry Ford Hosp. Med. Journal
Vol. 18, No. 2, 1970

NOTES AND COMMENTS

In Vitro Kidney Preservation Techniques

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The advantages of adding hyperbaria, low flow perfusion, and protein colloid to hypothermia at 0° C for kidney preservation are described. The possibilities of longer preservation at higher temperatures are discussed.

The preferred method of handling the donor kidney during transplantation continues to be rapid cooling by flushing the kidney's vascular bed with cold solutions, then holding its temperature at 0° C by surface cooling. With certain adjuncts, this technique offers good preservation of renal function for eight hours. A longer period of reliable preservation would allow for transportation and wider use of potential human donor kidneys. Reports of successful 24-hour preservation, using continuous plasma perfusion and oxygenation at temperatures of 8° to 10°C,[1] have encouraged studies of these techniques and various modifications of them.

Hypothermic preservation in its simplest form consist of flushing the kidney with cold isomotic electrolyte solution such as Ringer's lactate. Heparin is added to prevent intravascular clotting. After the blood has been flushed from the vascular bed, the kidney is immersed in a partially frozen saline solution or "saline slush." It is stored in this solution for periods as long as six to eight hours until anastomosed to the vessels of the recipient. In our experience the kidney thus handled does not retain all of its potential function. Certain additives and adjuncts have improved the immediate response and final function of the kidney.

Table I

Agents added to initial kidney flushing solution for preservation at 0 C.

ADDITIVES	RATIONALE
Albumin	Normal oncotic pressure
Potassium Phosphate (or Chloride)	Reduce extra-intracellular ion gradients
Magnesium	To conserve mitochondrial ATP and DPN*
Phenoxybenzamine	Reduce arterial spasm
Glucose and Citrate	Oxidative substrates
Hydrocortisone Chlorpromazine (Thorazine)	Stabilize lysozome and cellular membranes

* Adenosine Triphosphate and Diphosphopyridine Nucleotide

Albumin or plasma is used to maintain a normal oncotic pressure gradient between the extra and intracellular compartments. This minimizes cellular swelling during hypothermia. This is better accomplished with albumin*

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This work was sponsored in part by The McGraw Foundation.

* In practice, concentrated "Albumisol" or other human albumin is added to the intracellular electrolyte solution to give a six gram percent protein solution.

than with dextran. Kidneys perfused with albumin solution at 0° C have an immediate uniform pink color when revascularized. On the other hand, kidneys perfused with dextran in an electrolyte solution have a persistent bluish cast and do not warm rapidly, indicating poor perfusion. Also, angiograms of the kidneys perfused with dextran indicate obstruction of small arteries, particularly in the medullary portion.[2]

Composition of the Perfusate

Potassium phosphate or chloride is added to reduce the intra-extracellular gradient of sodium and potassium. This is done to conserve the energy normally expended to maintain these gradients. Also, it has been shown that intracellular potassium is rapidly lost from cells into cold perfusing solutions.[3] Magnesium is added to reduce intracellular losses, to inhibit ATPase, and to minimize the loss of ATP and DPN from mitochondria. Free magnesium is also necessary for the activity of many respiratory enzymes. Phenoxybenzamine is a long acting alpha-adrenergic blocking agent used to prevent arterial spasm. Recent proponents for the use of phenoxybenzamine now advocate giving it systemically to a cadaver donor. The use of phenoxybenzamine, magesium, and potassium is taken from the recent work of Collins, Bravo-Shugarman and Teraski.[4] This "intracellular" solution has been used successfully as a flushing solution but not yet as a continuously flowing perfusate. Hydrocortisone and chlorpromazine are sometimes added to reduce the breakdown of lysozome and cell membranes. Glucose is added as an anaerobic supply of DPNH (hydrogen ions) through glycolysis. Citrate provides a Kreb's cycle substrate.

For preservation longer than two hours, intermittent low flow perfusion and hyperbaria are carried out with the simple Swenko* apparatus. Until now, this perfusate has been a saline base solution with only four mEq/l of magnesium and no added potassium. A flow of 200 milliliters per hour under three atmospheres of oxygen delivers 8 milliliters of dissolved oxygen. This is more than is used by the human kidney of average weight at 0°C to 1°C. Aside from increasing oxygen delivery, hyperbaria somehow extends preservation through its pressure effect, possibly by minimizing cellular edema. The slow flow of perfusate also supplies constant substrate and lowers the very high PCO_2's which occur. The uniformly better results with perfusion and hyperbaria suggest that the kidney at 0°C is capable of respiration and sufficient energy production to keep its molecular organization intact. Figure 1 shows a typical response of the BUN in a patient after receiving a kidney stored under hyperbaria.

