Simple Kidney-storage Technique*

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Brit. med. J., 1964, 1, 407-410

Several years ago we did not seriously consider removing kidneys from healthy living people and transplanting them to uraemic patients. The obvious plan was to store kidneys for subsequent transplantation. The problems involved in storage were not underestimated. It was pointed out: "If the immunological problems and other problems involved in procuring a normal functioning organ were solved our difficulties would be by no means over. There would still remain the problem of storage of organs. This presents many difficulties. While there is no difficulty in preserving skin and blood-vessels in the frozen state, other organs, such as kidney, would require more delicate handling. It is possible that an organ-perfusion apparatus may turn out to be the only safe method of preserving delicate organs ready for homotransplantation at any given moment" (Dempster, 1951). However, the results of transplanting cadaveric kidneys were disappointing because of a high rate of immediate anuria or oliguria, although occasionally function was resumed several days later (Hume *et al.*, 1955). The pioneering effort of Oeconomos *et al.* (1953), using a fresh kidney, impressed us and influenced our attitude to the donor problem for several years.

A scientific contraindication, we think, to continuing with live donors at this juncture is the high incidence of a curious complication of obliterative and other vascular lesions involving kidneys some time after homotransplantation and leading inevitably to anuria and death of the patient.

A blood-perfusion machine presents many difficulties, and unless oxygenated blood is used perfusion can be definitely harmful because the perfused organ becomes oedematous (Nizet and Cuyper, 1962). Even when oxygenated blood is used vasoconstrictor substances are liberated and damage to the isolated kidney consistently develops after a period of about three hours.

Perfusion of the whole cadaver would overcome many problems involving vasoconstrictor substances. There would appear to be better prospects for this method of perfusion than for perfusing the isolated kidney. However, both methods involve teams of workers who must be prepared at any moment to go into action. This may not be practicable in some hospitals and hence our attempt to reassess cooling.

Simple methods, such as storage in a refrigerator at 4° C., have often been employed, but the results have been inconsistent (Kiser *et al.*, 1961). Several years ago we were unable to confirm the results of Lefebvre (1951), who used refrigerator storage. Lefebvre and Nizet (1952) reported and illustrated very well the total medullary damage which we found consistently and which they encountered in some of their experiments (see Fig.).

Lapchinsky (1960) reported that autotransplanted kidneys can be perfused in a blood-flow circuit within a refrigerator. However, his technique was not successful for homotransplanted kidneys, which is our main concern at the moment.

Materials and Methods

Surgical Procedures

The dogs used were greyhounds, weight 22-25 kg. The average weight of the kidneys was 85 g.

Autotransplants.—Six autotransplants of kidney were performed. The kidneys were removed by a formal nephrectomy technique. The interval between removal and delivery to the cold environment was about 20 minutes. This is considerably less than the interval imposed by the conditions of the homotransplant experiments.

Homotransplants.—Kidneys were removed from six dogs after death from exsanguination. In order to simulate a possible clinical situation a delay after death of about 30 minutes was allowed before the kidneys were removed.

Transplantation Technique.—Kidneys were transplanted to the carotid-jugular circulation by a technique previously described (Dempster, 1953). In both sets of experiments the recipients were unilaterally nephrectomized because it was anticipated that the initial function of the stored kidneys would be poor. After the anastomoses had been completed the circulation through the kidney was controlled by a tape round the carotid artery so as to allow only a small flow during the first five minutes. The capsular vessels become filled very quickly because they arise from the extrarenal portion of the renal artery. Gradually the flow was increased over the next five minutes until no constriction was applied proximal to the renal artery. A gradual establishment of the flow is, in our opinion, of vital importance in preventing medullary and cortical necrosis. (see Fig.). As soon as possible mannitol was infused intravenously and usually urine started to flow at once. Twenty-four hours later an intravenous infusion of "rheomacrodex" and 2% saline was given so that the ability of these kidneys to respond to diuretic agents could be assessed.

