IN VITRO KIDNEY STORAGE

I. HYPOTHERMIA AND HYPERBARIC OXYGEN

J. R. W. ACKERMANN, M.B., CH.B., AND C. N. BARNARD, M.D., M.MED., M.S., PH.D., F.A.C.S., *Department of Surgery, University of Cape Town*

Currently, progress in organ transplantation is limited by three major obstacles—technical limitations, immunological problems and the acquisition and storage of organs.

Certain transplantation techniques have become standard experimental procedures and, more recently, a growing number of medical centres have produced well-documented clinical reports. Investigation of the immunological barriers which exist between all human beings (uni-ovular twins excepted) is widespread. The present rate of progress will soon create a need for successful organ storage.

With hypothermia, successful kidney storage is possible for up to about 6 hours and this method is used in human cadaver graft programmes.[1-3] However, for large-scale clinical homotransplantation, prolonged storage for 24 hours or longer is essential. During this storage period it may eventually be possible to attack graft antigenicity and to assay function of the organ before transplantation.

This report concerns experiments performed in an attempt to achieve reasonable physiological function in a kidney after 24 hours of *in vitro* storage using hypothermia and hyperbaric oxygenation.

MATERIALS AND METHODS

Operative Procedure

Adult mongrel dogs, weighing from 25 to 45 lb., are anaesthetized with intravenous pentobarbitone sodium, intubated and manually ventilated. The right kidney is used because of anatomical suitability. Three different surgical procedures are involved:

1. *Right nephrectomy*. A right, muscle-splitting incision is used with careful dissection of the ureter, renal artery and vein. The vessels are ligated as close to the aorta and inferior vena cava as possible. The ureter is transected approximately 10 cm. from the hilum of the kidney.

2. *Reimplantation of the kidney* (after 24 hours' storage). The renal artery and vein of the graft are anastomosed to the common carotid artery and external jugular vein, respectively, of the same dog. A muco-cutaneous ureterostomy is created in the mid-line of the dog's neck.[4]

3. *Left nephrectomy*. The dog's left kidney is removed through a left-sided, muscle-splitting incision, either immediately after reimplantation or 2 - 3 weeks later.

Perfusion (Fig. 1)

The isolated kidney is perfused twice—immediately after the initial nephrectomy and just before reimplantation into the dog's neck.

Perfusate: 500 ml. of Rheomacrodex in normal saline, 50 ml. of 1% Leostesin and 15 mg. of heparin, at a temperature of 2° - 5°C.

Method. The renal artery is cannulated with a size 15 blunt needle connected to a syringe. The kidney is perfused under low pressure (80 - 120 mm.Hg) until the return from the renal vein is clear (150 - 250 ml.). Before reimplanta-

tion, at least 100 ml. of fluid is perfused through the already bloodless organ at very low pressure (40 - 60 mm.Hg). No antibiotic is added to the perfusate.

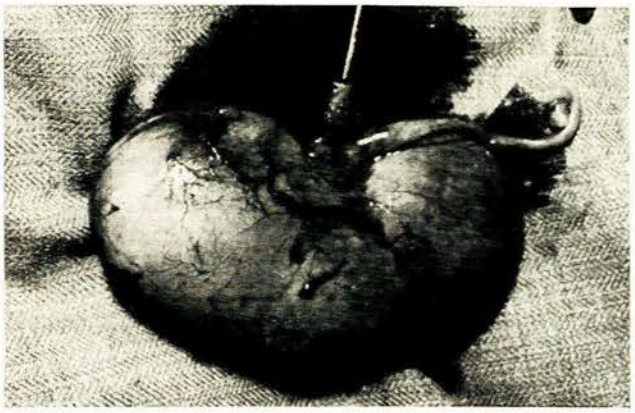

Fig. 1. Immediate cold perfusion of the isolated kidney after nephrectomy, by renal artery cannulation using a blunted size 15 needle attached to a syringe.

Storage

During storage, the kidney is totally immersed in a specially prepared solution. This solution is precooled to the required temperature.

Immersate: Rheomacrodex in saline, 0·92 G/100 ml., potassium chloride, 0·042 G/100 ml., calcium chloride, 0·024 G/100 ml., sodium bicarbonate, 0·015 G/100 ml., and dextrose, 0·10 G/100 ml. This, in effect, is a low-molecular weight dextran—Ringer's balanced physiological solution. No antibiotic is added to the immersate.

