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Daniel Dall'Olmo

Stanley G. Dienst

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Prolonging oxygen consumption during preservation of canine kidneys by the addition of continuous dialysis

Daniel Dall'Olmo* and Stanley G. Dienst, MD**

Kidneys were preserved by continuous pulsatile perfusion and their metabolic rate monitored by O_2 consumption. Experiments were carried out to determine if dialyzing the perfusate to supply metabolic substrates would aid in kidney preservation. A control group was compared against two experimental groups at 25°C. Average preservation time before fall off of oxygen consumption differed for each group: control – 8 hours, dialysis with amino acids – 6 hours, dialysis with amino acids and cofactors – 11 hours. Results indicated that, if suitable concentrations of metabolic substrates and cofactors were used, the preserved kidneys maintained higher levels of O_2 consumption for longer periods of time.

* Summer Research Fellow in Renal Preservation Laboratory

** Department of Surgery, Section of Transplantation

Address reprint requests to authors at Henry Ford Hospital, 2799 West Grand Boulevard, Detroit MI 48202

NUMEROUS experiments have been undertaken to extend the period of organ preservation by continuous pulsatile perfusion. Variations in the temperature and composition of the perfusate have been investigated.[1,2,3,4] To date, the perfusate recommended by Belzer et al (from 5-7°C) yields the most consistent results.[5] Using the same perfusate, this study seeks to determine if continuously dialyzing the perfusate will extend the period of organ preservation.

Method

Female mongrel dogs weighing between 14 and 24 kg were anesthetized with sodium pentobarbital (40 mgm/kg) and intubated prior to surgery. Through a midline abdominal incision, one kidney was removed, placed in an ice bath, and flushed with cold heparinized Ringer's lactate. After flushing, the kidney was immediately placed on the MOX-100 Renal Preservation Console. The total ischemia time was always less than two minutes. The pressure, PO_2, and pH of the arterial and venous perfusates, as well as the flow and temperature, were measured at intervals of 15-30 minutes.

The renal vein was cannulated with a large cannula (16.6 mm^2) for the purpose of measuring the partial pressure of oxygen. The oxygenation of the plasma was maintained by a Lande-Edwards Membrane Oxygenator (1M^2 surface area). The perfusate was cryoprecipitated canine plasma prepared by the Belzer method.[5] A diagram of the perfusion circuit is shown in Figure 1. Three types of tests were performed during the course of these experiments.

A. *Determination of oxygen consumption as a function of change in temperature.*

Starting the perfusate at 5°C, oxygen consumption measurements of each kidney were made for each 5° increment rise in the temperature of the perfusate until 37°C was reached. For every five degree increase in temperature, the arterial and venous PO_2 levels were allowed to stabilize for ten minutes before readings were taken. The pressure ranged from 60 mm/Hg at 5°C to 100 mm/Hg at 37°C. This was done to increase flow and provide the additional oxygen required at higher temperatures.

B. *Determination of O_2 consumption measured at a constant temperature–controls.*

Several perfusions were carried out at the selected temperature of 25°C while monitoring O_2 consumption. This was plotted against the duration of the perfusion period in hours.

C. *Dialyzing perfusate at 25°C.*

As in B, oxygen consumption was recorded throughout the procedure. In this case continuous counter current dialysis of the perfusate was carried out through a commercial dialyzer.* Two dialysates were prepared. The first was an electrolyte solution prepared from a clinical dialyzing concentrate** plus dextrose 400 mgm%, sodium citrate 12 meq/L, and an amino acid hydrolysate*** 0.25% final concentration. The remaining volume was made up with sterile water. The pH of the dialysate was brought to 7.4 at 25°C by adding disodium phosphate.

The second dialysate was a solution of tissue culture media**** (diluted 10:1 with sterile water) and sodium citrate added 6 meq/L. A dialysate pH of 7.4 was achieved by adding 2.2 grams per liter of sodium bicarbonate.

*Cordis Dow Artificial Kidney

**Renalyte 45/Cobe—Final concentration: Na 140 meq/L, C1 106 meq/L, Ca 3.5 meq/L, K 3.0 meq/L, Mg 1.5 meq/L, and Acetate 42 meq/L

***Amigen—Baxter: 5% protein (Casien) hydrolysate. Bisulfite as preservative.

****Eagle's 199—Grand Island Biological—20 amino acids, 18 vitamins, nucleotides, purines, and pyrimidines, dextrose and ribose and inorganic salts in Ringer's bicarbonate and Ca 1.3 meq/L and iron.[6, 7]

