PRESERATION OF CANINE KIDNEYS:
EVALUATION OF BIOCHEMICAL AND HISTOLOGICAL PARAMETERS*

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The management of terminal chronic renal failure using renal allografts, especially from a
cadaver source, has placed increasing emphasis on the development of techniques for
prolonged extracorporeal kidney storage. Most previous work on the effects of long term
renal ischaemia during extra-corporeal preservation has been based on renal function
following reimplantation. Although survival on reimplantation is the ultimate test, in those
that fail the cause of failure is usually not apparent. In order to evaluate different protocols
rapidly and, also to dissect, in biochemical terms, what was usually occurring in ischaemic
kidneys we studied in cortical slices a transport dependent system (PAH uptake), ri-
bonucleic acid and protein synthesis, and mitochondrial dependent oxidation using suc-
cinate. These parameters were compared with histology of the slice as well as subsequent
post-reimplantation histology.

METHODS

A. Experimental protocols

Adult mongrel dogs were anaesthetized with Nembutal, at a constant dosage adjusted to
body weight, by a single intravenous injection.

1. In a first group of experiments, canine kidneys were removed and stored in contact with
blood at 37°C. Cortical slices were taken at hourly intervals (and also at 30 minutes for
PAH) and studied for histology and metabolic parameters.

2. Similar studies were made on kidneys stored at 2°C after an initial five minute perfusion
with cold Robinson’s solution to remove blood and cause cooling. Cortical slices were
obtained immediately at the onset of ischaemia and then at 6 and 12 hour intervals.

3. In a third series of experiments, also at 2°C, renal toxicity of varying concentrations of
Dimethylsulfoxide (DMSO) from 10 vol.% to 30 vol.% in Robinson’s solution was
evaluated using the same parameters of histology and biochemistry. In this series of
experiments both kidneys were removed from dogs, one kidney was stored in Robinson’s
solution and biopsies obtained for normal control values. The other kidney was stored
in Robinson’s solution with 10, 20 or 30 vol.% of DMSO. In all instances the kidneys
were initially perfused for five minutes with these solutions.

4. In a fourth series, using perfusion, and refrigeration equipment described below, the
whole kidney was cooled to —8°C using progressively increasing concentrations of
DMSO (up to 20%); the process was reversed on re-warming (5% DMSO to +1°C —
10% DMSO to —1°C — 15% DMSO to —3°C — 20% DMSO to —8°C). Two perfu-
sion solutions were used: (a) Robinson’s solution and (b) an ICF solution containing
150 mEq/L potassium, 40 mEq/L magnesium, 10 mEq/L sodium, and 2 mEq/L calcium
(Uldall et al., 1966). After re-warming to 1°C biopsies were taken for histology and
metabolic studies.

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5. Reimplantations of kidneys were performed after 24 hours of storage at 2°C and after perfusion and cooling down to —8°C. These reimplanted kidneys were then evaluated histologically one to four weeks later.

B. Refrigeration perfusion equipment

Equipment consisted of a liquid nitrogen refrigeration unit, cooling coil, thermistor needles and recorder for monitoring temperature. Assembly of this equipment was made possible through co-operation of the Research Division of Canadian Liquid Air. With this unit it was possible to accurately control cooling temperatures and DMSO concentrations of perfusion solutions.

C. Biochemical methods
1. \(O_2\) utilization with succinate standard Warburg technique for slices.
2. \(O_2\) utilization with succinate oxidative phosphorylation uncoupled with DNP \(5 \times 10^{-5}\) M.
4. RNA and protein synthesis with \(C^{14}\) uridine (1 μc uridine \(C^{14}/3\) ml media—specific activity 30 mc/mM), and \(C^{14}\) leucine (1 μc leucine \(C^{14}/3\) ml media—specific activity 30 mc/mM), using an as yet unpublished modification from our laboratory (Dr. Jung Oh) of the technique of Schneider (1945).

RESULTS

1. The results of the biochemical studies at 37.5°C are summarized in Figure 1. Plotted on the ordinate is the mean percentage activity of the different biochemical parameters. In the composition of these graphs, the actual values were expressed as percentages of the zero values of each individual experiment. Then a mean was derived from the sum of these percentage values of any given point along the time abscissa. The absolute values immediately at the onset of the ischaemia (zero time) representing 100% activity were as follows: (a) succinate DNP — 6.7 μL \(O_2\)/mg wet weight/hour; (b) protein synthesis \(1.4 \times 10^{4}\) c.p.m./g dry weight; (c) RNA synthesis \(5 \times 10^{5}\) c.p.m./g dry weight. It can be seen that the accumulation of PAH in the slice is severely depressed by half an hour of ischaemia.

