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Metabolic Evaluation of Preserved and Reinserted Canine Kidneys

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Twenty-four hour preservation of kidneys has been achieved without perfusion, and in some instances without oxygen in a hypothermic hyperbaric environment (Groenewald et al., 1969). These experiments were designed to evaluate the survival of the preserved kidneys and the success of the preservation techniques.

MATERIALS AND METHODS

Kidneys were obtained from healthy adult male and female mongrel dogs housed and cared for at the Department of Experimental Surgery, Roswell Park Memorial Institute. Kidneys were preserved and subsequently reinserted into their original host. All kidneys were preserved in a Swenko² hyperbaric hypothermic chamber at 3 atm. and 4° C (Hesse et al, 1969). With the exception of the initial washout of blood after removal, none of the kidneys was perfused. The washout was accomplished with test solution with the addition of 2,000 U Heparin and one cc 1% procaine. The test solution was either defibrinated dog plasma or defibrinated plasma to which a respiratory stimulating extract, procytoxid (PCO)³ was added (Murphy et al, 1969). This agent is known to stimulate respiration of tissue slices of liver and kidney in vitro (Cook and Walter, 1941).

During the preservations, the chamber was pressurized with either 100% oxygen or 100% helium gas. After completion of the preservation period, the kidneys were reinserted into the host animals. Contralateral kidneys were removed 14 days later.

At the time of reinsertion, seven days after reinsertion, and at the time of contralateral nephrectomy, open renal biopsies were obtained for manometric studies of renal cellular respiratory activity (Melby et al, 1968; Murphy and Schirmer, 1966). Control biopsies of unpreserved kidneys from normal dogs were also obtained.

Biopsy specimens were immediately put into iced saline and were kept on ice until the reaction flasks were attached to the

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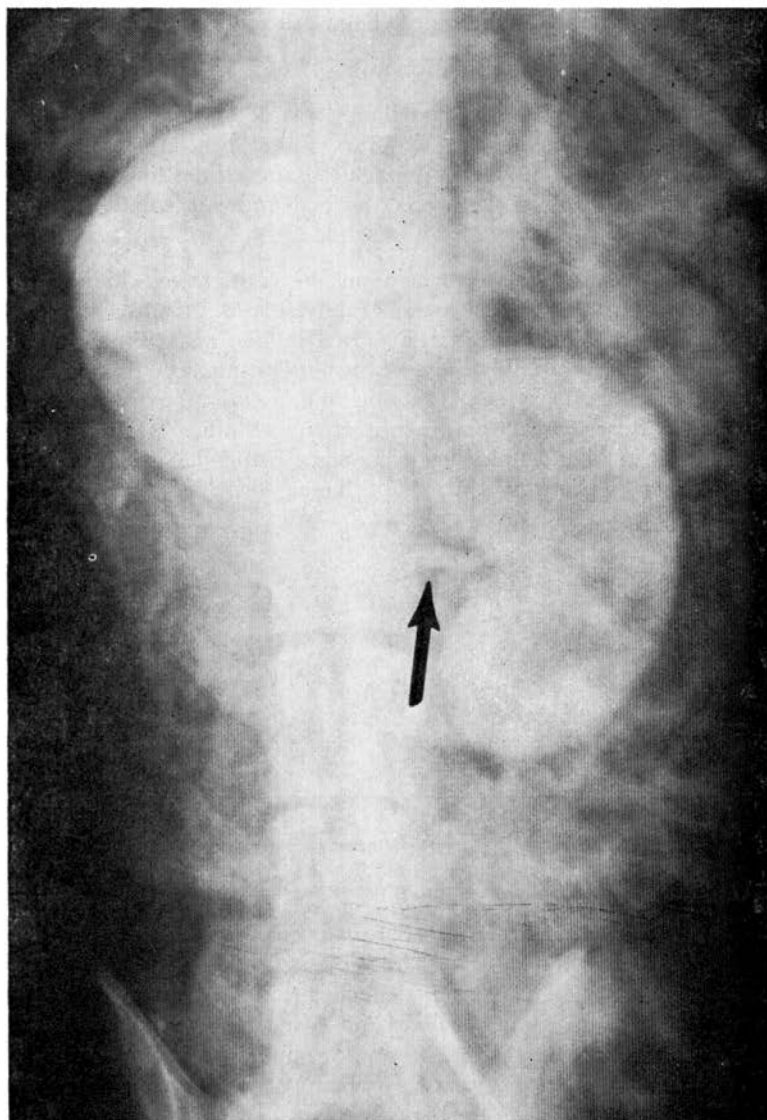


