LONG-TERM PRESERVATION OF THE KIDNEY

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The introduction of renal transplantation has made long-term preservation of kidneys desirable. The present paper describes our attempts to achieve this by perfusing the kidney with substances to lower the freezing point of the tissues so that they can be subsequently stored at —79°C, without ice-crystal formation.

Above 0°C, it is possible to preserve the mammalian kidney in good condition for several hours (Pegg, Calne, Pryse-Davies and Brown, 1964; Markland and Parsons, 1963). But, in order to preserve cells for months or years, very low temperatures are essential. Such preservation can be achieved with single cells (Smith, 1961) but is not yet possible with such a complex organ as the kidney.

A new approach to long-term preservation of tissues was made in 1965 by Farrant (1965). He took thin strips of smooth muscle and lowered their temperature to —79°C in solutions containing various concentrations of dimethyl sulphoxide (DMSO) but whose electrolyte concentration was maintained constant. By one method ice-crystal formation was allowed, by another it was prevented. The strips of muscle were then rewarmed by reversing the procedure. Both methods protected the smooth muscle equally from damage so that these strips contracted when stimulated with histamine. The muscle cells retained an almost normal morphological appearance (Farrant, 1966). These experiments confirmed the theory of Lovelock (1953) that the damage caused by cooling is due to ice-crystal formation itself, but to the increase in electrolyte concentration with which it is associated.

In order to lower the temperature of tissues to —79°C without freezing a 55% concentration of DMSO has to be reached. Unfortunately, high concentrations of DMSO are toxic to most cells at +37°C. However, the toxicity appears to decrease as the temperature is lowered and toxic effects may be avoided if the concentration of DMSO is gradually increased as the temperature is lowered. During rewar ming the procedure was reversed.

| **Composition of perfusion fluid (gassed with a mixture of 95% O₂ and 5% CO₂)** |
|----------------------------------|----------------|--------|
| KCl                              | 0.4            | g/litre|
| MgCl₂, 6H₂O                      | 0.2            | g/litre|
| NaH₂PO₄, 2H₂O                    | 0.05           | g/litre|
| CaCl₂, 6H₂O                      | 0.4            | g/litre|
| NaHCO₃                           | 1              | g/litre|
| Glucose                          | 1              | g/litre|
| Heparin                          | 3,000          | units/litre|
| Procaine hydrochloride           | 2              | g/litre|
| Low Molecular Weight Dextran ‘Lomodex’, 10% in saline | 450 | ml/litre |
| NaCl (in addition)              | 4.45           | g/litre|
| Distilled water                  | 550            | ml/litre|

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**Fig. 1.** Farrant's principle applied to kidney perfusion. During the cooling phase of the perfusion, DMSO is added to the perfusion fluid in increasing concentrations. After storage, DMSO concentration in the perfusion fluid is decreased while the temperature is raised.

**Methods**

This principle of tissue preservation has been applied in the present study (Fig. 1). In all instances the donor animal received Low Molecular Weight Dextran i.v., 10 ml per kg and the renal pedicle was infiltrated with procaine prior to nephrectomy.

After nephrectomy the kidney was perfused at a pressure of 100 to 200 mm Hg. using a Watson-Marlow roller pump. The composition of the perfusion fluid is shown in Table I.

As the temperature of the perfusion fluid was lowered DMSO was added increasing its concentration in steps of 5% until a final concentration of 55% was reached at —30 to —40°C. Lower temperatures for perfusion with this concentration of DMSO were not possible because of high viscosity. The temperature of the kidney was then lowered rapidly to —79°C., at which temperature the kidney was stored.

The kidney was rewarmed until its temperature reached —40°C. Perfusion was then restarted and the concentration of DMSO was decreased by steps of 5% until at 0°C. the concentration of DMSO in the perfusion fluid was zero.

**Results**

The first stage was to obtain a reasonable clearing of erythrocytes, as this indicates an evenly distributed perfusion. This was obtained after perfusion for half an hour using the technique already described. Examination of histological sections, specially stained with saffron du gatinais, showed less than one erythrocyte per five glomeruli.