![Graph](image)

**Fig. 1.** Comparison of biochemical parameters of renal ischaemia at 37.5°C. Mean values of 5 experiments.
two hour point, uncoupled and normal succinate respiration have comparable values which suggests that the oxidate phosphorylation is no longer occurring. Protein and RNA synthesis are depressed to within 30-50% of their activities at one hour and the depression is more severe at two hours.

2. Similar studies at 2°C are shown in Figure 2 in which it should be noted that the time scale on the abscissa has been changed. In addition, one standard deviation of each of the two succinate curves is plotted which indicates that there is no difference between them after the 24 hour point. This again indicates a breakdown of oxidative phosphorylation after 24 hours. It is also seen that PAH accumulation is markedly depressed at 12 hours and has ceased after 24 hours of storage. Again protein synthesis and RNA synthesis decrease at a rate intermediate between those of PAH and succinate. Also note that all points of the unmodified succinate curve are expressed as a percentage of the zero time value of the uncoupled succinate curve.

3. The studies of the toxicity of different concentrations of DMSO at 2°C on protein synthesis are shown in Figure 3. Ten and 20% DMSO resulted in approximately 30% depression of protein synthesis while 30% DMSO at this temperature caused almost complete suppression of all protein synthesis. The effects on PAH uptake were quite similar while succinate O₂ utilization was little affected.

4. Studies of the preservation of metabolism of renal cells after they have been perfused and cooled to −8°C and then re-warmed are shown in Figure 4. Two solutions were used, one an extra-cellular type, the other an intra-cellular type. The metabolic parameters were better preserved using the extra-cellular type fluids, but the reason for this will be discussed further, as vascular perfusion may be a factor.

5. Kidneys have been reimplanted after storage at 2°C for 24 hours, and after gradually cooling to −8°C and subsequent slow re-warming, using either ECF-type or ICF-type fluids in which to carry DMSO.

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Fig. 2. Comparison of biochemical parameters of renal ischaemia at 2°C initial perfusion and storage in Robinson’s solution. Mean values of 5 experiments.
Fig. 3. Effect of varying concentrations of DMSO on protein synthesis in kidneys ischaemic at 2°C.

Of five reimplanted kidneys which had been stored at 2°C for 24 hours in Robinson’s solution none provided sufficient function to sustain life and all animals died of uraemia following delayed contralateral nephrectomy.

Histological section 3-4 weeks following reimplantation showed varying degrees of interstitial fibrosis, tubular casts and varying degrees of tubular atrophy. Of 3 reimplanted kidneys stored at 2°C for 24 hours in ECF buffered with 14 millimoles/litre of Tris one had

Fig. 4. Biochemical parameters of ischaemic kidneys stored at −8°C. Comparison of Robinson’s solution with high K⁺ high Mg²⁺ perfusion solution.
a normal serum creatinine one month following contralateral nephrectomy. This may be significant and further evidence to support it will be given in the discussion.

Of five kidneys reimplanted after cooling to $-8^\circ$C in high concentration of $K^+$ and $Mg^{++}$ all kidneys became markedly cyanotic on reanastomosing the vessels and good cortical blood flows could not be established. Histological sections 1-3 weeks later revealed severe tubular necrosis with only an occasional intact tubule. Three kidneys were reimplanted after cooling to $-8^\circ$C with DMSO in ECF. In these kidneys as well cortical blood flow could not be established, the kidneys became cyanotic and subsequent histology revealed severe tubular necrosis with only an occasional intact tubule.

DISCUSSION

Initial experiments at 37.5$^\circ$C and 2$^\circ$C were primarily concerned with development of biochemical indices of survival.

At 37.5$^\circ$C most kidneys will survive one hour of clamping of the renal pedicle, but with two hours ischaemia at this temperature, none will survive (Gettler et al., 1961). At 2$^\circ$C, most kidneys will survive 12 hours of hypothermic storage and most will not survive 24 hours. We confirmed this latter point; none of the five reimplanted kidneys survived with sufficient function to maintain life.