Method of Cooling

A standard, easily regulated refrigeration system is used to attain temperatures of 2° - 10°C. For sub-zero temperatures, a special deep-freeze unit is used.

Both perfusate and immersate are pre-cooled, the former to 2° - 5°C and the latter as required. The donor animal is not surface-cooled.

Rewarming

At the end of the 24 hours' storage, at whatever temperature used, the kidney is perfused for the second time at 2° - 5°C. While the vascular anastomoses are completed, the kidney is placed in a previously-fashioned sac in the dog's neck, deep to the panniculus carnosus. The kidney surface is thus exposed to body temperature and is thus rewarmed during the 20 - 30 minutes while the blood supply is reconstituted.

Hyperbaric Oxygenation

A hyperbaric oxygenation chamber was manufactured locally to our own specifications (Fig. 2).* This chamber stands on a funnel-shaped pedestal to ensure stability. It is circular, 12 inches high and has an internal diameter of 9 inches. The dished lid is secured by means of 4 swing bolts of ample capacity. A dished head closes the lower end of the chamber, which is made from high-grade stainless steel.

Within, there is a perforated, removable bottom plate below which the thermometer projects. Two nipples of 2 mm. internal diameter are positioned in continuity with 2 similar nipples on the exterior of the chamber, and are easily blocked if required.

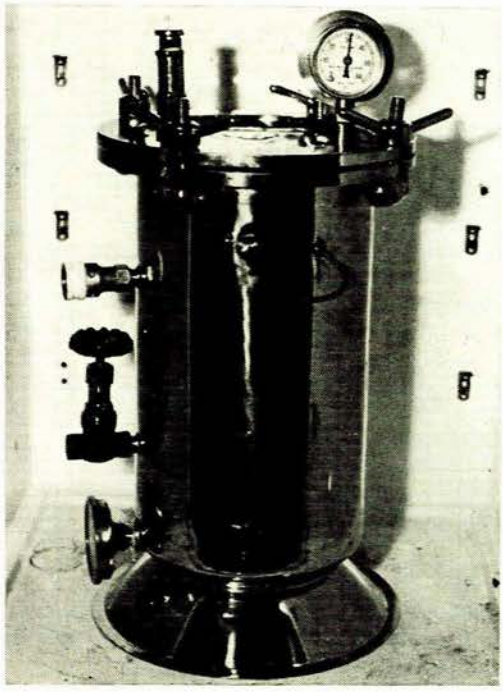

Fig. 2. The hyperbaric oxygen chamber which is compressed to 3 atmospheres absolute. Starting at the upper end, the fittings on the side-wall are: quick-coupling connector, exhaust valve and low-reading thermometer. The pressure gauge (on the lid) shows that the chamber is compressed to 3 ATA.

The external fixtures consist of a pressure gauge reading up to 60 lb./sq.in., a 40° - 110°F thermometer, a quick-coupling oxygen connector for recompression, an exhaust valve and a safety valve which operates at 52 lb./sq.in. (2 lb./sq.in. above working pressure). The chamber is tested to 75 lb./sq.in. and has a working pressure of 50 lb./sq.in.

The whole unit is sterilized with the lid off and is resealed immediately, the inside remaining sterile. Precooling is effected and the chamber is not removed until the isolated kidney has been perfused for the first time. The kidney is then immersed in the pre-cooled solution in a glass beaker, and is placed in the chamber. After compression, the apparatus is returned to the refrigerator system, where constant temperature and pressure are maintained throughout the 24 hours' storage.

The chamber is compressed using a standard oxygen cylinder at 2,000 lb./sq.in., by means of a reducing valve. In all experiments using hyperbaric oxygenation a pressure of 3 atmospheres absolute is used. Decompression is done over 5 - 10 minutes, by means of the single exhaust valve.

Experimental Groups

Group I—9 dogs. Cooling to 2° - 5°C; 24-hour storage. Four dogs underwent immediate left nephrectomy after reimplantation and five 2 - 3 weeks later.

Group II—4 dogs. Freezing at –5° to –10°C; 24-hour

*Acecor (Pty) Ltd, Parow, C.P.

storage. Left nephrectomy was not possible in any of these animals.

Group III—4 dogs. Cooling to 5° - 10°C with 3 ATA oxygen; 24-hour storage. As in group II, left nephrectomy was not possible.

