The Microcirculation of Hypothermic Kidneys during Perfusion with Blood-free Nutrient Solutions

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The aim of the present experiments was to determine the optimum conditions for temporary hypothermic kidney perfusion, with the ultimate aim of obtaining preservation by means of superficial cooling.

METHODS
To this end, 72 freshly removed dog kidneys were perfused with blood-free solutions using pulsatile and non-pulsatile flows. To prevent vascular collapse in the kidney, heparin was given into the renal artery during nephrectomy and light haemostats were applied to the renal artery and vein so that a blood-filled kidney with an isogravimetric residual pressure of about 10 - 15 mm Hg was perfused.

Non-pulsatile perfusion was achieved by using the pressure head generated by a column of perfusate 100 cm in height; this is approximately equivalent to 74 mm Hg. For the pulsatile perfusion we developed an open pumping system including a hose pump, and a damping and pressure control with volume regulation. The effective medium pulsatile pressure was adjusted to 74 mm Hg with a stroke frequency of 92/min and an amplitude of 40 mm Hg.

Three perfusates were tested (Table I).

Perfusate I
In Perfusate I the electrolyte concentrations were equivalent to those of the blood plasma.

Perfusate II
In Perfusate II the ion content was adjusted so that of the intracellular fluid as recommended by Collins.

Perfusate III
In Perfusate III, five per cent of low-molecular dextran (Note: not five per
Table I. Perfusates used for the temporary perfusion of dog kidneys

<table>
<thead>
<tr>
<th>Perfusate I</th>
<th>Perfusate II</th>
<th>Perfusate III</th>
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<tbody>
<tr>
<td>K⁺</td>
<td>5 mEq/l</td>
<td>15 mEq/l</td>
</tr>
<tr>
<td>Na⁺</td>
<td>140 mEq/l</td>
<td>85 mEq/l</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>5 mEq/l</td>
<td>15 mEq/l</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>3 mEq/l</td>
<td>10 mEq/l</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>103 mEq/l</td>
<td>60 mEq/l</td>
</tr>
<tr>
<td>CH₃COO⁻</td>
<td>50 mEq/l</td>
<td>25 g/l</td>
</tr>
<tr>
<td>glucose</td>
<td>25 g/l</td>
<td>glucose</td>
</tr>
<tr>
<td>mannitol</td>
<td>50 g/l</td>
<td>mannitol</td>
</tr>
<tr>
<td>glycocoll</td>
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pH 7.2 (20°C) 350 mOsmols

cent dextran!) and 0.33-molar glycocoll were added to this solution. We dispensed with heparin and vasodilator agents so as to be able to assess the purely circulatory effect of these solutions. We found that intrarenal vascular resistance increased markedly at temperatures below +5°C to +6°C in hydrostatic low-temperature perfusion and therefore chose this range of temperature for our investigations.

The effects of the perfusion were assessed using hydrodynamic measurements, chemical analyses of ureter, vein and lymph flow samples collected in fractions, and histological and zymo-histochemical studies. The micro-circulation could be examined after the introduction of coloured dyes into the solution towards the end of the perfusion.

RESULTS

Figure 1 shows original curves of the arterial pressure of perfusion, and of the needle tissue pressure when using Perfusate I. The traces at the top correspond to the values of the total and vascular resistances in the dimension dyn/min/cm⁻⁵ calculated according to Ohm's law. These curves show that in hydrostatic perfusion the tissue pressure rises continuously reaching – on an average – 27 ± 8.8 (standard difference) mm Hg after 15 minutes in the presence of a large individual range of fluctuation. The total resistance was considerably higher than the vascular resistance, and increased in a similar way. In pulsatile perfusion (lower traces) the increase in tissue pressure was significantly less than in gravity perfusion. The medium value was 19 ± 4.2 (standard difference) mm Hg. The total and vascular resistances were markedly less than in gravity perfusion.

There was no significant differences in the increase in the tissue
pressure and total renal resistance when Perfusates I and II were used. This increase could not be substantially reduced by the addition of vasodilator agents.

In pulsatile perfusion with Solution III no measurable increase in the tissue pressure could be established during the control period (Figure 2). Thus, the total resistance was equivalent to the vascular resistance.

Figure 3 shows that in kidneys perfused with Solution II there was incom-
plete filling of the glomerular and extraglomerular capillaries. Similar patterns were found after perfusion with Solution I. In oedematous kidneys
the perfusate obviously flows through certain main tracts while such capillaries as are situated in the shunt collapse. The precapillary vessels appear
to be greatly dilated.

The lymphatics are likewise filled to the extreme, and there is a lymphorrhoea (Figure 4).

When angiography of oedematous kidneys was carried out after 24 hours of low-temperature storage at a temperature of +8°C to +6°C, and the colouring redox matter triphenyltetrazolium chloride was injected into the renal tissue, the tubular cytoplasm in the vicinity of filled capillaries showed drop-shaped red colourations. In contrast, in the vicinity of collapsed vessels

Figure 3. Vasography of the subcapsular cortex area of an oedematous kidney after perfusion with Solution II

301
Figure 4. Incomplete filling of capillaries, and dilated lymphatic vessels in the region of medullary rays (same kidney as in Figure 3)

Figure 5. Zymo-histochemical demonstration of tubules with an active and an inactive metabolism depending on the capillary filling after 24 hours of preservation
Figure 6. Changes in the concentrations of potassium, sodium, protein, LAP, LDH and urea-N in fractions of 1.5 ml of urine specimens collected in series in perfusions with Solutions I-III. Continuous lines using pulsatile perfusion, interrupted lines using hydrostatic perfusion.
(Figure 5) there was either only slight plasmatic enzymatic activity or none at all.