The biochemical parameters also showed quite similar changes between one and two hours, at 37.5$^\circ$C and between 12 and 24 hours at 2$^\circ$C which we interpret as follows:

a. PAH accumulation, while it is a very sensitive index of ischaemic damage, is too sensitive to make the distinction between reversible and irreversible damage.

b. Succinate respiration, be it coupled with oxidative phosphorylation or uncoupled by DNP, is not significantly changed by 2 hours at 37$^\circ$C or by 24 hours at 2$^\circ$C to be a reliable index. It is not sensitive enough. This is in contrast to our previous work using homogenates, not slices, where there was a significant depression of respiration by two hours ischaemia at body temperature (Lannon et al., 1967).

c. Protein synthesis and RNA synthesis seem to give an index of viability. Thus, less than 30% activity is probably representative of irreversible damage whereas 30-50% activity of these two parameters is the range where survival may occur. Above 50% residual activity it is most probable that survival will occur. There is a striking similarity of these five curves at the two temperatures when one makes adjustment only in the time scale of the abscissa.

It is of interest, but of questionable importance, to note that implied oxidative phosphorylation ceases after the critical time for survivability, i.e., at the point where the two succinate curves become not significantly different from each other. Of course, if ATP synthesis were measured directly it might prove to be a sensitive index of survival.

Further support to the above conclusions was proved by an experiment where both kidneys were stored at 2$^\circ$C and tested in an identical fashion, except that one was in contact with a much better buffered solution (14 mM Tris). The latter kidney gave results for protein synthesis that were well inside the criteria for probable survival for 24 hours, whereas the other gave values within the range of doubtful survival and were comparable to the values depicted in Figure 2.

Although reimplantation after 24 hours at 2$^\circ$C using Robinson’s solution was unsuccessful, in one instance out of three with 24 hour storage when the storage solution contained 14 mM Tris a dog had a normal serum creatinine one month after contralateral nephrectomy. The implication is that further studies with better buffered solutions might give adequate 24 hours survival at 2$^\circ$C.

In comparing histologic sections of stored kidneys with simultaneous metabolic parameters, it was evident that the latter showed significant changes before the former when
using standard stains with light microscopy. This indicates the limitation of standard histology for assessing storage techniques.

With the metabolic criteria in mind, it is clear from the DMSO toxicity studies in Figure 3, that both 10% and 20% DMSO depress protein synthesis and 30% DMSO is highly toxic at 2°C. There is evidence to indicate that DMSO toxicity is reduced at lower temperatures. Also, it has been suggested that electrolyte solutions of high K and Mg concentrations may lessen the toxicity, especially when high DMSO concentrations and prolonged periods of perfusion are required (Uldall et al., 1966). Further, there is evidence that DMSO toxicity is reversible at least in regard to some isolated enzyme preparations (Rammler, 1967).

We then combined these concepts to determine if gradual increases in DMSO concentration at progressively lower temperatures would allow metabolic activity to survive 20% DMSO at —8°C. Two sets of metabolic experiments were done, using both kidneys of each individual dog; one was cooled with ICF-type fluid, the other with ECF-type fluid. For comparison, 6 kidneys were reimplanted after use of ICF-type fluid, and 3 reimplanted after ECF-type fluid. In these sub-zero experiments, the tissue is not frozen. The ischaemic time was about seven hours, three hours for cooling, three hours for re-warming, and one hour at —8°C.

We can conclude from the data in Figure 4 that the tissue perfused with ECF-type fluid showed fairly well preserved metabolic activity indicating probable cell survival, whereas ICF-type fluid caused profound metabolic depression, except for oxygen utilization. The difference between these two sets of data is unlikely to be due to increased toxicity from DMSO. We have some evidence from experiments at 2°C to show that our ICF-type fluid may be toxic in itself, but an important additional factor is increased vascular resistance to perfusion. This observed difficulty in perfusion resulted in incomplete removal of DMSO on re-warming. In none of the ICF-type perfused kidneys could we establish good cortical blood flow after reimplantation, and a patchy cortical necrosis occurred in each instance; also, when using ECF-type fluid, revascularization was still imperfect on reimplantation, though extracorporeal perfusion was normal.

The maintenance of adequate and even perfusion of the organ when re-anastomosed into the animal’s own blood stream still constitutes a problem in kidneys that have undergone prolonged extracorporeal perfusion with DMSO.

We believe the problem of inadequate revascularization explains the discrepancy between preservation of metabolic activity in these experiments using ECF-type fluid, and the total failure of the organ to survive on reimplantation.

REFERENCES