Figure 1. The early renal angiographic appearance of PCO/oxygen preserved kidneys. Good perfusion at 3-4 days after reinsertion correlated well with survival after contralateral nephrectomy. The arrow points out the renal artery.

manometric apparatus. Any remaining capsular material was removed from the biopsy specimens and the cortex was separated from the medulla. Slices of cortex and medulla were made with a

Stadie-Riggs hand microtome. These slices were suspended in Krebs-Ringer phosphate solution and $Q_{O_2}^0$ ($\mu\uparrow 1\downarrow$ oxygen consumed per mg dry weight of tissue per hour) was determined using standard manometric techniques (Umbriet et al, 1964). All manometric determinations were made in duplicate. Paired slides for histological assessment of the biopsies were obtained and studied under light microscopy.

Additional studies performed on the autografted kidneys included retrograde femoral arteriography, Figure 1, and ^{131}I orthoiodohippurate renograms, as illustrated in Figure 2. These studies were particularly useful prior to contralateral nephrectomy in evaluation of individual renal function and as an aid to prognosis of survival after contralateral nephrectomy. Studies performed on periodic peripheral blood specimens included: erythropoietin (ESF) assay (Murphy et al, 1970), hematocrit, hemoglobin, blood

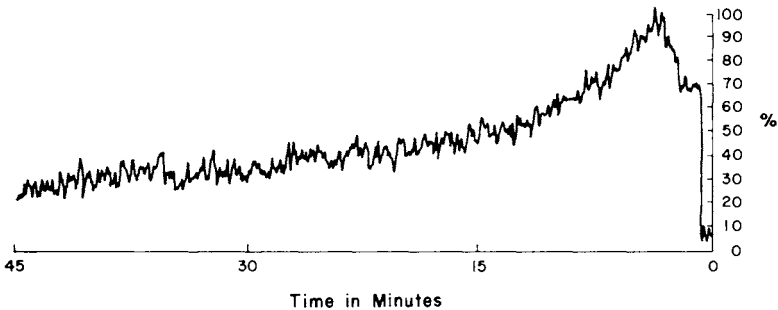


Figure 2. Radioisotopic renograms enable the repeated and early assessment of renal plasma flow. These studies correlated with renal perfusion and survival after contralateral nephrectomy.

urea nitrogen (BUN), total leukocyte count, total erythrocyte count, and renin assay (Woods and Michelakes, 1968). Periodic endogenous creatinine clearances were obtained on many animals. Femoral blood pressure was determined at the time of surgery, and at other intervals during the experiments.

RESULTS

Survival—Table 1 illustrates the survival rates of eight experimental preservation groups. The last four of these groups are the object of this report.

It can be seen from this table that PCO was associated with improved survival in all cases where it was used. Dogs with plasma oxygen PCO preserved kidneys enjoyed the highest survival rate of

TABLE 1. RENAL PRESERVATION RESULTS

Group	Preservation Fluid at 4°C	Gaseous Environment	Additives	No. Done	Survived Contralateral Nephrectomy		Successfully Preserved	
					#	%	#	%
1	Tis-U-Sol	Oxygen	None	7	6	85.7	2	28.6
2	Tis-U-Sol	Helium	None	8	7	87.5	3	38.5
3	Tis-U-Sol	Oxygen	PCO	9	7	77.8	7	77.8
4	Tis-U-Sol	Helium	PCO	8	7	87.5	6	75.0
5	Plasma, defibrinated	Oxygen	None	10	10	100.0	6	60.0
6	Plasma, defibrinated	Oxygen	PCO	10	7	70.0	7	70.0
7	Plasma, defibrinated	Helium	None	4	2	50.0	1	25.0
8	Plasma, defibrinated	Helium	PCO	6	2	33.3	2	33.3
TOTAL:				62	48	77.4	36	58.1

the four groups. The poorest survival rate was seen with those animals with plasma helium (PCO non-treated) preserved kidneys. These survival rates range from 70% for the plasma oxygen PCO group to a low of 25% for the plasma helium preserved group.

Biochemical, Hematological Alterations—None of the animals exhibited anemia (Hct < 30 vol %) postoperatively. Hemoglobin and erythrocyte counts remained normal. A transient leukocyte elevation was present in all treatment groups. A transient rise in BUN was noted following reinsertion. Moderate elevations of BUN followed contralateral nephrectomy. Maximal BUN increases, ranging from 46 mg % to 61 mg % in all groups, were evident for several days following contralateral nephrectomy. Animals with successfully preserved kidneys had reductions to normal levels (< 20 mg %) following this period.

