IN VITRO KIDNEY STORAGE

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GENERAL INTRODUCTION.

HISTORICAL REVIEW.

It has long been man's dream to replace in some way the diseased parts of his body. The first allusion to transplantation is to be found in "The Golden Legend", written by Jacobus de Voraigne in the 13th Century. His story involves the Saints Cosmos and Damian and their successful use of a cadaver limb to replace one affected with malignancy in a devoted member of the Early Church. Another "early success" is recorded by the 15th Century poet Elisio Calenzio, in the legend of the "Sympathetic Slave". These transplants apparently were dependent on some mystical sympathy between graft and host, which ceased at death.

In 1771, the great John Hunter evidenced an interest in transplantation, even to the extent of attempting heterotransplantation.

Experimental studies of renal homotransplantation reported in the literature lend themselves to division into two periods. The first covers the era from 1902 to 1949, when interest was focussed on technical detail and to proving that autotransplants would function well enough to sustain life whereas homotransplants would function only briefly. The second period dates from 1950 to the present, when interest shifted to more precise definition - or redefinition - of auto- and homograft function, to the immunological mechanisms disposing to homograft rejection, and to the acquisition and storage of suitable grafts.

It is the last mentioned aspect of homotransplantation which is the concern of this thesis.

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T E R M I N O L O G Y.

The terms listed below, many of which are used in this thesis, imply the degree of antigenic similarity between donor and host and, in some instances, identify the transplantation procedure employed.

Dempster (1955) suggested that the term "implantation" or "grafting" be used only to denote the transfer of pieces of tissue or skin which do not effect an immediate blood supply, while "transplantation" be used to denote whole organ transfer. Although sound, this scheme has not been adopted generally because the terms have been interchanged so often in the literature that to redefine them at this stage would lead to greater confusion.

AUTOTRANSPLANTATION — The transfer of an organ from its original site to some other part of the same body, with anastomosis of its vascular supply to some other artery and vein.

REPLANTATION — The removal of an organ and replacement in its original site with reunion to its original vessels.

EXPLANTATION — The moving of an organ to another site without division of its vascular pedicle.

ISOTRANSPLANTATION — The transfer of an organ, or tissue, from one individual to another of identical genetic make-up, e.g. monozygotic twin.

HOMO-ISOTRANSPLANTATION — The transfer of an organ, or tissue, between genetically similar (closely inbred) although not identical animals.

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PRIMARY HOMOTRANSPLANTATION — The transfer of an organ or tissue from one animal (person) to another of the same species without close genetic similarity as a consequent of repetitive in-breeding or development from the same ovum.

This definition thus includes chimeras and animals with acquired tolerance following in utero injections of donor tissue, but excludes closely in-bred strains (homo-isotransplants) and identical twins (isotransplants).

SECONDARY HOMOTRANSPLANTATION — The transplantation, to the same recipient of primary homotransplantation, of a second organ or tissue from the donor of the prior primary transplant.

SECOND HOMOTRANSPLANTATION — The transplantation to the recipient of a second organ or tissue from a donor other than the donor of the primary homotransplant.

RETRANSPLANTATION or RE-IMPLANTATION — The removal of the homotransplant from the recipient and replacement in the original donor.

HETEROTRANSPLANTATION — The transfer or an organ or tissue from one animal to another of a different species.

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INTRODUCTION TO KIDNEY STORAGE.

In the rapidly expanding field of transplantation of today, suitable renal grafts are acquired from three sources:

(1) LIVE DONORS - from closely related and therefore consanguineous donors, or from totally unrelated donors;

(2) CADAVERS - Here consanguinity is impossible and the considerations for selection depend on a multitude of factors related to age, anatomical suitability, cause of death and rapidity of the dying process;

(3) PRIMATES - I.e. heterogenous grafts - limited use has been made of both baboon and chimpanzee kidneys.

The difficulties of antigenic dissimilarity attendant upon the use of totally unrelated homografts (whether from cadavers or live donors) or of live heterografts, are not the concern of this thesis. Vitally important factors govern the use of live donors as opposed to cadaver grafts but, so limited has been the experience of live heterografts and so poor the results, that the practicability of large scale clinical use appears unlikely.

