X. COMMUNICATIONS BIOPHYSICS

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V. Tom
L. Tung

A. NORMOTHERMIC PERFUSION OF CANINE KIDNEYS — THE EFFECT OF PERFUSATES

C. H. Conrad, R. G. Mark

[With B. P. Kekis, C. D. Derry, and M. Slapak, Sears Surgical Laboratory, Boston City Hospital.]

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†Also at the Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Cambridge, Massachusetts.

‡On leave — Department of Biophysics, University College, London.

**Also Instructor in Medicine, Harvard Medical School, Boston, Massachusetts.

††Also Instructor in Preventive and Social Medicine, Harvard Medical School, Boston, Massachusetts.

‡‡Research Affiliate in Communication Sciences from the Neurophysiological Laboratory of the Neurology Service of the Massachusetts General Hospital, Boston, Massachusetts.

***Visiting Scientist from Institute for Perception Research, Eindhoven, The Netherlands.

†††Visiting Scientist from University of Stuttgart, Germany.

‡‡‡Department of Physiology, Harvard University, Cambridge, Massachusetts.

1. Introduction

Although hypothermic perfusion is now used as the basis of all long-term methods of organ preservation, a period of seven days is the longest for which preservation has been successfully achieved.[1] By using intermediate living hosts, perfusion at 37°C with whole blood has allowed kidney preservation for periods of more than three weeks.[2] At present, however, limitations imposed by the materials used for artificial preservation systems prohibit the perfusion of organs with whole blood for periods exceeding more than a few days, because of phenomena such as cell aggregation and hemolysis.

In a recent study[3] it was shown that a 25°C 24-hour kidney perfusion could be successfully achieved with life supporting function after transplantation by the use of either platelet-poor or platelet-rich plasma. The advantages of plasma as a perfusate in a normothermic system are obvious. It contains many of the substrates required by the kidney and its proteins confer appropriate buffering and osmotic properties. The use of plasma also eliminates problems of hemolysis and sludging. Although protein denaturation might still be damaging, the use of a membrane oxygenator and the minimization of the air-plasma interface area might minimize this problem. Inherent in the use of plasma as a perfusate at 37°C, however, is the question of whether it is capable of carrying enough oxygen to support a normothermic kidney. Theoretical consideration of the oxygen consumption and blood flow of normal kidneys in vivo would suggest that it is. At the normal flow rate of 3-4 ml/gram of kidney tissue/minute, a very small arteriovenous oxygen difference is found,[4] of the order of 2 ml/100 ml as compared with 4 ml/100 ml for systemic venous blood. Since it has been shown[5] that renal oxygen consumption during normal sodium filtration is of the order of 0.08 ml/gm/min, the 2 ml/100 ml of oxygen dissolved in oxygen-saturated plasma[6] might be enough to support the kidney, provided that sufficiently high flows could be maintained. Thus, by using a 97% oxygen 3% CO_2 mixture and flow rates of 3-4 ml/gm/min, an adequate supply of oxygen should theoretically be available.

The purpose of the present study, then, was to ascertain whether or not a normothermic kidney could be supported by using plasma as the perfusate. The methods and results have been described in detail elsewhere.[7]

2. Method

The experiment involved perfusion of kidneys from 19 apparently healthy 10-20 kg mongrel dogs. Three groups of 3-4 perfusions were done. Group A (9 kidneys) was perfused with whole blood, Group B (4 kidneys) with low hematocrit blood (4-7%), Group C (6 kidneys) with plasma. Anesthesia was induced with sodium thiamylal at an approximate dosage of 20 mg/kg, and additional thiamylal was given intravenously as needed. After intubation, a midline incision was

made, and the left kidney was dissected free after infiltration of the surrounding tissue with 5% Cyclaine (topical hexylcaine hydrochloride, 50 mg/ml). The artery, vein and ureter were freed and the ureter divided and cannulated with polyethylene tubing.

The dog was maintained in a diuretic state with a fluid load of 5% dextrose in normal saline or 25% mannitol in 5% dextrose in normal saline. The renal artery and vein were then clamped and divided and both cannulated with siliconized glass cannulae. The kidney was then weighed and transferred to the perfusion apparatus while the donor animal was maintained under light thiamylal anesthesia until reimplantation.