Erythropoietin, Renin Levels—Erythropoietin (ESF) is released from the canine kidney in response to hypoxic stimuli. Successfully preserved kidneys, Table 2, exhibited some elevations in ESF levels, but to a lesser degree than unsuccessfully preserved kidneys. From 1 to 7 days after reinsertion, maximum elevation of ESF was noted. From 7 to 14 days after reinsertion, ESF levels had declined, and the levels of PCO treated groups were lower than those of PCO non-treated groups. At 15 days and longer after reinsertion, ESF levels were much lower. PCO treated groups displayed lower levels than PCO non-treated groups. Oxygen preserved groups had levels lower than helium preserved groups. As shown in Table 3, long term survivors with PCO preserved kidneys had persistently lower ESF levels, reflecting an apparent lack of major hypoxic stimuli.

Renin, a component of a complex pressor mechanism, is released from the kidneys under the influence of reduced pulse pres-

TABLE 2. ERYTHROPOIETIN (ESF)* LEVELS AFTER 24 HOUR RENAL PRESERVATION

Group	Pre-reinsertion	Days after Reinsertion		
		1-7	7-14**	15+
Plasma + Oxygen	0.44 ± 0.26	1.05 ± 0.08	1.87	1.19 ± 0.31
Plasma + Helium	0.95 ± 0.09	9.28 ± 6.26	3.67 ± 1.34	1.46 ± 0.22
Plasma + Oxygen + PCO	0.65 ± 0.14	2.20 ± 0.43	1.04 ± 0.10	0.30 ± 0.06
Plasma + Helium + PCO	0.85 ± 0.04	1.54 ± 0.22	1.33 ± 0.45	0.72 ± 0.23

*24 hour ⁵⁹Fe % uptake.

All values are mean ± 1 S. E.

**Contralateral nephrectomy on 14th day.

TABLE 3. ERYTHROPOIETIN (ESF) LEVELS* IN LONG SURVIVING DOG WITH PRESERVED KIDNEY.**

Pre-reinsertion	Days after Reinsertion					
	1-7	7-14	15-30	30-59	60-89	90-119
0.88	1.02	0.77	0.97	0.63	0.71	1.64
	±0.26	±0.01	±0.11	±0.17	±0.15	±0.55

*24 hour ⁵⁹Fe % uptake.

All values are mean ±1 S. E.

**Using 100% Helium gas, plasma and PCO (procytoxid).

TABLE 4. RENIN ASSAY RESULTS IN PRESERVED KIDNEYS.*

Environmental Gas Treatment	Pre-reinsertion	Days after reinsertion		
		1-7	7-14**	15+
Plasma + Oxygen (3 dogs)	1125 ± 143	1292 ± 65	806 ± 39	924 ± 68
Plasma + Helium (3 dogs)	615 ± 32	991 ± 33	1074 ± 56.5	474 ± 30
Plasma + Oxygen + PCO (7 dogs)	540 ± 24	838 ± 71	659 ± 37	402 ± 19
Plasma + Helium + PCO (5 dogs)	892 ± 63	693 ± 37	685 ± 38	-

*ng% plasma renin activity.

All assay values are mean ±1 S. E.

**Contralateral nephrectomy on 14th day.

sure or ischemia. As can be seen from Table 4, renin assay results were similar to ESF assay results. The greatest elevation of renin activity was seen through the postoperative period. PCO preserved groups had lower renin activity than PCO non-treated groups, and oxygen preserved groups had lower levels than helium preserved groups.

Arteriography, Renography—Figure 1 is an example of an arteriogram of a reinserted kidney three days after reinsertion. Adequacy of renal circulation was clearly demonstrated by the filling of the renal vasculature with the radiopaque substance. The renal artery was patent as shown by the arrow.

¹³¹I orthiodohippurate renograms were successfully used to evaluate renal function post-reinsertion. ¹³¹I labeled hippuran was

injected intravenously at time zero and the rate of removal of the hippuran by the kidney was monitored by a scintillation detector located over the kidney. The renogram depicted in Figure 2 shows a rapid removal of hippuran, and indicates a normally functioning kidney.

Blood Pressure, Histologic Appearance—Hypertension (BP > 160/110, femoral artery) was not observed in non-azotemic and successfully preserved kidneys in longer surviving animals.

Unsuccessfully preserved kidneys were readily identifiable by light microscopy. These features have been previously described in detail (Weber et al, 1969).