Without doubt, the clinical results of the use of live donor grafts are far superior to those obtained with cadaver organs. Rather than reflecting immune responses of greater virulence from cadaver grafts, this is the result of the extent of ischaemic and anoxic renal damage present. The ischaemia results largely from

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the time lag between nephrectomy of the cadaver and completion of the vascular anastomoses in the host, when a proper blood supply is reconstituted to the transplanted kidney. This delay is unavoidable and necessitates some method of storage to obviate anoxic damage.

The questions which spring to mind are: why not transplant live donor kidneys only, and what, if any, are the indications for using cadaver grafts?

ETHICAL CONSIDERATIONS.

A surgeon who proposes to remove a healthy kidney from a living donor for transplantation assumes a tremendous moral responsibility. It is a tribute to human nature how often the relatives and friends of a dying uraemic patient will offer to donate one of their own healthy kidneys if there is even an infinitisimal chance of the transplant succeeding.

The probabilities of the success of any operation can only be estimated by the surgeon and, in most surgical decisions, the likelihood of success has only to be considered in relation to the sick patient himself. However, in the case of renal transplantation from a living donor, the risk to the sick patient must be assessed and then must be related to the risk of unilateral nephrectomy in the healthy donor - a far more difficult task for the clinician. Even after carefully determining that both the donor's kidneys function efficiently, the risks of nephrectomy though small are not negligible; there is also the possibility that disease may occur in the donor's remaining kidney in later life.

There is no precedent to guide the surgeon in this extremely difficult decision.

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If the results of homotransplantation ensured a complete cure, good reason would exist for using live donor organs. However, in proceeding with transplantation in a situation less satisfactory, the surgeon shoulders an excessive moral burden.

NUMERICAL CONSIDERATIONS.

It is impossible to accurately assess the number of patients in a given population whom renal transplantation might benefit, if the biological problems were solved. The number is doubtless considerable, as the following considerations show, even if allowance is made for the unknown errors which are possible in data collection.

The Statistical Review of England and Wales for the year 1959 (published by the Registrar-General in 1961) shows that between 6,000 and 7,000 persons died that year out of a population of about 45 million. Uraemia is thus responsible for about 1% of all deaths (for comparison, carcinoma of the stomach accounts for about 2%). Forty per cent of deaths from nephritis and nephrosis in England and Wales occurred between the ages of 5 and 55 years. These figures give no indication of the number who would have been suitable for operation. No comparable South African figures are available.

If 2,500 people (40%) who died from uraemia in England and Wales during 1959 had been eligible for transplantation, and live donor kidneys had been transplanted, a significant population of unilaterally nephrectomised individuals would arise. Equally, were this the only source of kidney grafts, the whole concept of renal transplantation in man would lose its practicability and would be limited to a highly selected, numerically small, group of patients. A limiting factor like this would reduce the value of transplantation almost entirely.

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In contrast, the availability of cadaver organs is unlimited. With proper preparation and storage facilities, these could and should meet the demand, however large the number of potential recipients.

A scientific contra-indication to further consideration of live donor grafts, which may be introduced at this juncture, is the high incidence of a curious complication of obliterative and other vascular lesions. Involving kidneys some time after homotransplantation, this inevitably leads to anuria and to the eventual death of the patient (Dempster et al, 1964).

The most significant reason precluding the use of live donor organs is the slender chance of any lasting success. At present, the results of prolonged dialysis are comparable with those of transplantation. This suggests that transplantation is still far from being a curative procedure. In this light, to subject a normal, healthy individual to a unilateral nephrectomy is strongly felt to be unthinkable.

Thus, there are many reasons which point to the inclusion of cadaver organs in any transplantation programme. Successful storage methods are essential to the existence of the future of this exciting new science.

In the world today, there is a small number of highly selected patients who have received renal grafts. The number has been restricted not for lack of suitable recipients or organs, but because of the disappointing results. Very recently these results have improved and, in view of the fantastic pace of research into the attenuation or abrogation of the rejection phenomenon, the outcome of renal transplantation procedures can be expected to improve steadily. More and more transplants will be performed and this will make the development of suitable organ acquisition and successful storage essential.

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In practice, there are three different periods during the transplantation procedure when the kidney to be grafted is exposed to anoxia: three periods, therefore, when it is devoid of an adequate blood supply.