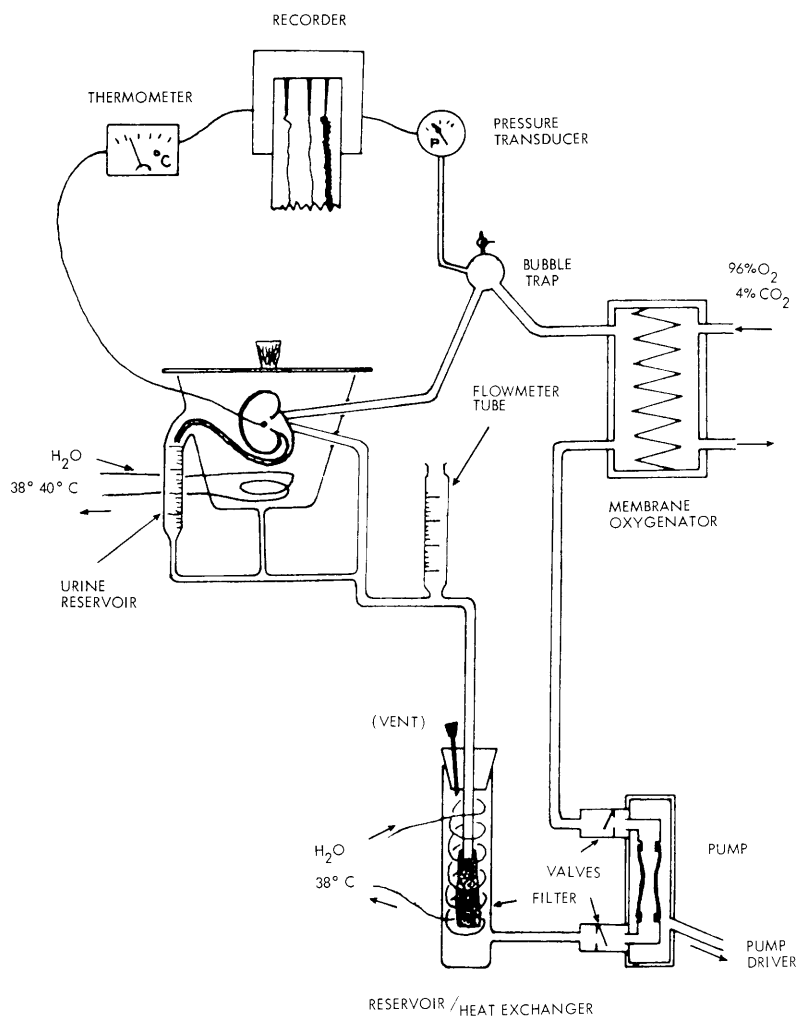The perfusion circuit is shown schematically in Fig. X-1. It included a pulsatile

Fig. X-1. The perfusion system. The silastic pump is driven by a pneumatic oscillator which has been described previously.[8]

pump, membrane oxygenator (silicone rubber), bubble trap, organ chamber, and venous reservoir/heat exchanger with an incorporated filter. The pump was a silicone rubber ventricle with one-way valves, driven by a pneumatic oscillator with adjustable systolic and diastolic intervals and pressures similar to the one described by Slapak et al.[8] The oxygenator was a Travenol 5M0321 membrane oxygenator ventilated by 97% oxygen with 3% CO_2 or 96% oxygen with 4% CO_2. There was a bubble trap in the arterial line, and a graduated tube in the venous line which made possible direct measurement of flow by occlusion of the venous line between the graduated tube and the reservoir. The venous return was filtered by a blood administration set filter as it entered the reservoir. The organ rested in a glass organ chamber covered by a moist sponge and supported by a silicone rubber coil through which water at 38°C was circulated. Urine was collected in a graduated reservoir and could be returned to the venous line if desired. The venous reservoir contained a glass coil through which 38°C water was circulated, to serve as a heat exchanger. The priming volume of the apparatus was approximately 200 ml.