Group IV—5 dogs. Normothermia with 3 ATA oxygen; 24-hour storage. In 1 dog a left nephrectomy was performed later.

Group V—10 dogs. Cooling to 2° - 5°C with 3 ATA oxygen; 24-hour storage. Four animals underwent immediate left nephrectomy and in 6 this was performed after 20 - 28 days.

Investigations

The following investigations are performed daily to assess the function of the reimplanted kidney and to ensure that no diagnostic criteria of threatened rejection manifest after storage.[5]

1. *Urine*—approximate volume, protein, stained urinary sediment smear,[6] catalase,[7] urinary urea.
2. *Blood*—urea, nitrogen.
3. *Local examination* of the grafted kidney, with special attention to its size and consistency.
4. *General examination* of the dog—pulse rate, temperature, blood pressure.

Treatment

Antibiotics are administered only for specific indications, and then for 5 days (routine: penicillin 1 million units daily and streptomycin 1 G daily).

Intravenous infusions either of half-normal saline or a balanced electrolyte solution are administered during operation and for 2 days postoperatively.

The animals are fed a standard diet and are exercised daily.

RESULTS

In these experiments 'ischaemic time' is the period during which the kidney was devoid of a blood supply, and excludes the 24 hours' *in vitro* storage. Ischaemic time is thus made up of the 3 - 5 minutes delay between nephrectomy and storage, plus 20 - 30 minutes rewarming while the vascular anastomoses are being completed after storage. The ischaemic time averaged 30 minutes.

True success is the survival of a bilaterally nephrectomized animal with a single, reimplanted, previously stored kidney. Any comparison of the relative merits of various storage procedures on histological grounds is arbitrary and is largely worthless. Although ischaemia has a special meaning in these experiments, in any attempt at *in vitro* storage the organ is deprived of a normal blood supply and is inadequately oxygenated to some extent.

In this series the relationship of functional capacity to pathology was inconclusive. The histology showed a widely varying picture, ranging from complete necrosis to tubular degeneration only, or to a picture indistinguishable from normal. Although the experimental procedure was standardized, significant microscopic variation was found within each group. This may partly be due to the fact that histological specimens were only taken from non-functioning kidneys, or those proven incapable of sustaining life, and the post-storage time factor thus varied. The only exception was one successful survivor, sacrificed only for histology. Renal biopsies were not performed.

In a small minority, left nephrectomy and reimplantation were simultaneous, with no set indication for doing this. Survival was accepted if the animal was alive and reasonably well 14 days after left nephrectomy, being sustained for this period by the reimplanted, stored kidney.

In those animals where a late left nephrectomy was performed, the criteria for the removal of the left kidney were:

(*a*) adequate urinary output volume, assessed both before and after an intravenous water load;
(*b*) absence of any marked abnormality of urinary sediment, particularly casts;
(*c*) proteinuria of 2+ or less;
(*d*) normal size, shape and consistency of the reimplanted kidney;
(*e*) urinary urea approximating that measured in the urine excreted by the remaining, normal kidney.

In these cases too, survival was accepted if the animal was alive and reasonably well 14 days after left nephrectomy. The over-all results of the experiment are depicted in Table I. Each group is discussed in greater detail below.

TABLE I. THE OVER-ALL RESULTS OF IN VITRO STORAGE IN THE 5 GROUPS, USING HYPOTHERMIA, FREEZING AND HYPERBARIC OXYGEN, SINGLY AND IN VARIOUS COMBINATIONS*

Group	*No. of dogs*	*Storage temperature*	*Oxygen availability*	*Staging of contralateral nephrectomy*	*Survivors*
1	9	2° to 5°C	—	4 immediately after reimplantation, 5 at 20–28 days	— 1
2	4	—5° to —10°C	—	These kidneys were functionless and left nephrectomy was never possible	—
3	4	—5° to —10°C	3 ATA	As in group 2	—
4	5	±37°C	3 ATA	1 at 26 days post-reimplantation	—
5	10	2° to 5°C	3 ATA	4 Immediately, 6 at 20–28 days	— 4

*The only significant survivors were obtained in group V after late contralatera nephrectomy.