1. The time lag which must exist between the death of the donor and the excision of the kidney.

In point of fact, this may include a variable pre-mortem episode of relative ischaemia, the result of hypotension, anoxia and low output cardiac states. There is also a delay in actual removal of the kidney once the diagnosis of death has been confirmed.

There has been much research into the proper preparation of cadavers where graft acquisition is contemplated, incorporating methods to combat anoxia, e.g. total extracorporeal circulation (Marchioro et al, 1963). Preferably, donors should be young, be free of infection, metastases or renal disease, and death should not be preceded by prolonged anoxia or hypotension, predisposing to acute renal failure.

Naturally, these requirements limit the use of cadaver material but opportunities are seen in a neurosurgical ward. Minor ethical problems exist with regard to consent. There may also be some indication for protection of the kidney by the use of Mannitol or one of the peripheral vasoconstrictors. However, these problems do not operate in experimental renal storage.

2. The second anoxic phase is concerned with temporary storage, once the kidney has been removed from the donor.

As the organ will already have been subjected to unavoidable partial, and some total, ischaemia, a technique of organ preservation is essential. As will be discussed later, the technique in general use is that of immediate hypothermic perfusion.

3. The third delay exists while the vascular anastomoses are completed post-storage.

Although this ischaemic period is significant, in comparison

with the other two already discussed it is of relatively short duration. Methods are available to counter anoxia at this stage (Markland and Parsons, 1963), but it is generally agreed that any delay at this time is insufficient to warrant the technical problems of resuturing.

For practical purposes, organ storage is concerned with two main sets of circumstances:

A. Short term storage:

This is necessary when the prospective recipient is hospitalised awaiting the availability of a suitable cadaver graft. When the kidney is obtained, functional viability must be maintained until operating theatre and patient have been prepared and the operative exposure has been performed. The total time should not amount to more than 5 hours; successful storage is possible for this period by means of simple hypothermia.

B. Organ "Bank Storage":

In the event of a patient presenting in renal failure, a kidney graft would be immediately available. Short term storage is not feasible to achieve this, unless the supply of cadaver grafts was unlimited. A successful "bank" depends firstly on the utilisation of every suitable cadaver graft, and secondly on sustaining sufficient kidneys for a period long enough to ensure the supply to an actively functioning unit.

Current research to prolong organ storage has been along in vitro lines. The predominant channels of investigation are hypothermia, perfusion, hypothermia with perfusion and, more recently, hyperbaric oxygenation with either one or both of the other methods mentioned. In the review of the literature it will be seen that there has been very little success in attempted storage of over 12 hours using these techniques, although in vitro methods for short term storage are already in successful practice.

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In most experimental projects, 24 hours has become the period for which successful storage is attempted. Although this period is not adequate for an organ "bank", if adequate techniques were available the unhurried and careful preparation of the patient (and also the exciting possibility of functional assessment of the kidney graft during storage) would be possible.

The storage of whole organs for transplantation is a recent development. However, a tremendous store of renal physiological data has been accumulated by the study of isolated kidneys maintained by in vitro techniques. Furthermore, the metabolic effects of hypothermia on isolated organs has also received close attention, using such methods as simple immersion cooling and hypothermic perfusion. All this information is obviously of great value in attempting any technique directed at the maintenance of normal or near-normal functional viability of an isolated organ. For this reason, the relevant physiological effects of hypothermic perfusion and hyperbaric oxygenation are presented in the review of the literature.

Another reason, too, has made organ protection necessary in modern surgery. The use of hypothermia (to lower tissue oxygen requirements and metabolic processes) is established both in neuro-surgery and intracardiac surgery. Of more direct application to kidney storage is the in situ hypothermic perfusion of kidneys required during the resection of abdominal aneurysms involving both renal arteries. Radical cancer surgery, too, may require such methods for the total clearance of involved tissues.

Irrespective of the storage method used, it must be strongly emphasised that post-storage function is the only true reflection of success of the method employed. For any storage procedure to be acclaimed truly successful, the stored kidney must be capable of sustaining life in a bilaterally nephrectomised animal. To gauge the relative merits of different storage procedures on

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histological grounds is arbitrary and is largely valueless. Although ischaemia denotes a specific entity in these experiments, in any attempt at in vitro storage the organ is without its normal blood supply and thus, to some extent, is inadequately oxygenated.