Metabolic Evaluation—Figures 3, 4 and 5 illustrate the results obtained from the metabolic study of biopsy specimens from four experimental groups. The degree of metabolic activity corresponds with the survival results shown in Table 1.

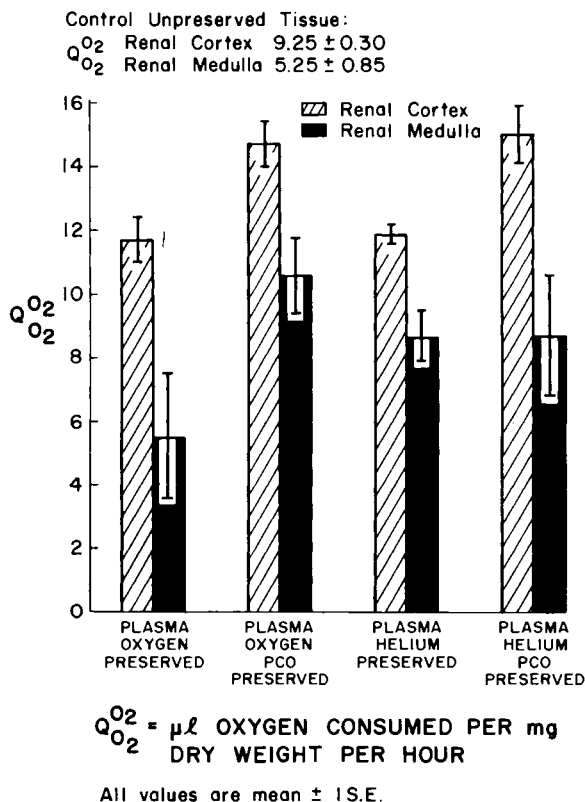


Figure 3. Oxygen consumption (Q_{O_2}) of renal tissue removed approximately one hour after reinsertion is shown. Cortical and medullary respiration from tissues of four preservation groups is compared.

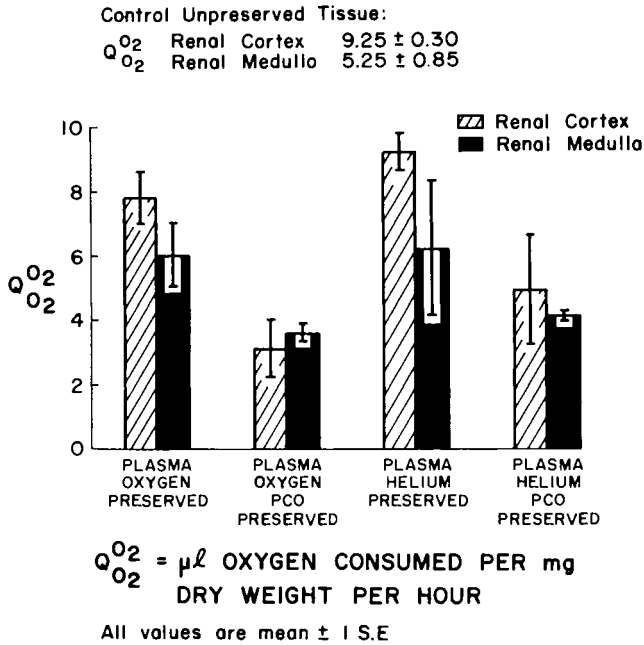


Figure 4. Q_{O_2} values of cortex and medulla of the four preservation groups seven days after reinsertion are compared.

Metabolic results from reinsertion day biopsy specimens, Figure 3, clearly differentiated between the PCO treated groups and the PCO non-treated groups. At this time, respiration levels of plasma oxygen and plasma helium preserved kidneys were nearly identical. The respiration levels of serum helium PCO and serum oxygen PCO preserved kidneys were significantly higher than those of the serum helium and serum oxygen groups. Serum helium PCO preserved cortical slices had slightly higher respiratory levels than serum oxygen PCO cortical slices. On the other hand, serum oxygen PCO medullary slices displayed higher respiratory levels than serum helium PCO medullary slices.

PCO treated groups showed better results at the time of reinsertion, and had better success rates. Renal biopsies taken at seven days after reinsertion showed a reversal of the early (zero day) and late (14th day) findings (Fig. 4).