Group I (9 Dogs): Simple Hypothermia between 2° and 5°C for 24 Hours

Immediately after storage, these kidneys were soft and white. After restoration of the blood supply there was a mottled, pink but relatively normal appearance. A size increase of one-third to half of normal occurred. In consistency the kidneys were tense to the point of rubbery hardness. There was an invariable ooze of darkish coloured blood from the area denuded by capsulotomy.

The reimplanted kidney was closely observed for 30 minutes after completing the vascular anastomoses. There was usually a noticeable colour alteration from pink to a darker, more congested, appearance, although both consistency and size of the kidney remained constant. Within this observation period, a few drops of clear urine were usually excreted.

The immediate flow of urine prompted the simultaneous left nephrectomy in the first 4 animals in group I. None of these survived longer than 3 days and all 4 deaths were due to uraemia—even though small amounts of urine were still passing at the time of death.

The remaining 5 dogs underwent contralateral nephrectomy between 20 and 28 days after reimplantation. At this stage there were no established criteria for assessing the

function of the stored organ. Had these criteria been available, left nephrectomy would have been considered in only 1 of these 5 dogs (the only survivor in group I). The other 4 succumbed to uraemia within 4 days, although there had been some urinary output (insignificant in amount as compared with the output excreted by the survivor).

Postmortem revealed a swollen, congested kidney, blue-black in colour, with apparent areas of massive subcapsular haemorrhage. Microscopically, whether death had occurred within 14 days or 3 weeks of reimplantation, the glomeruli were essentially normal. The predominant damage was to tubules and there was massive small-vessel thrombosis. In those animals where late left nephrectomy had been performed, there was evidence of regeneration with organization of small-vessel thrombi.

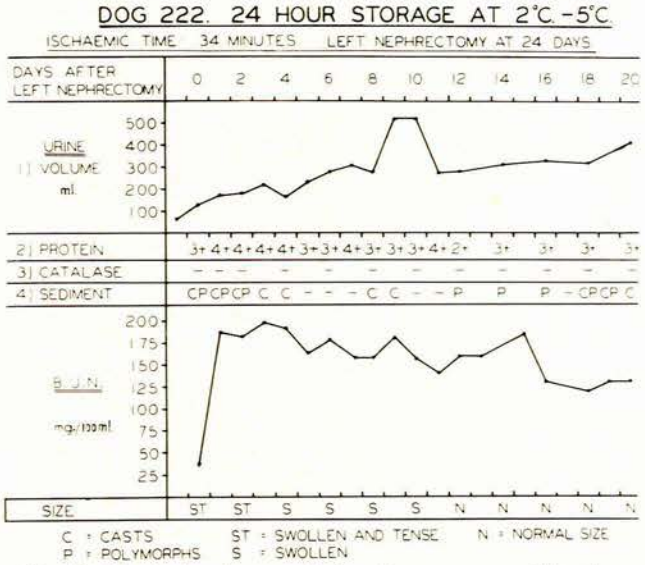

Fig. 3. Chart showing renal function as assessed in the only survivor in group I (after storage with hypothermia alone for 24 hours). Marked residual impairment of function is present 20 days after late contralateral nephrectomy (performed 24 days after reimplantation of the stored organ).

The results achieved in the sole survivor are detailed in Fig. 3. Although present, renal function is subnormal and at this stage damage must be presumed to be irreversible.

Groups II and III (8 Dogs): 24-hour Storage at −5° to −10°C of 4 Kidneys without Hyperbaric Oxygenation and of 4 Kidneys with 3 ATA Oxygenation

There was no difference between groups II and III in the pathological or functional results. There were no survivors. The functional indications for left nephrectomy never existed.

Before reimplantation, the kidney was frozen solid but during rewarming in the dog's neck, became soft and pale. Immediately after completing the anastomoses, the organ became tensely swollen and blue-black in colour.

During the 30-minute observation period, no urine was passed and no noticeable colour change occurred. There was no subsequent urinary output and, within 5 days, the kidney was soft and obviously totally ischaemic. At this stage it was removed.

Macroscopically and histologically, the entire kidney was necrotic, with destruction of cortex and medulla.

Group IV (5 Dogs): 24-hour Storage at 37°C (Room Temperature) with 3 ATA Oxygenation

In this group, before vascular anastomoses, the kidney was pale pink, altering to a normal, reddish-pink colour when the blood supply had been reconstituted. Swelling was moderate.