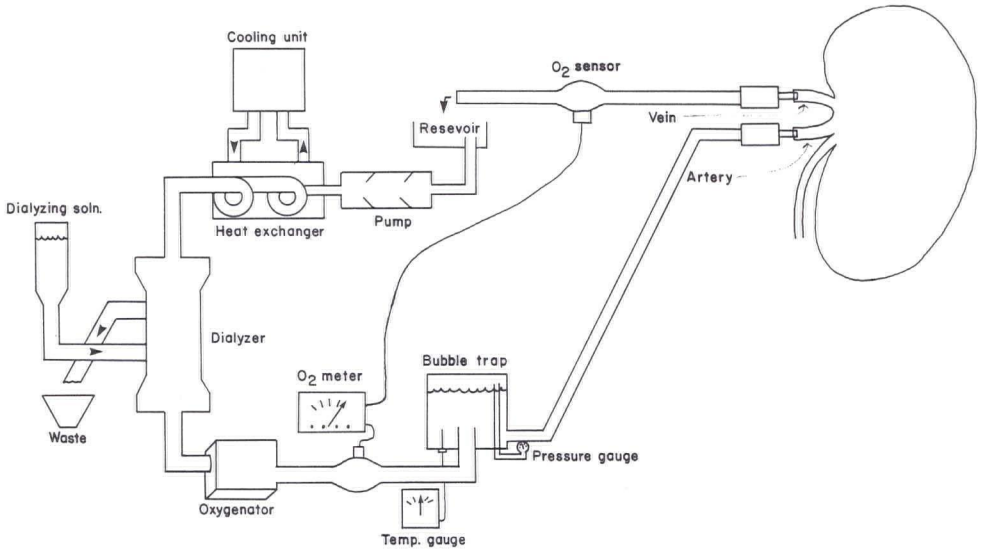

Figure 1
Kidney Perfusion Circuit

Results

Experimental groups:

Group A – All kidneys showed an increase in oxygen consumption with increasing temperature. Results of oxygen consumption as a function of temperature are shown in Figure 2.

Group B (Control) – In this group kidneys maintained at 25°C had constant oxygen consumption for six to seven hours after which the flow and A-V oxygen difference and subsequently the oxygen consumption decreased.

Group C – Dialysis of the perfusate with the electrolyte solution (plus amino acids) did not extend the period of oxygen consumption. Continuous consumption was present for only five hours. The perfusate flows in this experiment remained constant as A-V oxygen differences narrowed. On the other hand, dialysis of the perfusate with Eagle's solution gave longer sustained oxygen consumption as compared to controls. With the Eagle's, constant oxygen consumption and plasma flow was maintained for eleven hours. The A-V, PO_2 difference was sustained for a time even after flow decreased. It was only after the flow decreased (below 20 cc/min) that the A-V, O_2 difference disappeared. Table 1 shows the effect of the two dialysates on the length of oxygen consumption.

Discussion

Since the acceptance of cryoprecipitated plasma as a perfusate for kidney preservation, modifications of this solution have been studied in an effort to extend reliable preservation time. Tests have been made on the effect of substituting fresh plasma perfusate every 12 hours,[2] using silica gel treated plasma,[4] or using the albumin fraction (purified protein fraction) of plasma[3] as the perfusate. The objective of our study is to determine the length of effective metabolic preservation as influenced by continuously dialyzing the cryoprecipitated plasma perfusate during kidney preservation. The theoretical basis of this approach is to replenish and stabilize substrate and soluble cofactor concentrations for the actively metabolizing kidney cells. There is evidence that a lack of essential energy substrates can lead to irreversible tissue damage during organ preservation.[1, 2] Among the substrates implicated are unesterified fatty acids[2] (the preferred substrate for energy production in the kidney

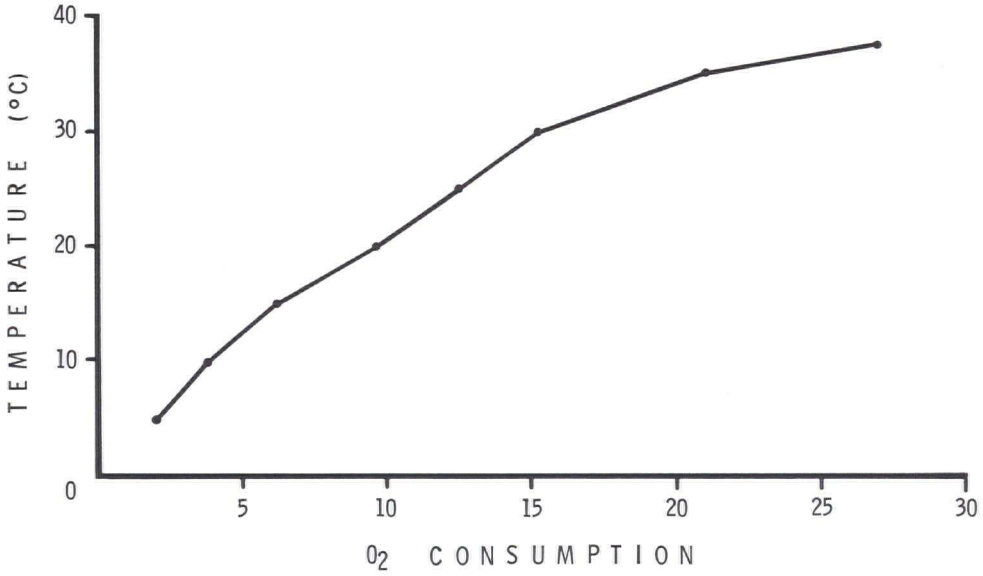

Figure 2
Changes in oxygen consumption (m-moles/hr/grm) as a function of increasing temperature (°C).

