Water Electrolyte and Metabolite Content in Cortical Tissue from Dog Kidneys Preserved by Hypothermia

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It has long been known that cooled kidneys may be stored for 6-12 hours without appreciable damage. In recent years the preservation time has been extended to one or more days by hypothermic perfusion or by hypothermic storage after flushing with a solution having an electrolyte composition resembling that of the intracellular fluid.

The effectiveness of the various methods of preservation has usually been evaluated after transplantation of the preserved organ. With a better understanding of the metabolism in the tissue more effective methods of preservation might be devised; moreover, metabolic data might serve as a measure of the viability of the preserved organ.

In the present study we have examined the content of water and electrolytes, adenine nucleotides and lactate in biopsy specimens from canine kidneys preserved in vitro by various methods. Preservation measures were introduced either immediately after removal of the organ or after normothermal ischaemia of 20 minutes duration.

MATERIAL AND METHODS

Biopsy specimens of superficial cortical tissue were repeatedly obtained for electrolyte and metabolite determinations. The following preservation methods were employed.

1 A Flushing with 100-200 ml of Ringer's solution at 5-8°C immediately followed by continuous perfusion with cryoprecipitated plasma in a thermostatically controlled perfusion circuit containing a single run-off oxygenator and a roller pump (manufactured by AB Aga Lidingö, Sweden) as described by Belzer et al (1967). The temperature was kept at 8-10°C and the flow rate at 0.2-0.4 ml/g/min.

1 B Flushing with 100-200 ml of a cold (5-8°C) electrolyte solution (ICS) with a composition resembling that of intracellular fluid and identical with
Collins' solution C4 (Collins et al, 1969) except that no phenoxybenzamine or procaine were added. After flushing the kidneys were preserved at 0-4°C.

1 C Flushing with 100-200 ml of a cold (5-8°C) solution (ECS) consisting of 5% dextran 40 in an extracellular type electrolyte solution (Pefadex) with the addition of 17 ml of 5% sodium bicarbonate, 10 ml of 1% lignocaine and 5,000 IU of heparin per 1,000 ml before use. After flushing the kidneys were preserved at 0-4°C.

2 A Preservation by continuous perfusion as in 1 A except that the preservative measures were introduced after arresting the circulation for 20 min in situ (about 37°C).

2 B Preservation by cold storage as in 1 B after arresting the circulation for 20 min in situ.

Electrolyte studies

Control specimens for electrolyte determination were obtained from 4 kidneys immediately after arrest of the circulation. Biopsy specimens were also obtained after flushing with 50, 150 and 300 ml of either ECS (2 kidneys) or ICS (2 kidneys). After storage for 24 hours 3 kidneys preserved with ECS and 3 kidneys preserved with ICS were flushed with 50 ml of cold 5% mannitol and biopsy specimens were taken for electrolyte determination. The mannitol flushing was introduced in order to minimise the effect of extracellular electrolytes on the determinations. These results were compared with results obtained from 2 fresh kidneys immediately flushed with 5% mannitol.

Water and electrolyte determinations were made in triplicate. Water was determined by weighing before and after drying at 90°C, neutral fat was extracted with petroleum ether and sodium, potassium and magnesium were determined by atomic absorption spectrophotometry (Bergström et al, 1971).

Metabolite studies

Control specimens for metabolite determination were obtained by freeze-drying of cortical tissue in situ with intact circulation (5 kidneys). The effect of warm ischaemia was studied in biopsies obtained after about 1, 20 and 60 min of circulatory arrest with the kidney remaining in situ (at approximately 37°C). The metabolite content was followed in biopsy specimens obtained at various times during preservation (3 kidneys with each preservation method).

Abbreviations: ATP = adenosine triphosphate; ADP = adenosine diphosphate; AMP = adenosine monophosphate; IMP = inosine monophosphate; ICS = preservation solution of intracellular type (Na+ 10 mM, K+ 115 mM, Mg++ 30 mM, phosphate 60 mM); ECS = preservation solution of extracellular type (Na+ 140 mM, K+ 6 mM, Mg++ 0.25 mM, phosphate 1 mM)
Specimens for metabolite determination were rapidly frozen in liquid Freon at its melting point. The material was freeze-dried and assayed for ATP, ADP, AMP, lactate, and other metabolites by enzymatic procedures based on those described by various authors in 'Methods of Enzymatic Analyses' (Ed: H. U. Bergmeyer).