Thirty minutes later the colour was more dusky, but still relatively normal. A small but significant amount of urine was passed during the observation period. In 4 animals this flow was not maintained and the storage kidney was removed within 5 days. In the remaining dog, urine secretion continued in small amounts and, though there were no specific indications, a left nephrectomy was performed 26 days after reimplantation. This animal died within 72 hours, from uraemia, though still passing small amounts of urine.

The pathological features in group IV were not dissimilar from those in group I, only being more severe. Glomeruli were normal on the whole, with extensive small-vessel thrombosis and massive tubular necrosis. There was little histological improvement in the longest-surviving kidney and no remarkable differences.

Group V (10 Dogs): 24-hour Storage at 2°-5°C with 3 ATA Oxygen

The post-storage appearance of these organs was indistinguishable from those in group I. With restoration of the blood supply, the colour immediately became pink and was still normal after 30 minutes.

A moderate size increase was noted during the 30-minute observation period, but was less pronounced than in the other groups. Urinary output was prompt and the urine clear.

Simultaneous left nephrectomy was performed in 4 dogs and all died from renal dysfunction within 6 days. In the other 6 animals, the criteria for contralateral nephrectomy were satisfied within 20 - 28 days after reimplantation. Two of these dogs died within 14 days of left nephrectomy, and are not considered survivors; in both, kidney sepsis was uncontrollable.

In the 6 dogs in group V where late left nephrectomy was performed, the parameters used to assess renal function before contralateral nephrectomy showed results far superior to those seen in any other group. When the animal was rendered wholly dependent upon the stored organ, renal function was impaired for some days (Fig. 4). In 3 of the 4 survivors there was apparent complete return of function eventually. These animals continued as though autotransplants, without any intervening storage period. The remaining survivor was alive and well 40 days later but renal function was definitely subnormal, and at this stage renal damage must be presumed to be irreversible.

Pathologically, group V can be subdivided into 3 groups:

1. The 4 animals which succumbed within 6 days of simultaneous left nephrectomy.

Macroscopically there was nothing of particular note. Microscopic examination revealed definite evidence of ischaemia—focal areas of tubular destruction, predominantly of proximal convoluted tubules. Slight interstitial

oedema was present and there was evidence of small-vessel thrombosis on a limited scale. There was also a suggestion of tubular cell vacuolation. The glomeruli were normal, as was the general renal architecture.

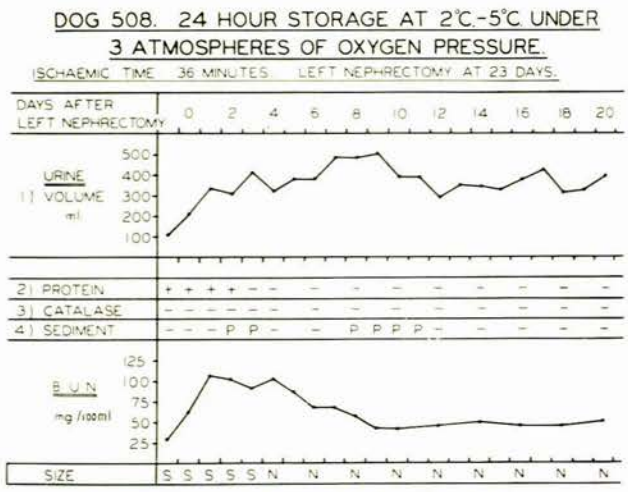

Fig. 4. Chart depicting renal function assessed in a typical survivor in group V (after storage with hypothermia and hyperbaric oxygen for 24 hours). Late contralateral nephrectomy was performed (23 days after reimplantation of the stored organ). Note the return of renal function to within normal limits 8 - 10 days after the opposite nephrectomy.

2. In the 2 animals which died within 14 days of a late stage left nephrectomy, sepsis precluded worth-while observation.

3. Into the third pathological subdivision falls 1 of the 4 survivors which was sacrificed at 35 days, expressly for histology. By the parameters of these experiments, renal function was within normal limits. Pathologically, too, this kidney was ostensibly normal, although there was still evidence of tubular regeneration.

DISCUSSION

It was stated earlier that the only true indication of successful kidney storage is the adequacy with which the single, stored kidney is able to sustain life. In these experiments, by the combination of hypothermia with hyperbaric oxygenation, it was possible to retain kidney viability during the 24-hour storage period, but its functional capacity after immediate contralateral nephrectomy was inadequate for the animal's survival.