TABLE I

Dialysate	Number of Experiments	Oxygen Consumption (average) (m-moles / gm x hr) Hour										
		1	2	3	4	5	6	7	8	9	10	11
None	2	18.2	16.4	16.7	16.1	16.1	17.3	16.0	10.0	6.0	—	—
Electrolyte solution plus Dextrose, Amigen and Citrate	5	37.0	36.7	26.6	20.5	19.5	6.1	4.1	2.0	—	—	—
Eagle's Tissue Culture Medium (199) plus Citrate	6	18.0	17.5	16.6	15.4	21.4	17.7	19.1	20.9	20.0	15.8	9.3

Changes in oxygen consumption over time comparing the two dialysates to the control group (without dialysis)

cortex), glycerol, and glucose. Another theoretical advantage favoring the incorporation of dialysis is the removal of metabolic end products. The kidney produces metabolites that accumulate during continuous perfusion.[1, 2, 8] One of these, lactic acid, increases the acidity of the perfusate and also leads to an increase in the production of ammonia.[2, 8] The increase in the concentration of metabolic products and an ultimate decrease in the oxidizable substrates may be limiting factors in extending kidney preservation.

Oxygen consumption was chosen as a measure of metabolic activity due to this event being the final step in cellular respiration. When oxygen is consumed, it indicates that the respiratory chain is intact. The arterial-venous oxygen difference is a direct measure of how rapidly respiration is being carried on. Therefore, oxygen consumption was used as a measure of kidney metabolism. When the temperature was increased, the oxygen consumption also increased. These results coincide with previously published data.[9]

To measure accurately the effects of dialysis, the temperature of 25°C was selected. At the usual 5-8°C, the activation energy of biochemical reactions is very high. Even in the presence of enzymes, they proceed very slowly. The activities of many of the glycolytic and tricarboxylic acid cycle enzymes particularly are reduced or arrested at lower temperatures.[10-12] Therefore, a temperature was selected at which enzyme activities would be close to normal. Looking at the glycolytic and respiratory enzymes together, the largest change in their activity (noted by Q^{10}) is 20-30°C. Therfore, 25°C was chosen. This temperature would provide sufficient activity to use substrates quickly if the substrates were to be the limiting factor. Table I shows the differences in the length of the preservation period between controls and the two dialysis groups. This suggests that the enzymes were more active at this elevated temperature and substrate concentration was a determining factor. By maintaining the substrate concentration, as the Eagle's solution does, metabolic activity is prolonged and therefore oxygen consumption is obtained for longer periods of time.

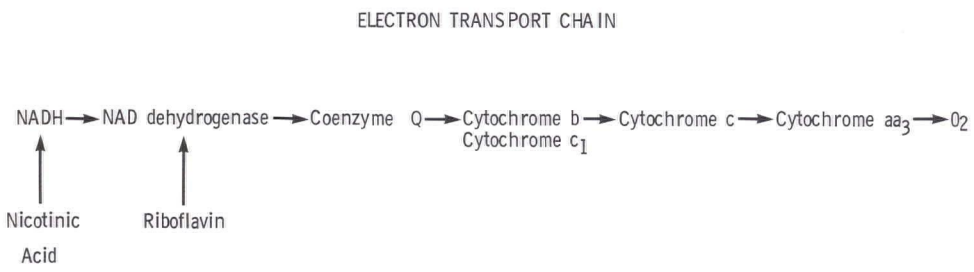

Figure 3
This figure indicates positions of sustained cofactor concentrations along an electron transport chain.

The effect of dialysis differed depending on the dialysate used. With the electrolyte solution, failure to achieve longer preservation could be due to loss of components such as glucose, adenosine, vitamins, and other soluble compounds into the dialysate. On the other hand, longer preservation periods were realized by dialyzing with Eagle's 199 solution. This could be due to a sustained concentration of compounds required for respiration. For example, the solution maintained concentrations of cofactors and substrate such as biotin (which functions in the decarboxylation of pyruvate), nicotinic acid (which is a precursor of NAD), and riboflavin (the precursor of FAD and FMN). These are all necessary for cell respiration. Deficiencies in these would result in lowered cell respiration. Another molecule whose concentration was maintained in the perfusate by dialysis was ATP. Figure 3 shows the sequence from NADH to oxygen in the electron transport chain. Positions where cofactors were sustained by dialysis are indicated by arrows. Maintaining the cofactor and substrate concentrations by dialysis with Eagle's 199 solution resulted in prolonged oxygen consumption. Therefore, it is suggested that maintenance of a relatively high concentration of cofactors as well as oxidizable substrate may be important in extending safe organ preservation.

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