RESULTS

Electrolyte studies

The effect of an initial perfusion with 150 ml of preservation fluid is shown in Figure 1. With ICS the sodium content decreased to less than 25% of the

![Graphs showing electrolyte content changes](image)

**Figure 1.** The effect of an initial flushing with cooled electrolyte solution of intracellular type (ICS) and extracellular type (ECS) on electrolyte and water content in kidney cortex. mean ± SD. **FFS** = fat free solids
initial content; the potassium and magnesium content rose considerably. Flushing with ECS resulted in a substantial loss of potassium (15-25%) indicating a depletion of cellular potassium; the magnesium content was unchanged and the sodium content increased.

After 24 hours storage followed by flushing with 50 ml of 5% mannitol (Figure 2) the difference in potassium and sodium content between kidneys initially flushed with ICS and ECS still persisted.

![Figure 2. Electrolyte and water content in cortex from kidneys preserved for 24 hours at 0-4°C after initial flushing with ICS or ECS. The kidneys were flushed with 50 ml 5% mannitol immediately before sampling](image)

**Metabolite studies**

Figure 3 shows the effect of different preservation methods on the ATP, ADP and AMP content in cortical tissue. Warm ischaemia resulted in a rapid reduction of the ATP content, more than 50% disappearing within 1 min. At 20 min ATP and ADP were low but AMP increased considerably. After 60 min ADP and AMP had also decreased and the total adenine nucleotides (ATP + ADP + AMP) were also low. After continuous hypothermic perfusion for 24 hours (1 A) ATP was reduced by 50%; ADP and AMP were also reduced as was the total adenine nucleotides. After 48 hours' perfusion ATP tended to be higher than after 24 hours' perfusion, indicating the efficiency of the perfusion in maintaining an active metabolism.

Cold storage at 0-4°C after an initial flushing with electrolyte solution resulted in much lower ATP concentrations than was obtained after continuous perfusion. However, there was a difference depending on the type of solution used for the initial flushing. After flushing with ICS significant amounts of ATP were still present after 24 and 48 hours, whereas after initial flushing with ECS the ATP was practically exhausted.
Figure 3. The effect of warm ischaemia and of various preservation methods on the ATP, ADP and AMP content in kidney cortex. Mean values. For further explanation, see text.

Figure 4. The effect of warm ischaemia and of various preservation methods on the lactate content in kidney cortex. Mean values. For further explanation, see text.
When continuous perfusion was started after warm ischaemia for 20 min (2 A) there was evidence of a rise in ATP after 24 hours, although to a lower level than in group 1 A (without warm ischaemia). On the other hand, cold storage after warm ischaemia for 20 min (2 B) resulted in a very low ATP level after 24 hours.

The lactate concentration in cortical tissue (Figure 4) increased rapidly during warm ischaemia. After 24 and 48 hours' preservation by continuous hypothermic perfusion the lactate concentration was only slightly increased above the basal level. After cold storage for 24 hours the lactate concentration rose about threefold being slightly higher in group 1 B (ICS) than in group 1 C (ECS). A further increase occurred after 48 hours. Higher lactate values were found in group 2 A than in group 1 A, indicating the role of the initial warm ischaemia for the accumulation of lactate.

DISCUSSION

The findings of this study bear out earlier observations that cooling greatly delays the reduction in the ATP level in the tissue resulting from ischaemia (Schmidt-Mende & Brendel, 1967). This course varied considerably, however, depending on the method of preservation used. The ATP level was best retained and the lactate was lowest when the kidney was perfused continuously with oxygenated plasma, probably because of the constant supply of substrate. When the kidney had first been exposed to warm ischaemia subsequent continuous perfusion was even accompanied by some recovery of the ATP level.

It is more difficult to account for the difference in ATP concentration between the kidneys preserved by hypothermia after flushing with ICS and those where ECS was used. It is conceivable that this difference is due to the difference in electrolyte composition. Our electrolyte determinations reveal that the initial flushing with ECS rapidly depletes cortical tissue of potassium and overflows it with sodium which presumably enters the cells in exchange. A further reduction in intracellular potassium and increase in sodium takes place during storage; it is known that both anoxia and hypothermia bring about such effects (Calkins et al, 1954; Hashish, 1958; Keeler et al, 1966). Flushing with ICS, on the other hand, will rapidly rinse out 80% of the sodium content and increase the extracellular potassium concentration. This should tend to stabilise the intracellular potassium concentration and prevent the entrance of sodium in the cells.