There are many instances of the inconclusive correlation of functional capacity with the pathological picture present. The effects of renal ischaemia are recognised both clinically and pathologically as "acute tubular necrosis". Pathologically, a particularly wide range of changes are recognised, ranging from changes seen only on electronmicroscopy to total destruction of all the elements of renal architecture: all being denoted as "acute tubular necrosis". Perhaps the most important point in this respect is the well-recognised entity of a normal histological picture despite the presence of gross functional impairment, which culminates in the death of the patient.

In clinical kidney homotransplantation, it is vital that the stored organ functions satisfactorily immediately after it is transplanted. The patient must derive maximal immediate benefit from the kidney graft and, more important, function must be sufficient to facilitate the positive diagnosis of threatened rejection, without any doubt, when it occurs.

Two further points, perhaps not so important, to stress the need for immediate post-storage function stem from the necessity for bilateral nephrectomy of the recipient, either before or at the same time as the homotransplantation is performed. If the kidneys are left in situ the transplanted kidney may develop the same disease as was originally the problem. Alternatively, if the patient is hypertensive, this in turn might affect the vasculature of the transplanted kidney.

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Experimentally, the theory of counterbalance has very special application to the kidney. It would seem that its relationship in man is less significant but, to ensure the proper function of the transplant, it is obviously desirable to exclude any possibility of counterbalance by performing a simultaneous bilateral nephrectomy in the patient.

EXPERIMENTAL AIM.

The aim of these experiments is to establish a method to ensure that homotransplanted kidneys are capable of life-sustaining function immediately after 24 hours of in vitro storage.

REVIEW OF THE LITERATURE.

THE PHYSIOLOGY OF RENAL HYPOTHERMIA.

1. KIDNEY SURVIVAL WITHOUT HYPOTHERMIA.

The extent of ischaemic renal damage is extremely important in cadaver kidney transplantation and probably has been responsible for more failures than immunological reactions.

In 1880, Litten demonstrated that the renal pedicle may be occluded for 1 hour before microscopic damage can be observed. Eisendrath and Strauss studied the kidneys of rabbits in 1910, and found residual evidence of injury 4 weeks after a period of 15 minutes' temporary anoxia. Prather (1934), in suggesting the use of a rubber-shod pedicle clamp to occlude renal hilar vessels in renal surgery, cautioned that this should be released at 8 to 10 minute intervals.

Using previously unilaterally nephrectomised dogs, Van Slyke and his associates (1944) studied longer periods of renal anoxia. In their experiments, occlusion of the renal artery and vein for 3 hours resulted in a brief period of anuria followed, after 2 to 3 days, by a gradual rise of the blood urea ~~nitrogen~~ to approximately 150 mgm./100 ml. By the 4th day, however, return to normal levels was noted. Survival in this group was close to 100% but when the anoxic period was extended to 4 hours 50% of the animals died, and after 6 hours' anoxia the mortality was 100%.

There is little doubt that occlusion of both artery and vein is better tolerated than occlusion of the vein alone (Vermeulen, 1949; Mundth et al, 1964).

In the course of renal transplantation, once the kidney is removed from the donor it starts to cool towards room temperature. If (as was the case in Van Slyke's experiments in 1944) the kidney is kept at body temperature, greater tubular damage occurs.

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In a preliminary series of experiments in rabbits (Calne et al, 1963) one kidney was removed and the opposite renal pedicle was clamped for varying periods, the kidney remaining in the peritoneal cavity at body temperature. With 1½ hours of ischaemia 4 out of 9 animals survived. Proximal tubular necrosis was observed in the rabbits that died within the first 3 weeks.

Similar results have been reported by others in the rabbit (Sheehan and Davis, 1959), in the dog (Hamilton et al, 1948; Bogardus and Schlosser, 1956; Moyer et al, 1957; Stueber et al, 1958; Birkland et al, 1959; Kiser et al, 1961; Cleveland et al, 1964) and in sheep (Mitchell and Woodruff, 1957).

The following conclusions emerge:-

1. If the total ischaemic period is less than 60 minutes, adequate renal function may be expected immediately after operation with only temporary abnormalities;
2. If the ischaemic period is between 1 and 2 hours, a varying degree of tubular necrosis is probable;
3. If the ischaemic period is over 3 hours, irreversible damage is probable and the kidney may never function sufficiently well to maintain life.