Current Techniques

During the 20 to 40 minutes of vascular anastomosis, the kidney is gradually warming toward the temperature in the operative field. The greatest temperature increase is evidenced in the kidney cortex, which normally has the highest metabolic rate. To prevent this warming during the ischemia period, a double wall silastic kidney cooling jacket has been designed. This allows for the circulation of cold solution around the kidney during anastomosis.

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* Swenko Incorporated, Minneapolis.

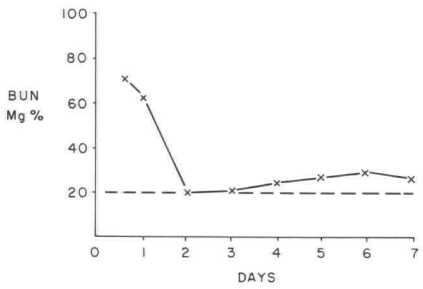

Figure 1

Chart shows the ability to clear urea of the donor kidney stored under hyperbaria.

Its ability to preserve function in the transplanted kidney is being evaluated in the laboratory. A human size cooling jacket* has been used during transplant of one cadaver (five hours total storage) and one living related kidney donation. Both patients had receive Mannitol. There was prompt diuresis of over a liter during the first hour. Blood urea nitrogen and serum creatinines were normal within 36 hours and both kidneys later had above normal creatinine clearances.

The use of the "intracellular" solution before storage at 0°C[4] for a period of 24 hours has not been uniformly successful in the laboratory. After autotransplantation, these canine kidneys perfused well and produced urine immediately. However, even the best results show moderately elevated BUNs and creatinines which began to climb rapidly after five to seven days. The final specimens showed diffuse parenchymal damage.

Perfusion of the kidney at higher temperatures appears to be the method which is now evolving for longer preservation. Figure 2 outlines a system incorporating oxygenation and dialysis of the perfusate. In this Extracorporeal circuit only cryoprecipitated plasma, after the method of Belzer,[1] or heavily citrated plasma or serum will flow through the kidney for 24 hours or more. In our experience microclotting is an important factor in the vascular obstruction which is prone to occur. Also, perfusion pressures over 50 to 60 millimeters of mercury are associated with increasing edema and resistance to flow. Generally, low pressures and low temperatures (less than 8°C) yield the best perfusion.

Effects of Temperature

Temperature seems to be the most important variable in these experiments. With plasma perfusate, the temperature is limited to less than 14°C because plasma alone cannot transport the larger volumes of oxygen required at higher temperatures. This narrow range, 6°C to 14°C, has the disadvantage of retarding respiration and

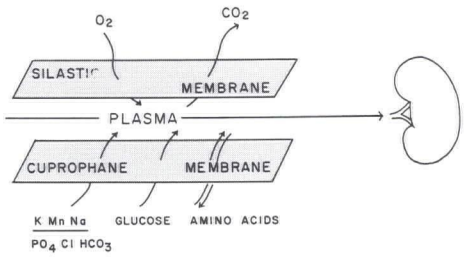

Figure 2

Diagrammatic presentation of the method for perfusing kidneys at 10°C. The perfusate is serum or more commonly cryoprecipitated plasma which is continuously oxygenated and dialyzed.

* Extracorporeal, New Jersey.

phosphorylation and yet does not give the full stabilizing effect of keeping these systems near freezing. This compromise may prove to be the limiting factor in the method.

Current experiments are designed to measure respiration by oxygen consumption while varying the temperature. The temperatures will be carried to between 20°C and 28°C by adding red cells or free hemoglobin to the perfusate for increased oxygen transport.

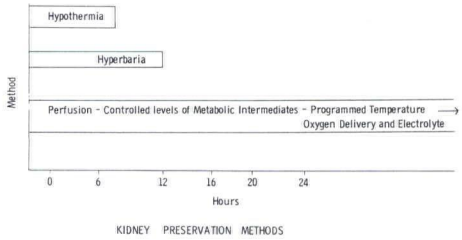

Figure 3

Effective preservation time which can be achieved with different techniques. Perfusion at physiological flow rates and moderately reduced temperatures appears to be the most promising method for reliable preservation beyond 12 hours.

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Henry Ford Hospital
Detroit, Michigan 48202

Printed in U.S.A.

Vol. 18, No. 2

HENRY FORD HOSPITAL MEDICAL JOURNAL