Cellular potassium depletion with simultaneous increase of intracellular sodium (as probably occurs during storage after flushing with ECS) may be deleterious as in this condition several enzymes may be inhibited, some of these being involved in carbohydrate metabolism (Losert, 1968). An increase of intracellular sodium concentration also stimulates the sodium
pump by activation of Na-K-dependent ATPase (Skou, 1957; Baker & Connelly, 1966) which hydrolyzes ATP, thus providing energy available for ion transport. After flushing with ICS the intracellular electrolyte environment is conceivably maintained with less effort by the sodium pump so that the ATP consumption is lower.

The decrease of total adenine nucleotides observed with all preservation methods used was most pronounced after 60 min of warm ischaemia and after 24-48 hours of storage after flushing with ECS, both representing conditions when the kidney may be considered to be irreversibly damaged. The reason for this is most probably that AMP is deaminated to IMP, a reaction known to occur in tissues during anoxia. The role of depletion of total adenine nucleotides for graft viability remains to be determined.

The changes in the cortical ATP concentration are fairly closely correlated with the effectiveness of the various methods of preservation, as reflected in the results of functional studies after autotransplantation. Immediate life-supporting function has been observed after 72 hours' continuous hypothermic perfusion (Belzer et al, 1967), whilst after flushing with electrolyte solutions of the intra- or extracellular type hypothermic storage has given a longest preservation period of 36-48 hours (Collste et al, 1970a) or 16-24 hours (Collste et al, 1970b). It would thus appear that the ATP concentration in the cortex might serve as a measure of the viability of the preserved kidneys.

ACKNOWLEDGMENT

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REFERENCES

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OPEN DISCUSSION

W J KOLFF (Salt Lake City, Chairman): Now, some would like to perfuse kidneys for storage and some would rather just store them in the cold. I will ask you all two questions. Supposing that you had the facilities to do it, which of you would elect to perfuse kidneys rather than use cold storage alone? Will you raise your hands? (Massive response – Eds.) Now, those of you who would just like to do cold storage without perfusion, will you raise your hands? Did I count three or four, perhaps five?

L E GELIN (Gothenburg): No, I think Mr Chairman that this question must be divided into two. If you do not need more than up to eight or ten hours storage you do not need to have continuous perfusion, but if you have to exceed ten hours of storage you really do need it. I think you should keep things simple as long as you can; simplicity, I think, is the key to success.

J MOORHEAD (London): Dr Kolff, when you asked which of those in the audience would prefer perfusion and which did not want perfusion, did you mean prolonged continuous perfusion or initial perfusion and then cold storage?

KOLFF: Well, I did not want to make it too complicated but I will be willing to see a show of hands of those that would like to have an initial brief perfusion followed by storage in the cold with, perhaps after 24 hours, another brief perfusion and further storage in the cold. Who would like that? (Poor response – Eds.) You do not get much support here.

L WIBELL (Uppsala): How long does it take in practice to get an answer on the viability of the kidney if you measure the ATP content of the cortex? You have a laboratory ready to do this quickly?

BERGSTROM: I have been asked this before and think it should be possible to do this in one or two hours. At present, however, it takes about a month because we are developing the analytical system and have the samples frozen a long time before the assay.

F PARSONS (Leeds): I do not know whether to ask it to you Dr Kolff or to the author here. Cold perfusion, yes, but could we ask the author whether he would like an intracellular fluid or an extracellular fluid, or should I ask
you if you would like an extracellular fluid, or an intracellular fluid with your cold perfusion?

KOLFF: That is a good question and we will have one more vote. Which of you wants to perfuse with an extracellular fluid with very little potassium? Now, who wants to poison the kidney with an intracellular fluid and a lot of potassium? I think the poisoners win!

BERGSTRÖM: We have measured the potassium in the kidney after the initial flushing and after putting the kidney into the continuous perfusion system with plasma in it. Plasma is an extracellular type of solution and we have found that the potassium content of the kidney cortex rises again after the initial depletion, probably as the result of a good metabolic state.
PART IV

ROUND TABLE DISCUSSION
ON
RENAL TRANSPLANTATION

with particular reference to the value of tissue
typing in cadaver donor transplantation

Chairman: L-E Gelin, Gothenburg,
Sweden

Participants: J Anderton, Edinburgh, UK
S-E Bergentz, Malmö, Sweden
J Dormont, Paris, France
H Festenstein, London, UK
W Kolff, Salt Lake City, USA
J May, Sydney, Australia
M Mebel, Berlin, German
Democratic Republic
J Moorhead, London, UK
L Röhl, Heidelberg, German
Federal Republic

Speakers from the floor: M G McGeown, Belfast, UK
C M Kjellstrand,
Minneapolis, USA