Preparation of the Kidneys for Refrigerator Storage

Under sterile conditions the aorta and vena cava were sectioned above and below the level of the kidneys. A block of tissue containing kidneys, ureter, aorta, and vena cava was dissected free. The proximal aorta was cannulated. The distal aorta and proximal vena cava were ligated. Any branches of the aorta which were visible were ligated; the block of tissue was now a closed circuit except for a caval outlet. The kidneys were then flushed through slowly with about 400 ml. of heparinized dog plasma plus 150 ml. of rheomacrodex, and into this solution was added 0.01 mg. of papaverine sulphate, 20 mg. of procaine hydrochloride, 5 mg. of dipyridamole ("persantin"), and 0.5 g. of streptomycin. The temperature of the fluid was about 37° C. The head of pressure of the perfusate was 1 metre. The effluent from the kidneys very soon became clear, and this we attributed to the antisludging property of rheomacrodex, because flushing with plasma alone had resulted in slow clearing. At the end of the flushing the distal vena cava was ligated before all the perfusate had passed through the system so as to trap fluid under slight tension in the kidneys. This was an attempt to hold the vessels open.

The block of tissue was then placed in a sterile plastic bag containing 1 litre of normal saline at 4° C. This bag was

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sealed and placed within another, which was also sealed. The bags were then placed on soft pads in a metal dish and put in a refrigerator at 4° C. for six hours. Dog plasma has to be obtained from blood-donor dogs. A greyhound of 25 kg. will yield about 500 ml. of plasma. A fresh supply of dog plasma is required before any storage experiment is started.

Rewarming Process

After six hours the tissue block containing the kidneys was removed from the cold environment and immediately placed in a bath of normal saline at 37° C. The ligature on the distal vena cava was removed and the proximal aorta was cannulated. A solution identical to the one described above was slowly allowed to run in over a period of about 10 minutes. During this time the renal vessels were carefully dissected ready for sectioning. When the perfusate had run through, the renal vessels of one kidney were sectioned and the kidney was removed for transplantation. The other kidneys were kept as controls.

Arteriograms ; Blood Flow

Arteriograms were made of the transplanted kidneys one hour after the establishment of the new circulation and also at 48 hours prior to removing the kidneys for histological examination.

The blood flow was determined directly by cutting the renal vein and measuring the flow over a given period of 15 seconds.

Histology

The homotransplanted kidneys were removed while functioning 48 hours after the operation. It was considered that kidneys examined at this stage would show the maximum amount of tubular damage if cell damage had in fact occurred. The whole kidney was sectioned carefully. Histological examination was made of several parts of the kidney. The autotransplanted kidneys were removed on the fourth day.

Control Kidneys

A series of six kidneys were cooled in the refrigerator but not transplanted. In these kidneys recordings were taken of the core temperature after storage at 4° C. Histological examination was made to determine if the flushing of the kidneys was efficient in removing red cells and preventing red-cell aggregation.

Results

The core temperature of kidneys stored at 4° C. for six hours with this technique varied between 5.5° and 6° C. All six homotransplanted kidneys functioned within 15 minutes of opening up the new circulation. They continued to function during the observation period of 48 hours. Arteriograms indicated good cortical concentration. The quality of the urine produced was the usual low-specific-gravity type associated with neck kidneys (Dempster and Joekes, 1953). The volume of urine was low compared with fresh autotransplanted kidneys. These kidneys were, in fact, oliguric, since the volume per day did not exceed 100 ml. and the response to diuretic agents was poor.

The renal blood flows of six consecutive homotransplanted kidneys varied between 160 and 280 ml./min. at 48 hours. (The average for the normal greyhound is about 300 ml./min.) These values correspond to the blood-flow values established in previous experiments with fresh homotransplanted kidneys (Dempster, 1955). On removing the kidneys, inspection indi-

cated a virtually normal cortico-medullary junction. Areas of mild medullary congestion could be seen sometimes in some parts of these kidneys in the early part of the series. Histological examination of kidneys removed at 48 hours indicated some scattered tubular necrosis. Some tubular lumina were filled with casts. Mild congestion in the cortico-medullary area was apparent in some areas. In view of the good blood flow through these kidneys it is suggestive that little in the way of irreversible cell damage had been produced by storing at 4° C. for six hours. In our previously unsuccessful experiments the blood flow was about 40 ml./min. at 24 hours, indicating considerable intrarenal damage.