2. HISTORICAL REVIEW OF RENAL HYPOTHERMIA.

Although the protective effects of hypothermia have long been recognised, more recently the introduction of total body hypothermia as an adjunct to both cardiac surgery and neurosurgery has redirected attention to the benefits of local cooling in reducing kidney damage during ischaemia of this organ. There is no doubt that cooling the kidney decreases anoxid damage and permits a longer ischaemic period (Birkland et al, 1959; Kerr et al, 1960; Kiser et al, 1960; Macksood et al, 1961; Mitchell, 1959; Schloerb et al, 1959;

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Stueber et al, 1958; Matloff and Glower, 1962; Jones and Politano, 1963; Murray and Holden, 1954; Archibald and Cawley, 1956).

As early as 1924, Avramovici transplanted dog kidneys which had been preserved at cold temperatures for varying periods of time, up to 30 hours. One kidney thus preserved for 8 hours was then homotransplanted, one of the host's kidneys being removed, and the animal is said to have lived for 36 more days. The lack of detailed studies and the failure of others to approach the level of success reported by Avramovici render these results highly suspect, however.

In 1948, Oudot placed canine kidneys on ice at 4°C for as long as 8 days before homotransplanting them. He obtained uniformly poor results, all kidneys infarcting as soon as the blood flow was restored.

Using dogs, Lefebvre in 1951 removed the kidney from the donor and perfused the organ with a saline solution at a pressure of 140-160 mm.Hg until the effluent from the renal vein was clear. The kidney was placed in physiological fluid on ice for from 3 to 24 hours, sometimes being cooled to as low as 1°C, and was then re-implanted in the neck. Urine flow was less rapid than in an immediate transplant. Sometimes a bilateral nephrectomy was done, or urea was injected, to stimulate diuresis. The best urea concentration obtained was five times that of blood.

Lefebvre concluded that the kidney may be kept at a low temperature for up to 24 hours and still show a return of function, although recovery is always less than normal. These experiments were all short term, over a few hours, and no information is available as to whether recovery would have been progressive.

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3. THE EFFECT OF HYPOTHERMIA ON GLOMERULAR AND TUBULAR FUNCTION.

In homeothermic animals biochemical processes function in harmony at 37°C, and below this temperature there is an imbalance of interdependent reactions. As the body temperature of mammals is lowered, the various metabolic functions slow at different rates. At 30°C, for example, respiration ceases although the heart beat continues. At 15°C the heart stops but the cells of the body still function and require oxygen and metabolites.

Enzymatic activity continues at temperatures far below 0°C. For example, blood cells decay significantly in a month, even at -80°C. If enzyme activity is allowed to continue, cells are killed by autolysis or self-intoxication (Joslyn, 1952). One of the problems of the use of hypothermia is to suspend the vital processes by rapid cooling to profound hypothermic levels, and yet to prevent the destruction of the cells by freezing the tissue fluids.

Bickford and Winton (1937) observed that cooling an isolated blood-perfused canine kidney to as low as 3°C does not abolish the concentration gradient of creatinine between urine and serum. They reported a minimum urine/plasma creatinine concentration ratio of 1.5 Cr. Their results suggest several possible reasons for the failure to wash out all tubular fluid formed at higher temperatures. There may be some residual active transport of water, or the re-absorption of water may be the result of osmotic gradients between the capillary plasma and protein-free tubular fluid.

The same workers also found that, at temperatures below 18°C, the chloride concentration of urine becomes indistinguishable from the chloride concentration of serum. They suggested that the tubular transport system for chloride is more markedly influenced by temperature than is water transport.

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Cort and Kleinzeller (1958) studied the effect of temperature on the transport of sodium and potassium by dog kidney cortex slices. On incubation after leaching, they found that the slices extrude sodium at a faster rate than they accumulate potassium, and sodium extrusion was more affected by temperature changes than potassium uptake.