It appears that kidneys stored in this way require 2 - 3 weeks for the regeneration of traumatized tissue before reasonable function can be expected. However, using these 2 methods in combination, it is possible to prevent irreversible damage incompatible with life.

Once specific indications had been recognized, the timing of the contralateral nephrectomy became easier to determine. These criteria were based on simple parameters, with the exception of comparative assays of the urinary urea of the stored organ and the dog's normal kidney. Differential urinary secretion tests were of significant value in gauging function in these experiments.[8]

Both here and elsewhere, the relative merits of various perfusates have been studied.[5,9] Generally, it is agreed that immediate perfusion of the resected kidney with a pre-cooled solution is essential:[10-13]

(*a*) to remove any remaining blood, preventing vascular aggregation and thrombosis,

(*b*) to lower renal core temperature and so reduce the metabolic processes and requirements of the tissues, and

(*c*) to minimize or to prevent vascular spasm.

A Rheomacrodex/Leostesin/heparin perfusate gives good results.[14]

Two points merit emphasis: Firstly, the kidney should be perfused as soon as it is removed; secondly, the perfusion pressure should not exceed 150 mm.Hg.[15,16] Both these points were strictly observed in this series.

In any storage procedure, the organ must be immersed in some fluid during the period of total ischaemia. We used a physiological solution with Rheomacrodex in saline as the base, as in the perfusate. A tremendous amount of literature is available on the many advantages and fewer disadvantages of this low-molecular weight dextran solution. Although no comparison was made with other solutions, Rheomacrodex was considered most suitable and has not been disappointing.[17]

Although survival of the animal with a single, stored kidney is the outstanding feature of successful storage, other parameters of renal function were also studied.

Reimplantation of the kidney after storage constitutes autotransplantation and thus the rejection phenomenon was never a complicating factor in assessing function. Clinically, however, renal function of the stored organ must be sufficient to make possible the diagnosis of threatened rejection. In this series therefore, in addition to the assessment of ordinary kidney function, it was also necessary to investigate the possible exclusion by storage of any of the important criteria indicating threatened graft rejection.[5] Storage produced no definite signs which could confuse the diagnosis of graft repudiation.

The successful use of hypothermia during certain surgical procedures led to the investigation of its effects on individual organ systems.[18-20] Where operations involve prolonged renal ischaemia, hypothermia tends to preserve kidney function by depressing a variety of tubular mechanisms.[21,22]

The reduction of oxidative metabolism during hypothermia is a manifestation of the decreased velocity of chemical reactions which occurs with temperature reduction.[23] Hypothermia exerts a far greater depression of oxidative metabolism in the kidney than in the body as a whole.[23,24] The relative changes in renal oxygen consumption are greater than the alterations in total body oxygen consumption.[25,26] It has been estimated that at 5°C the oxygen requirement of the kidney is approximately 5% of normal, an almost incredible diminution.[27]

There are many reports of successful hypothermic kidney storage, usually for short periods of 6 - 8 hours.[3,28,29] In addition, there are isolated reports of hypothermic storage for up to 24 hours.[30] In our series, there was one survivor after contralateral nephrectomy at 24 days, an animal which behaved so differently from the others in its group that no significant conclusions could be drawn. This dog was still alive 60 days after left nephrectomy but renal

function remained markedly impaired.

Hypothermia—especially in combination with other techniques—is the most promising method to preserve viable material required to achieve a state of animation subsequently.[27,31]

It is well known that tissues cannot survive long periods in the ordinary frozen state,[32-34] but methods have been evolved to alleviate the traumatic effects of freezing.[35-37] Currently, many tissues (including skin, glands, and cell suspensions of semen and blood) may be stored by freezing.[38-44] As yet, no one has successfully frozen and resuscitated a large organ such as a kidney or a heart.

Without expounding on the theory of injury through crystallization,[43] temperatures of –5°C to –10°C were used on the tentative supposition that hyperbaric oxygenation might protect the organ during thawing.[45] However, both with and without hyperbaric oxygenation, when the kidney was subjected to sub-zero temperatures, the cells were destroyed.

The use of 3 atmospheres absolute of oxygen was empirical. Most clinical and experimental work is done at this level.[45-48] The obvious reason for supplying increased oxygen tensions to a stored organ is to maintain satisfactory oxygenation with total ischaemia. The actual supply of oxygen to the kidney depends on gaseous diffusion into the immersate—and so to the organ. Whether any oxygen actually reaches renal cells via this route has not been shown, to our knowledge. It has been proved, though, that the kidney requires only 5% of its normal oxygen supply at 5°C.