Medullary necrosis after six hours' cooling. The new circulation was established quickly. Homotransplanted kidney removed after 24 hours.

The autotransplanted kidneys started to secrete within 15 minutes of establishing the new circulation. The daily output was usually considerably over 300 ml., but two kidneys were oliguric and produced less than 100 ml. The concentration was low, as is usual in the early post-operative stage. Calne and colleagues (1963) have recently reported that autotransplanted kidneys may recover after 12 hours' preservation.

The histology of the autotransplanted kidneys differed in one important respect from that of the homotransplanted kidneys. None of the autotransplanted kidneys showed tubular necrosis. In two cases there was widespread distribution of casts, and this correlated well with oliguria. Autotransplanted kidneys which functioned well showed only a few casts and an apparently normal histology.

Histological examination of the control kidneys revealed that the red cells were not entirely removed from the vascular spaces. No red-cell aggregations were seen. It is a fair assumption that rheomacrodex and heparin together prevented sludging and red-cell aggregation.

Clinical Application

After the experimental technique had been established opportunity arose to apply it clinically.

A woman of 55 died suddenly of a subarachnoid haemorrhage. During the terminal stages her blood-pressure was normal. Permission from the relatives having been given, the kidneys were removed immediately after death (Mr. D. Crosby). The left kidney, together with a cuff of aorta and vena cava, was flushed out with 2 litres of plasma-rheomacrodex solution at 37° C. as previously described. After the flushing the kidney was put into a sterile bag containing normal saline at 4° C. and placed in a refrigerator at 4° C. The time taken after death to complete this phase was 45 minutes. After a period of about four hours the kidney was removed

and rewarmed as previously described for 10 minutes. The kidney was then anastomosed to the right iliac vessels of a young uraemic male. The total time of renal ischaemia was approximately five hours. The circulation was very carefully controlled for the first 10 minutes, after which time a full flow was gradually allowed. During this control period 2 ml. of dipyridamole was injected into the external iliac artery above the site of the end-to-side anastomosis. Before this was done a bulldog clamp was reapplied to the external iliac artery distal to the anastomosis so as to allow the full injection to flow direct into the kidney. The recipient received 80 ml. of rheomacrodex and 80 ml. of mannitol intravenously after the blood flow was restored to the kidney. The kidney remained anuric, however, and the ureter did not undergo peristalsis. About nine days later a diuresis of 2 litres a day started, but over a two-weeks period the concentration of urine urea did not exceed 500 mg./100 ml. Unfortunately the kidney was rejected in the fourth week in spite of giving antimetabolic drugs to the recipient. There were minimal signs of damaged tubules that were impossible to differentiate from the rejection process, which appeared to involve the vessels in a strange fibrinoid necrosis.

Discussion

On several occasions over the last 12 years we have attempted to store kidneys in the refrigerator but without success. The outstanding lesion was widespread medullary and cortical necrosis (see Fig.). In a recent reassessment of the problem it occurred to us that there might be various causes for this kind of damage.

Cell death due to perfusion with physiological solutions such as Tyrode's or Ringer-Locke solutions.—In agreement with many other workers we find that perfusion with this type of solution does positive harm in causing oedema and electrolyte disturbances within the cells. It was decided, therefore, that the flushing fluid for these experiments must be heparinized plasma.

Cell death due to interference with enzymatic systems as a result of prolonged ischaemia.—Further work is required to enable us to correlate biochemical changes with the ultimate test of viability in our system—the production of urine and ultimately good function.