Both of the PCO treated groups had greatly reduced Q_{O_2} at seven days, with metabolic activity of cortex more severely depressed than that of medulla. The plasma oxygen and plasma

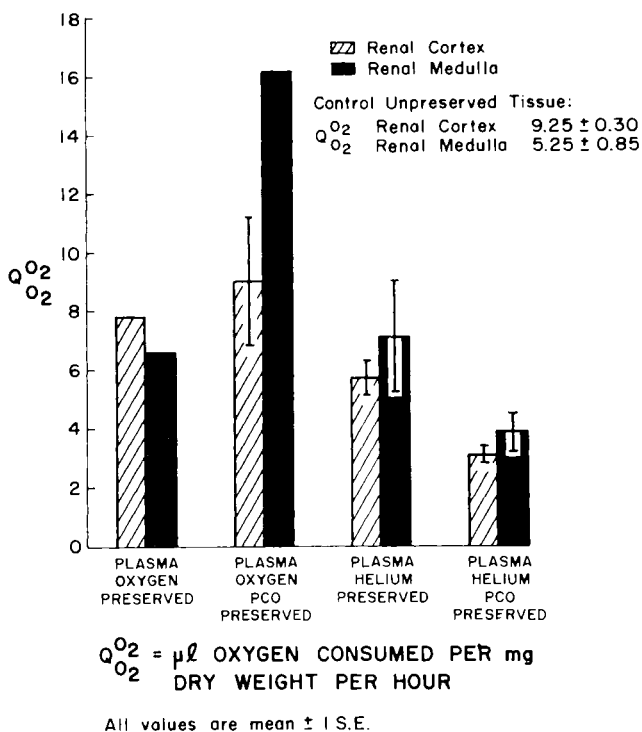


Figure 5. Q_{O_2} values at 14 days after reinsertion are compared.

helium (PCO non-treated) groups had nearly normal respiratory levels at this time, with slightly elevated activity seen in medullary respiration of both these groups. Cortical respiration of the plasma oxygen group was lower than normal seven days after reinsertion.

Figure 5 shows respiratory values at 14 days after reinsertion. At this time, plasma oxygen PCO preserved kidneys had the highest respiratory values. Q_{O_2} of cortex of these kidneys was nearly normal. Medullary levels, however, were elevated to nearly three times normal. Plasma oxygen preserved kidneys showed cortical respiration rates somewhat lower than normal and the medullary rates were elevated.

Plasma helium and plasma helium PCO preserved groups showed depressed cortical respiration, with that of plasma helium PCO more severely depressed. Medullary respiration of the plasma helium group was elevated, while that of the plasma helium PCO group was depressed.

DISCUSSION

In a program designed to develop means of preserving viable kidneys for transplantation it is desirable to evaluate the preservation techniques by as many criteria as possible. The health and survival of the experimental animal is the ultimate indication of success or failure. Tests can be performed to determine the health of the organ. Biochemical and hematological tests give an indication of the total health of the animal, and in some cases indicate how or if the reimplanted organ is functioning.

Arteriography can show if the reimplantation technique was successful by demonstrating vascular patency, or lack of it. Renograms give some indication of how well the kidney is performing its excretory function.

Metabolic evaluation of the reinserted preserved organ may be a more direct means of evaluating the viability of the organ. The current results of renal respiratory evaluation correspond with the other means of testing the success of the experimental procedures.

The respiratory stimulating effects of procytoxid (PCO) have been described previously (Murphy et al, 1969, Cook and Walter, 1941). The present experiments have affirmed that PCO has similar respiratory stimulating effects on kidneys in vivo following 24-hour hyperbaric hypothermic preservation.

It is not likely that PCO begins to affect the kidney under the hyperbaric (3 atm.) hypothermic (4 C.) conditions of storage. The stimulating effects were greatest shortly after ($\frac{1}{2}$ to 1 hour) restoration of renal circulation in the host animal. $Q_{O_2}^0$ of PCO preserved kidneys was greatest at this time. The respiratory distinctions between PCO treated vs. PCO non-treated groups are also most notable shortly after reinsertion. The mechanism by which PCO influences respiration is not precisely known. PCO has direct effects at a cellular level and may also operate directly on the renal vasculature. The lower levels of renin and ESF in animals with PCO preserved kidneys suggests that PCO suppresses the effects of ischemia and hypoxia on the kidney.

The results of the metabolic evaluations, ESF and renin assay, and more conventional tests, encourage us to consider PCO as an adjuvant in clinical renal preservation systems.

SUMMARY AND CONCLUSIONS

A number of methods have been employed to evaluate renal preservation. Respiratory evaluation of preserved, reinserted organ tissues has been found to be a valuable aid in evaluation of the preservation methods. The results obtained from metabolic respira-

tory tests correspond with results from radiographic, radioisotopic, biochemical, and hormonal assessments. The metabolic evaluations go beyond the other methods in that they give a direct indication of the degree of viability of the preserved tissue.

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