Another reason for the use of hyperbaric oxygen was with the idea of minimizing damage resultant upon thawing,[45] though unsubstantiated experimentally. This theory did not apply as no improvement occurred, either pathologically or in functional result.

In the conditions of this experiment, hypothermia is singly of more value than hyperbaric oxygenation, to conclude from the functional rather than the pathological results. However, 3 ATA oxygen was of some benefit, as shown when combined with hypothermia. However, whether hyperbaric oxygen was used or not, once the kidney was frozen, the injury to the cells caused by freezing and thawing proved lethal.

The relationship of pathology to function is particularly applicable when considering the histological differences between kidneys evincing a transient diuresis and totally anuric kidneys. Studies by Oliver *et al.*[49] indicate that the renal tubules sustain the major damage in the ischaemic kidney. The regeneration of this type of renal damage involves the restoration of morphological and functional integrity to the tubular units. Transient diuresis is interpreted—and histologically confirmed—as evidence of tubular damage, whereas glomerular damage can also be shown in anuric kidneys.[24]

The importance of immediate renal function has already been stressed. In clinical practice it is essential not only to diagnose threatened rejection without doubt, but also for the patient to derive maximum immediate benefit from the transplanted organ. Neither of these requirements would have been satisfied by the clinical application of these experiments.

Currently, perfusion techniques are combined with hypothermia and hyperbaric oxygenation in an effort to improve immediate functional results, so that stored organs will be capable of life-sustaining function as soon as implanted. This work will be the subject of another article.

SUMMARY

A system for storing canine kidneys for 24 hours is presented. Using hypothermia with hyperbaric oxygen, immediate post-storage function is inadequate. Only if contralateral nephrectomy is delayed for 2 - 3 weeks is animal survival possible with the stored kidney alone.

We wish to thank Prof. J. H. Louw of the Department of Surgery for his continued and enthusiastic support of this work; also Dr. M. S. Barnard and Mrs. I. du Toit for technical assistance; Messrs. G. McManus and C. C. Goosen for help with the illustrations; and the other personnel of the surgical research laboratories for assisting in various ways. We are indebted to the C. L. Herman Bequest to the University of Cape Town and the J. S. Marais Surgical Research Fund for financial support of this work.