The arterial perfusion pressure and organ surface temperature were recorded continuously. Flow, arterial and venous pH, PCO_2 and PO_2 were monitored periodically, as were hematocrit, plasma glucose, and urine flow. Samples of perfusate and urine were also taken periodically for determination of osmolarity, sodium, potassium and creatinine. After termination of perfusion, the kidney was flushed with cold lactated Ringer's solution and reweighed. It was then reimplanted by end-to-end anastomosis of the renal artery to the external iliac artery and end-to-side anastomosis of the renal vein to the external iliac vein. Total ischemic times were from 25 to 35 minutes. The ureter was implanted directly in the bladder and contralateral nephrectomy was performed immediately following the implantation. Postoperatively, blood samples were drawn every few days for measurement of BUN, serum creatinine, sodium, potassium, and osmolarity. Since in all groups venous PO_2 was above 150 mm Hg, a value at which hemoglobin remains fully saturated, oxygen consumption was calculated from the dissolved oxygen alone by using the formula

$$O_2 \text{ CONSUMPTION (ml/gm/min)} = \text{FLOW (ml/gm/min)} \times \frac{\Delta O_{2_{AV}} \text{ (ml/100 ml)}}{100},$$

where FLOW is the perfusate flow, and $\Delta O_{2_{AV}}$ is the arteriovenous oxygen difference, given by

$$\Delta O_{2_{AV}} \text{ (ml/100 ml)} = \frac{\left(PO_{2_{art}} - PO_{2_{ven}}\right)}{760} \times \alpha \text{ (ml/100 ml/atm)}$$

where α is the solubility of oxygen in plasma as given by Sendroy et al.[6] The solubility was corrected for temperature and for hematocrit, since α_{cells} is greater than α_{plasma}.

Table X-1. Viability after perfusion, and oxygen consumption, creatinine clearance, and sodium reabsorption during perfusion of the perfused kidneys. Note in Groups A, B, and C that 6, 2, and 2 kidneys, respectively, were not reimplanted or function was not evaluated because of unrelated technical failure.

	Dog No.	Function after Reimplantation	Oxygen Consumption (ml/gm/min)	Creatinine Clearance (ml/100 gm/min)	Sodium Reabsorption (μEq/gm/min)
PLASMA (Group C)	6873	—	.024	—	—
	6711	—	.020	—	—
	6890	U	.035	—	—
	6924	U	.038	2.7	3.7
	6946	U	.021	3.6	4.7
	6760	U	.041	—	—
	MEAN ± S.D. (n)		.030 ±.009(6)	3.1 ±.7(2)	4.2 ±.7(2)
LOW HEMATOCRIT (Group B)	6977	—	.039	20.2	29.0
	6206	—	.049	44.3	63.5
	6978	S	.043	17.1	24.4
	6990	U	.041	—	—
	MEAN ± S.D. (n)		.041 ±.008(4)	27.2 ±(3)	39.0 ±21.4(3)
WHOLE BLOOD (Group A)	6856	—	.050	—	—
	6860	—	.042	—	—
	6875	—	.038	—	—
	6872	—	.039	—	—
	6903	—	.028	—	—
	6925	—	.037	19.0	28.3
	6885	S	.025	5.5	7.9
	6945	S	.040	16.8	25.0
	6966	S	.034	12.3	17.3
	MEAN ± S.D. (n)		.037 ±.007(9)	13.4 ±6.0(4)	19.6 ±9.1(4)

Notes: — No reimplant, or function not evaluated.
S Survival
U Failure (uremic).

2. Results

Results are summarized in Table X-1.

a. Viability

None of the 4 kidneys perfused by plasma were functional after reimplantation. Futhermore, at reimplantation, these kidneys were dark and sometimes mottled. They
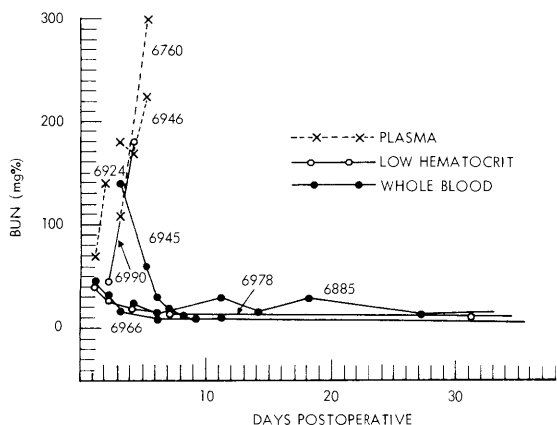

Fig. X-2.