Cell death due to ischaemia suffered in the interval between death and refrigeration.—In our experiments the autotransplants functioned better than homotransplants after six hours' preservation. We attribute this to the fact that the period of ischaemia between removal and delivery into a cold environment was about 20 minutes compared with about 60 minutes for the homotransplants. The longer the period of ischaemia prior to preservation the more damage is a kidney likely to suffer. Thus a technique which gives satisfactory results with autotransplants may not give equally satisfactory results with homotransplants removed some time after the death of the animal.

Damage by cold to the vascular endothelial and muscular system of the kidney.—No doubt this kind of damage could occur if kidneys were stored at temperatures below 0° C. We regard as absurd, at this juncture, any attempt to store kidneys below a temperature range of 4–10° C. Below 0° C. there are all the problems involved in dealing with ice-crystal formation within the tubule cells. It is known that, in the course of hypothermia for cardiac surgery, patients can be reduced to a whole body temperature just about 20° C. and the heart to about 10° C. with impunity. We considered it to be safe to keep just below this latter range, and we could find no evidence which suggests that permanent vascular damage follows storage at 4° C.

Incomplete removal of red cells, particularly from the renal capillaries by cold perfusing solutions.—It was decided to use solutions at 37° C. and to employ plasma together with rheomacrodex (an antisludging agent) and vasodilators. The effluent quickly appeared clear with this technique.

Too quick a return of the blood flow after anastomoses of the renal vessels leading to overloading of the juxtamedullary blood flow, congestion, and stasis in the medullary flow.—When a kidney is cooled to about 4° C. its vessels tend to be in spasm. This means that in the arteries beyond the arcuate vessels and juxtamedullary afferent arterioles there is an increased tonus and markedly increased resistance. Blood is thus prevented from travelling freely to the outer cortex and is accordingly diverted in too great a quantity and too quickly into and through the juxtamedullary afferent arterioles. Since the juxtamedullary afferent arterioles and their capillaries (vasa recta) are also in cold spasm, and because the latter are rather delicate long capillaries and sudden overloading will lead to congestion, then to rupture and haemorrhage. This leads to medullary necrosis. The flow in the inner cortex is similarly impeded and overburdened and congested, and cortical necrosis follows. A consideration of this possibility led us to start the rewarming process by slow flushing with plasma and rheomacrodex at 37° C. before the anastomosis was started. This can only remove the chill from the renal vasculature, but we consider it an essential step prior to opening up the new circulation.

The opening-up process must be gradual, and be timed deliberately by an assistant. The kidney must never be allowed to become turgid.

In spite of all these measures our stored human kidney transplant was anuric for about nine days. Except by taking successive biopsies one will not be in a position to assign a cause for the immediate anuria. We have noted hyaline casts in the lumina of the tubules in dog kidneys. We interpret this as protein which has leaked through the filter as the result of ischaemia. Casts in the human kidneys may be more widespread than we have encountered in dog kidneys, and this may be one explanation of anuria.

Persistent Intravenous Vascular Spasm

It is possible that for some hours after opening the new circulation a functional ischaemia continues owing to persistent increased tonus in the renal arterial system not yet adjusted to a new norm, and because of this some degree of moderate but reversible tubular necrosis develops. This is a concept for which we are grateful to Friedman *et al.* (1954) and Finckh *et al.* (1962). In an attempt to overcome this continued increased tonus we have used vasodilator drugs in the flushing fluid and intra-arterially after the new circulation has been established. The persistent afferent vascular spasm may be caused by an increased venous pressure in the kidney. Some evidence indicates (Haddy, 1956) that increased renal venous pressure, produced experimentally, can reflexly cause afferent spasm. The cause of the increased venous pressure may be slow flow, sludging, and oedema.

Autotransplanted kidneys cannot provide the controls for the type of experiment required for cadaveric homotransplants. In practice, however, the autotransplants were of some value because they were removed from dogs with normal blood-pressure and quickly stored. The period of ischaemia between removal and refrigeration was only 20 minutes compared with 30 minutes after the death of slowly exsanguinated dogs in the case of homotransplants. This difference in technique, whereby the period of pre-cooling ischaemia is reduced, could account for the better function of the autotransplants. This is a relevant point in clinical application. If, for example, the blood-pressure of a dying patient has been low for some time or if the period of ischaemia between death and refrigeration is much in excess of 45 minutes—permanent and irrecoverable damage may ensue. The conditions in which the present technique could be expected to be moderately successful are exactly those obtaining in our one human case. There is, however, no guarantee that any human kidney will recover function after this kind of storage, and one may only be able to assess the

situation in retrospect. In order to assess more accurately the time interval between death and cooling which is compatible with subsequent function one will be obliged to include this information in protocols.