We found homogeneous perfusion of the renal cortex and medulla only when performing pulsatile perfusion with Solution III. When the concentrations of biochemical parameters were measured (Figure 6) it appeared that Perfusate I led to an exponential mobilisation of urea-N and creatinine (not shown in the diagram). The potassium and sodium concentrations approximated those of the perfusate. Initially, there was a highly increased enzyme activity and proteinuria. In most cases the changes in concentration occurred more rapidly in pulsatile perfusion than with hydrostatic perfusion.

When using Perfusate II similar findings were obtained. Here, too, the potassium and sodium concentrations approximated those of the perfusate.

In pulsatile perfusion, however, Solution III did not effect leucine aminopeptidase (LAP) activity, and only brought about a slight initial increase in lactic dehydrogenase (LDH). There was no proteinuria. The excretion of urea-N during the control period was considerably less than it was when Perfusates I and II were used. The potassium and sodium concentrations reflected the perfusate concentrations less accurately. The hilar and capsular lymph also showed an initial increase in the enzyme, protein and potassium contents as well as a subsequent protracted decrease as compared with the original values established in situ in the perfusion with Perfusates I and II. In the perfusion with Perfusate III there was, however, only a gradual drop in concentration.

In the 'paravenous' outflow, too, we found higher original values and a correspondingly greater mobilisation of these substances than we did in the pulsatile perfusion using Solution III.

**INTERPRETATION AND CONCLUSIONS**

The results show that, due to the fact that the effective colloid-osmotic pressure was too low, hypothermic perfusion with multi-electrolyte solutions of the Types I and II leads to an increased capillary filtration. As a result of the interstitial accumulation of fluid the hydrostatic tissue pressure increases, and – to a growing extent – presses the veins and capillaries together. The strong outflow resistance conditioned thereby leads to an increase in the filtration pressure in the arterial capillary loop, and to dilatation of the capillary 'pores' while the absorption in the venous loop is blocked.

Thus, a growing stream of capillary fluid begins to flow into the tissue which – due to its 'washing-out effect' and its pressure effect – leads to the destruction of the membrane potential mainly of the tubular cells.

The hydrostatic vascular compression affects the renal capillaries differently thus leading to irregular perfusion which – in kidney preservation – may result in the failure of individual nephrons.
In pulsatile perfusion, the extent of the interstitial congestion of fluid is considerably less. This is due to the greater pressure-passive dilatation of the peripheral renal vessels, and the rhythmic 'kneading movements' acting on the renal tissue.

The colloid-osmotic pressure of Perfusate III is obviously strong enough to prevent the formation of oedema in pulsatile flow. But since the extracellular fluid is depleted of protein and other substances not present in the perfusate, further experiments will have to be carried out to determine if the prospects of success of low-temperature preservation can be increased by adding further amino acids, nucleotides, soluble vitamins etc to the nutrient medium.

**OPEN DISCUSSION**

D N S KERR (Newcastle): I wonder why in your first model some of the capillaries were blocked and not others, and would like to ask you a technical question. When we were first trying pump perfusion of kidneys, we found a rising perfusion pressure and similar blockage of some capillaries. This was, eventually, traced to our method of perfusion – the urine which was formed was put back into the perfusion solution to keep its electrolyte content constant and the filter system did not remove desquamated tubular cells. Do you separate the urine off from your perfusion or do you allow it to re-enter?

ERDMANN: I can give you a practical demonstration. If you are free we can go now to our Research Department and look at this perfusion system which is running at the moment.

W J KOLFF (Salt Lake City, Chairman): This is getting extremely interesting. Here we have one group that wants to perfuse the kidney and another that does not want to perfuse it. I have always believed that any pump does damage, but this morning I saw with the speaker, in his laboratory, a kidney that had been preserved for four days and was still producing urine. It is hard to argue with success.

L GELIN (Gothenburg): I also had the chance to see that kidney perfused yesterday, and was very impressed. Apparently a Type III perfusate was being used and the great value of this work is that it has shown that plasma is not essential as has been believed in the past and that this type of perfusate is suitable for long-term perfusion. The introduction of a perfusate which can be manufactured and stored should remove many of the practical problems of continuous perfusion.
M SNEILL (London): I would like to ask the speaker if he has had any problem with precipitation of magnesium phosphate. We have used a very similar perfusate in hyperbaric conditions, and found that unless we removed the magnesium sulphate from the solution we got precipitation with blockage of the cannulae after one or two hours.

ERDMANN: Precipitation occurs if the temperature of the perfusate is too high – 12-15°C. We use a temperature of 4°C and have seen precipitation in about 30% of cases. We add the magnesium to the perfusate just before the start of perfusion.

KOLFF: If I understand you correctly we have the strange situation that to do the kidney some good we must perfuse it with an almost deadly amount of potassium and magnesium!