Postoperative renal function after reimplantation for dog numbers indicated, measured by blood urea nitrogen values. Serum creatinine values not shown were similar in their behavior.

produced little, if any, urine. In contrast, all of the three kidneys perfused by whole blood and reimplanted regained normal color and were immediately life-sustaining; the animals were sacrificed some months later with normal renal function, as shown in Fig. X-2. One of the two kidneys perfused with low hematocrit blood was life-sustaining.

b. Oxygen Consumption and Other Results

There were no statistically significant differences in the oxygen consumption of the three groups. Figure X-3 shows the relationship between Na reabsorption and O_2 consumption in the three groups. There was, however, a statistically significant difference ($P < .05$) in favor of the whole blood and low hematocrit groups for sodium reabsorption and creatinine clearance, although all groups showed impaired filtration. It was found in all three groups that renal venous PO_2 during perfusion was greater than 150 mm Hg, arterial PO_2 averaging approximately 560 mm Hg.

3. Discussion

The data indicate that inadequate oxygen supply or consumption does not seem to provide a convincing explanation of the striking difference in the viability between the plasma- and whole blood-perfused kidneys, although there is a possibility that failure of
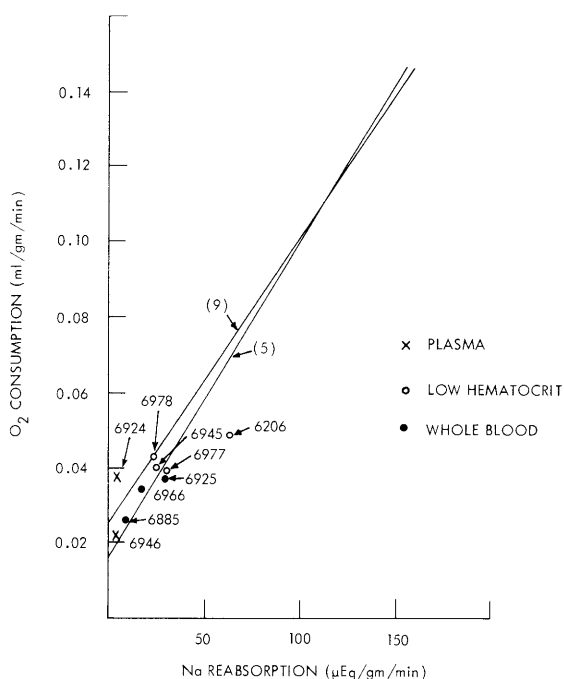
Fig. X-3. Oxygen consumption and sodium reabsorption of perfused kidneys. Note that although oxygen consumption is not significantly different in the three groups, Na reabsorption values in the plasma group (shown by X's) are much lower. The straight lines indicate the relations obtained by other researchers.[5, 9]

the plasma-perfused kidneys is related to oxygen supply or consumption. Renal cortical PO_2 is generally[10] below renal venous PO_2 and localized areas of hypoxia might exist even with a high venous PO_2. The presence of intrarenal "shunt diffusion" has been proposed to explain this effect,[11] although limited oxygen diffusion at the capillary level would produce the same effect. These effects would allow oxygen to bypass respiring tissue, and make the organ incapable of extracting all seemingly available oxygen. A shunt diffusion effect would be most significant with high arterial PO_2 because of the large gradient for diffusion that is thus created.

Thus, since the venous PO_2 was 150 mm Hg in all groups, the possibilities are (i) there was more than adequate oxygen; (ii) the oxygen could not reach the renal parenchyma because of a shunting effect; and (iii) oxygen diffusion at the capillary level was inadequate, or the cells were unable to utilize the available oxygen. Even if the plasma-perfused kidneys in these experiments did have adequate oxygen, the low creatinine clearances leave open the possibility that a normally filtering kidney perfused with plasma would become hypoxic as a result of increased sodium load and the consequent need for oxygen to provide energy for sodium reabsorption.

Work done by Gimbrone et al.[12] suggests that platelets might play a role in the maintenance of vascular endothelium. In their studies, organs perfused at 37°C showed diminished function and increased endothelial damage in the absence of platelets. In our experiment, platelets were present in Group A (whole blood) and Group B (low hematocrit), and the lack of platelets in Group C (plasma) might be responsible for the observed failure in this group. Although kidneys perfused with platelet-rich and platelet-poor plasma at 25°C did not demonstrate advantages of platelet-rich plasma,[3] results might differ at 37°C. Further experiments including a platelet-rich plasma group would be necessary to test this possibility.

4. Conclusion

These data show that perfusion with plasma at 37°C does not produce life-supporting function, whereas perfusion with whole or diluted blood can. The cause for the failure of the plasma-perfused group seems to be due to a fact or factors other than oxygen. The absence of platelets is one of the possible factors.

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