Harvey (1959) followed up this work with extremely well conducted experiments, studying the effects of cooling on an isolated canine kidney, using a heparinised dog as a source of arterial blood. He monitored blood flow, oxygen consumption, carbon dioxide production and the excretion of inulin, creatinine, PAH, sodium and potassium. Cooling was found to reduce blood flow and gaseous metabolism, and urine concentrations of tested substances approached plasma concentrations, with reversal of these changes on warming. The net movement of PAH into the tubule was found to be highly sensitive to cooling, indicating that the rate limiting reactions involved have high energy characteristics. Water and sodium net movements were more resistant to cooling: the least change resultant upon cooling was reflected in the glomerular filtration rate. The net movement of sodium out of the tubule, and the concentration gradient apposing this movement, do not remain proportional during hypothermia. It was also suggested that there may be some residua l active transport of water and other substances at temperatures below 15^{o}C.

Another physiological observation of possible importance, made by Smith et al (1965), is that the resultant combined metabolic and respiratory acidosis of total body profound hypothermia is not reflected by diminished urinary pH. To account for this, there is an increased excretion of bicarbonate, coupled with a rise in the output of inorganic phosphate.

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Isaacson et al (1964) studied renal function during and immediately following profound hypothermic cardiopulmonary bypass. They found the initiation of active bloodstream cooling to produce a sudden rise in glomerular filtration rate; however, on further cooling, this dropped markedly. Interestingly, where present, proteinuria diminished or disappeared during profound hypothermia, and then reappeared (or increased) during rewarming. This phenomenon was attributed to variations in glomerular permeability to protein, which diminished during cooling and increased during the rewarming phase of the extracorporeal procedure. Urinary osmolality, sodium, potassium, calcium, magnesium and PAH concentrations approximated those of the serum at 10°C - 12°C. This must reflect the inhibition of tubular mechanisms for reabsorption and/or secretion of these substances. The urinary inorganic phosphate concentration changed but little with cooling, and even at 10°C was far from approaching that of serum. The percentage excretion of filtered water, sodium, calcium and magnesium rose on cooling. That of filtered inorganic phosphate rose too, but to a strikingly lesser degree. These increases - like the alterations in urinary concentrations - must reflect the cold-induced inhibition of their reabsorptive mechanisms. Unlike the other electrolytes, they found it impossible to derive a consistent relationship between potassium excretion and body temperature.

All this data strongly indicates the marked inhibition of renal tubular function by cold. In fact, it has been amply shown that hypothermia depresses a variety of renal tubular functions (Andersen and Neilsen, 1955; Page, 1955; Segar et al, 1956; Hong, 1957; Morales et al, 1957; Blatteis and Horvath, 1958; Couch et al, 1958; Kanter, 1959; Hong and Boylan, 1959).

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4. RENAL OXYGEN CONSUMPTION.

One of very few studies in which the effects of hypothermia upon the rate of oxidative metabolism of the intact kidney were determined, was performed by Bergstrand and Sterky in 1954. They estimated renal blood flow in 4 dogs by clearance techniques. It was noted that, at the hypothermic levels produced (18°C - 20°C), the arterial pressure frequently dropped to levels which severely impeded the glomerular filtration rate. The resultant low amount of urine formation would tend to vitiate the estimation of renal blood flow, upon which the calculation of oxygen consumption is based.

In 1959, Levy employed an isolated kidney technique to permit a constant arterial perfusion pressure, and to allow the more direct measurement of renal blood flow. The changes in renal oxygen consumption were determined over a wide range of temperatures, from 6°C to 41°C. Lower temperature levels resulted in an appreciable reduction of renal blood flow at constaht arterial blood pressure; increased blood viscosity and vasoconstriction were both responsible for this reduction of flow. Hypothermia also brought about a reduction in arterio-venous oxygen difference, roughly proportionate to the centigrade temperature. Furthermore, hypothermia exerted a marked but reversible depression of the rate of oxidative metabolism. This renal effect was relatively more severe than the changes for the body as a whole at equivalent temperatures, reported by others. Thus, at 30°C the oxygen consumption was approximately 43% of the value at 39°C, at 20°C it amounted to only 16% of the control rate, and at 5°C oxygen consumption was less than 5% of normal.

In 1960, Semb et al showed the oxygen consumption of a kidney to vary essentially, in an exponential way with temperature. Their results indicate that the general oxygen metabolism can be suppressed

sufficiently ....

sufficiently by the use of a relatively simple method of local hypothermia.

Fuhrman and Field (1942) studied the influence of temperature on the oxygen consumption in rate kidneys and found an exponential decrease with decreasing temperatures, to the extent that renal cortical respiration dropped to almost unrecordable levels at 0.2°C.