REFERENCES

1. Cleveland, R. J., Lee, H. M., Prout, G. R. and Hume, D. M. (1964): Surg. Gynec. Obstet., **119**, 991.
2. Dempster, W. J., Kountz, S. L. and Jovanovic, M. (1964): Lancet, **1**, 407.
3. Kiser, J. C., Farley, H. H., Mueller, G. F., Strobel, C. J. and Hitchcock, C. R. (1960): Surg. Forum, **11**, 26.
4. Pierce, J. C. and Varco, R. L. (1964): J. Surg. Res., **4**, 275.
5. Ackermann, J. R. W., Terblanche, J., De Villiers, D. R. and Barnard, C. N. (1965): S. Afr. Med. J., **39**, 295.
6. Ackerman, J. R. W. and Barnard, C. N. (1965): *Ibid.*, **39**, 571.
7. Kench, J. E. (1964): Personal communication.
8. Dowdle, E. B. (1965): Personal communication.
9. Dottori, O., Ekeström, S. and Hansson, L. O. (1962): Acta chir. scand., **124**, 80.
10. Human Kidney Transplant Conference (1964): Transplantation, **2**, 147.
11. Starzl, T. E., Marchioro, T. L., Rifkind, D., Holmes, J. H., Rowlands, D. T. jnr. and Waddell, W. R. (1964): Surgery, **56**, 296.
12. Starzl, T. E., Marchioro, T. L., Brittain, R. S., Holmes, J. H. and Waddell, W. R. (1964): J. Amer. Med. Assoc., **187**, 734.
13. Murray, J. E. and Harrison, J. H. (1963): Amer. J. Surg., **105**, 205.
14. Hitchcock, C. R., Kiser, J. C., Telander, R. L. and Peterson, T. A. (1964): Surgery, **56**, 533.
15. Knight, P. R., Tomkiewicz, Z. M. and Couch, N. P. (1963): Surg. Forum, **14**, 171.
16. Kiser, J. C., Telander, R. L., Peterson, T. A., Coe, J. I. and Hitchcock, C. R. (1961): Arch. Surg., **83**, 502.
17. Hendren, T. H. and Hardin, C. A. (1964): *Ibid.*, **88**, 206.
18. Terblanche, J., Isaacson, L. C., Eales, L. and Barnard, C. N. (1961): Surgery, **50**, 869.
19. Shields, T. W. and Lewis, F. J. (1959): *Ibid.*, **46**, 164.
20. Sealy, W. C., Young, W. G., Brown, S., Smith, W. and Lesage, A. (1960): *Ibid.*, **48**, 432.
21. Isaacson, L. C., Terblanche, J., Eales, L. and Barnard, C. N. (1964): *Ibid.*, **56**, 957.
22. Ariel, I., Bishop, F. W. and Warren, S. L. (1943): Cancer Res., **3**, 448.
23. Karow, A. M. and Webb, W. R. (1964): Surg. Gynec. Obstet., **119**, 609.
24. Schloerb, P. R., Waldorf, R. D. and Welsh, J. S. (1959): *Ibid.*, **109**, 561.
25. Kameya, S., Oz, M., Neville, W. E. and Clowes, G. (1960): Surg. Forum, **2**, 190.
26. Kerr, W. K., Kyle, V. N., Keresteci, A. G. and Smythe, C. A. (1960): J. Urol. (Baltimore), **84**, 236.
27. Levy, M. N. (1959): Amer. J. Physiol., **197**, 1111.
28. Stueber, P. J., Kovacs, S., Koletsky, S. and Persky, L. (1958): J. Urol. (Baltimore), **79**, 793.
29. Bogardus, G. M. and Schlosser, R. J. (1956): Surgery, **39**, 970.
30. Slapak, M., Brooks, D. K. and Calne, R. Y. (1964): Brit. J. Surg., **51**, 469.
31. Humphries, A. L. jnr., Moretz, W. M. and Pierce, E. C. II (1964): Surgery, **55**, 524.
32. Lovelock, J. E. (1953): Biochim. biophys. Acta (Amst.), **10**, 414.
33. Lovelock, J. E. and Smith, A. U. (1959): Ann. N. Y. Acad. Sci., **80**, 487.
34. Walton, A. (1957): Proc. Roy. Soc. B., **147**, 508.
35. Stulberg, C. S., Peterson, W. D. jnr. and Berman, L. (1962): Nat. Cancer Inst. Monogr., **7**, 17.
36. Connaughton, P. J. and Lewis, F. J. (1961): Surg. Forum, **12**, 177.
37. Lovelock, J. E. and Bishop, M. W. H. (1959): Nature (Lond.), **183**, 1394.
38. Polge, C. (1957): Proc. Roy. Soc. B., **147**, 498.
39. Sherman, J. L. and Lin, T. P. (1958): Proc. Soc. Exp. Biol. (N.Y.), **98**, 902.
40. O'Brien, T. G., Haynes, L. L., Hering, A. C., Tullis, J. L. and Watkins, E. jnr. (1961): Surgery, **49**, 109.
41. O'Brien, T. G. and Watkins, E. jnr. (1960): J. Thorac. Surg., **40**, 611.
42. Billingham, R. E. and Medawar, P. B. (1952): J. Exp. Biol., **29**, 454.
43. Brown, I. W. jnr. and Hardin, H. F. (1953): Arch. Surg., **66**, 267.
44. Playfair, J. H. L. and Davies, A. J. S. (1964): Transplantation, **2**, 271.

45. Manax, W. G., Bloch, J. H., Longerbeam, J. K. and Lillehei, R. C. (1964): Surgery, **56**, 275.
46. Symposium on Hyperbaric Oxygenation (1965): Ann. N. Y. Acad. Sci., **117**, 647.
47. Meijne, N. G., Sluyter, M. E., Eloff, S. J. P., Schoemaker, G., Schripsema, L., Dean, L. and Boerema, I. (1964): Surgery, **56**, 519.
48. Humphries, A. L., Russell, R., Ostafin, J., Goodrich, M. and Moretz, W. H. (1963): *Ibid.*, **54**, 136.
49. Oliver, J., MacDowell, M. and Tracy, A. (1951): J. Clin. Invest., **30**, 1305.