Immediate function after cold storage of the canine kidney provides no guide to its subsequent behaviour. Several years ago, in attempting to confirm the results of Lefebvre (1951), immediate function frequently occurred for a few minutes or even hours but then ceased. Histological examination suggested that the damage these kidneys suffered was irreversible.

Arteriograms provide apparent proof of an adequately perfused kidney. However, they provided no hint of the presence of those foci of medullary congestion which were found in some kidneys removed after functioning well for 48 hours.

Kidneys stored under the conditions described above did not function well enough to maintain life immediately when transplanted. This was true for one human case (anuric for about a week) and the dogs. Since the dog homotransplant often does not survive beyond eight days and becomes involved in an oedematous process, it was not considered worth while following the homotransplants for longer than 48 hours. Our main objects were to assess cell damage at 48 hours and avoid medullary necrosis. The repair process of such damaged kidneys might take several weeks. Clinically, this is a risk that can be taken and managed accordingly.

If stored human kidneys are inevitably going to pass through a phase of mild or moderate tubular damage as a consequence of varying periods of ischaemia prior to cooling, the problem arises of the wisdom in modifying the recipients by administering antimetabolic and cytotoxic drugs. Other authors suspect that delay in tubular repair may be caused by purine analogues (Porter *et al.*, 1963). One report (Parsons *et al.*, 1963) indicates that this need not be an embarrassment to the kidney's recovery if the ischaemic period is about two hours. In our own case, an adequate diuresis occurred, but a steady recovery in terms of urine-urea concentration did not occur. It is not possible on the available evidence to judge confidently, but our present attitude would be to avoid using cytotoxic drugs to condition recipients for kidneys stored for several hours. An alternative method allowing full freedom to the tubule cells to recover from damage is obviously desirable.

Summary

A moderately successful technique is described for storing kidneys at 4° C. for six hours. Consistent results with dog kidneys have been found. A similar technique applied clinically resulted in recovery of function nine days after transplantation. The kidney had been stored for over four hours at 4° C. and the total period of ischaemia was about five hours. The period of pre-cooling ischaemia was about 45 minutes.

It has been suggested that the main problems to be overcome are not connected only with intracellular damage as a consequence of a period of ischaemia at body temperature but with vascular spasm and consequent inadequate perfusion of the kidney after the new circulation has been established. In addition, it has been suggested that inadequate flushing of the isolated kidney results in red-cell aggregation within the capillaries of the medulla prior to storage. This can aggravate vascular obstruction when the new circulation is effected. To overcome these problems the kidney is flushed out prior to storage at 4° C. with a plasma-rheomacrodex solution at 37° C. After storage and prior to performing the anastomoses of the renal vessels, the kidney is slowly rewarmed by a similar solution.

The new circulation must be gradually established so as not to overload the juxtamedullary circulation before the general cortical circulation is free from spasm.

It is suggested that for short-term storage a simple refrigerator technique may provide for some clinical centres a practical alternative to perfusion machines. Some damage to the stored kidney inevitably results, but when function returns the patient can be managed as a case in the diuretic phase of recovery from acute tubular damage. It is essential to reduce the period of pre-cooling ischaemia to about 40 minutes.

It is a pleasure to record our thanks for advice and discussion with Dr. J. F. Heggie and Dr. Audrey Smith. The expenses of the experiments were defrayed by a grant to one of us (W.J.D.) from the Wellcome Trust and a generous gift from Mr. W. Butlin. We are grateful to Boehringer Ingelheim Ltd. for a supply of persantin.

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