5. THE OPTIMAL LEVEL OF HYPOTHERMIA.

Having established that hypothermia affords protection to the ischaemic, and therefore anoxic, kidney - both by the application of observed physiological data and by more direct research, the consideration of the optimal level of hypothermia for renal preservation is the next obvious step in the sequence.

It is established that, after 6 hours of total ischaemia under normothermic conditions, all dogs die from acute renal failure (Stueber et al, 1958; Van Slyke et al, 1944). Stueber found that when the kidney of the same animal is cooled to 0°C to 5°C all animals survived, but at higher temperatures of 20°C to 25°C there was only 30% survival. It appears that cooling to temperatures above 25°C produces only minimal improvement (Mitchell and Woodruff, 1957; Moyer et al, 1957). At 15°C to 25°C the improvement is more useful, 2 hours' ischaemia being well tolerated (Bogardus and Schlosser, 1956; Mitchell and Woodruff, 1957; Dottori et al, 1962), and even after 6 hours' ischaemia at this temperature level some success has been reported (Stueber et al, 1958).

Although it is generally accepted that not until the temperature range of 5°C to 15°C is reached, that really significant improvements are obtained, there have been exceptions to this. Wickham and Taylor (1965), in a trial of various grades of hypothermia, concluded that the region of 20°C provides the most efficient protection of renal function during total renal ischaemia. Mitchell and Woodruff (1957)

recommended ...

recommended that the renal temperature should not be reduced below 10°C, because permanent physiological damage may be inflicted on the renal tubules below this level.

Within the range of 5°C to 15°C, 6 to 7 hours of ischaemia is tolerated in a high percentage of cases (Stueber et al, 1958; Birkland et al, 1959). At 0°C to 5°C the ischaemia may be extended to 8 hours, but not to 12 hours (Schloerb et al, 1959).

Studies using the isolated kidney with subsequent auto-transplantation and contralateral nephrectomy have been less extensive. Schloerb and his associates (1959) used this technique but were troubled by thrombosis in the re-implanted kidney. Nevertheless, they succeeded in preserving excised kidneys for 4 hours at 0°C. However, when attempting to prolong preservation to 24 hours, all the animals died - even though the opposite nephrectomy was delayed for 3 to 6 weeks.

Kiser et al (1960) used a blood-dextran perfusion technique to obtain a renal temperature of 1°C to 5°C, and achieved satisfactory function after 7 hours of ischaemia.

Lapchinsky (1960) preserved canine kidneys, cooled to between 2°C and 4°C, using perfusion techniques in some experiments and surface cooling in others. He obtained good function in some kidneys after 24 hours' ischaemia, but from his report it is not clear which experimental procedures were responsible for the satisfactory results.

Humphries et al (1962) perfused canine kidneys at 4°C to 10°C with Ringer-plasma mixtures, and obtained two functionally successful autotransplants after 24 hours' storage and late contra-lateral nephrectomy.

In 1963, Calne et al found that, after preservation for periods of up to 12 hours with surface cooling (ice), the kidneys could withstand the severe test of an immediate opposite nephrectomy.

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Between 12 and 17 hours of re-implantation the damage was sometimes partially reversible. However, in one experiment at 17 hours and in both experiments at 24 hours, severe and irreversible damage resulted.

F R E E Z I N G.

1. THE MECHANISM OF FREEZING.

The most promising method for the preservation of viable material is by means of a state of suspended animation through profound hypothermia. It is well known that tissue cannot long survive the ordinary frozen state, but methods have been evolved to alleviate the traumatic effects of freezing. Currently, many tissues - including skin, glands, and cell suspensions of semen and blood - may be stored by freezing. However, with the possible exception of Deshpande and Jacob (1963) no-one has successfully frozen and resuscitated a large organ, such as the kidney or heart.

There are three methods by which water may enter the solid state (Karow and Webb, 1964).

(1) Heterogenous nucleation, occurring when cooling is slow, with ice forming around large molecular inclusions of non-aqueous substances. The freezing ordinarily found in nature results from heterogenous nucleation, because the cooling rates rarely exceed 1°C per minute.

(2) Homogenous nucleation: this may result from faster rates of cooling (50 - 100°C per minute) when innumerable minute crystals develop around the natural molecular clumps of water.