The second stage was to be able to preserve a good histological picture of the kidney after preservation. Various factors were assessed in 70 experiments using the isolated rat kidney. It was found that it was important to keep the electrolyte concentration of the perfusion fluid constant. (For example, in making a solution containing 50% DMSO, equal quantities of DMSO and a solution containing twice the normal concentration of electrolytes are mixed.) While the rate of diffusion of DMSO into the kidney cells was not known, it was found empirically that it was necessary to perfuse the kidney with 5 ml. per g. kidney weight of each new concentration of DMSO (5% steps). Otherwise, the subsequent histological picture was poor.
Fig. 2. Kidney of the rat. Stored after perfusion for 24 hours at $-79^\circ$C. Then rewarmed by perfusion to $+4^\circ$C. (Saffron du Gatinais stain 130 $\times$) (reduced 40% for reproduction).

Fig. 3. The kidney depicted in Fig. 2 by phase-contrast microscopy in order to demonstrate the brush-border of the proximal tubules (300 $\times$) (reduced 40%).

Using these methods the kidney of the rat was cooled to $-79^\circ$C, stored for 24 hours and then rewarmed to $4^\circ$C. The histological picture is shown in Fig. 2 and in Fig. 3; the phase contrast is used to demonstrate the brush border.

The third stage was to study the function of kidneys preserved in this manner. Thirty experiments were performed using the kidney of the cat. Initially, after the storage procedure the kidney was connected to a perfusor animal by means of Teflon cannulae between the
blood vessels. Later the method of homotransplantation, devised by Carrel (1908), was used. It was here in Lyon that Alexis Carrel originally worked and laid the foundations of vascular anastomosis. In 1908 he recorded homotransplantation of both kidneys by means of segments of aorta and inferior vena cava.

In our experiments, difficulty was encountered in perfusing the kidney of the cat and thrombosis occurred soon after transplantation. On histological examination (Fig. 4) the tubules are seen to be relatively intact, but the glomeruli contain an amorphous material.

By contrast the kidney of the dog was perfused with ease. A canine kidney, which had been stored at \(-79^\circ\text{C}\) for a week, was transplanted into the neck of another dog and produced 5 ml urine within the first hour. In contrast to the cat, the glomeruli appeared normal while the tubules showed necrosis.

The present technique is not perfect. Protein, potassium and enzymes are lost from the kidney into the perfusion fluid during cooling, storage and rewarming, indicating that damage is occurring. While the measurement of renin and acid phosphatase is a relatively insensitive guide to damage, that of lactic acid dehydrogenase (LDH) has been useful. During cooling relatively little LDH is lost and few isoenzymes can be detected on enzyme electrophoresis. During rewarming a larger loss occurs and all five isoenzymes contained in the kidney are found in the perfusate. As these kidneys are often very similar to the kidneys of acute tubular necrosis in man, the loss of these enzymes into the perfusion fluid supports the view that in acute tubular necrosis the cause of the very high serum enzyme level is renal damage (Kemp, Lange, Laursen and Nielsen, 1964).

While you have placed our paper today before that of Dr. Uldall and his colleagues from Newcastle, we wish to say that they were before us in this field and that we have enjoyed the privilege of learning from their experience.
Summary

A procedure for long-term preservation has been elaborated. Dimethyl sulphoxide was added to the perfusion fluid so that the kidney could be stored at –79°C, without freezing. The brush-border lining of the tubules of the kidney of the rat was still virtually intact after storage in this manner for 24 hours. Kidneys from the cat and dog have been stored for up to one week with subsequent homotransplantation. In the cat, perfusion was difficult and thrombosis occurred. In the dog urine was produced, but there was tubular necrosis. The loss of protein, potassium and enzymes from the kidney into the perfusion fluid has been studied.

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REFERENCES

Farrant, J. (1966): Personal communication.