(3) Vitrification: entailing the transformation of water into an amorphous glass, rather than a crystal, this can only be achieved through ultra-rapid cooling (Meryman, 1956, 1957, 1960, 1962). Theoretically, vitrified water enters the solid phase without deadly effects on the tissues. To say the least, it is difficult to achieve as water must be cooled in 1 second to under -130°C,

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and maintained below that temperature. Only two well documented reports of this achievement are to be found and it is possible that vitrification was not complete even there (Burton and Oliver, 1935; Pryde and Jones, 1952).

2. THERMAL INJURY.

Why freezing should be lethal is still a mystery. Profound cooling per se seems to adversely affect the living organism. The irreversible and lethal effects of extreme changes in temperature, termed "thermal shock", appear to be the product more of rapid cooling than of thawing (Lovelock, 1953, 1959), and are unrelated to the differential slowing of biochemical reactions (Walton, 1957).

Motility, respiratory activity and glycolysis are reduced in spermatozoa cooled faster than 0.25°C per minute (Blackshaw and Salisbury, 1957). Red blood cells may undergo haemolysis (Lovelock, 1955) and even such relatively stable molecules as nucleoprotein may be affected (Lovelock, 1957). Lochte et al (1958) found that sudden chilling of human bone marrow mixed with glycerol slightly inhibits desoxyribonucleic acid synthesis, but repeated chilling depresses desoxyribonucleic acid synthesis "appreciably".

Many theories have been propounded to explain the mechanisms of thermal injury. However, none is wholly acceptable.

3. WHOLE ORGAN PRESERVATION.

The actual preservation of organs at sub-zero temperatures has been infrequently attempted. Techniques have been devised to store many mammalian tissues at temperatures below 0°C, such as spermatozoa (Polge, 1957; Sherman, 1957), ova (Sherman and Lin, 1958), blood (Brown and Hardin, 1953; Jones et al, 1957; O'Brien and Watkins, 1960; Weiss and Ballinger, 1958), skin (Billingham and Medawar, 1952), cornea (Brown and Hardin, 1953), dura

(Crawford ...

(crawford, 1957) and the thymus (Playfair and Davies, 1964).

The paucity of documented work on the super-cooling of whole organs is striking. It has been reported that investigators in Russia have perfected a technique for the viable storage of whole organs at +2°C for 27 to 48 hours (Berman, 1958, 1960).

Barsamian et al (1959) first approached the problem of organ preservation by vacuum dehydrating and super-cooling dog hearts. In 15 attempts, 7 hearts with 20-55% of their water removed could be resuscitated upon transplantation, even after 20 hours of storage at -8°C. These hearts functioned for an average of 40 hours. The organs thus treated were never actually frozen, however. Barsamian recognised that the method has only limited practicability and does not allow a significant extension of the storage period.

Webb and Howard (1958) found that dog hearts may be refrigerated at 4°C in a nutrient medium for at least 8 hours, and return to completely normal function upon homotransplantation.

Jacob and his associates (1958) attempted to store kidneys at low temperatures and, although this method did not achieve any functional success, it deserves attention. Through the arterial circulation, they introduced helium which had been chilled in liquid nitrogen, thus reducing the temperature of the organ to -180°C in 2½ hours. After the kidneys had been thawed with warm helium, clear urine formation was possible for up to 2 hours.

4. <u>WHOLE ANIMAL PRESERVATION</u>.

Although there have been almost no other attempts to preserve whole organs, much of the knowledge derived by freezing live mammals can be applied to organ preservation. In elaborate investigations, Andjus and Lovelock (1955), Lovelock and Smith (1956) and Smith (1956, 1957, 1961), have frozen and resuscitated mice, rats, hamsters and rabbits. Animals were cooled in chilled containers to

approximately ...

approximately 15°C, were then immersed in melting ice and finally were immersed in cold baths to below 0°C. Extensive studies on the hamster revealed that resuscitation is possible, even if over 45% of the total body water is converted to ice. If heterogenous nucleation is initiated above -3.5°C survival is possible, but if ice formation is initiated at temperatures below that level the prognosis is poor.

Using hamsters, four methods of warming were studied by Lovelock and Smith (1956). Some animals were allowed to warm spontaneously at room temperature, others were warmed either by immersion in a warm water bath at 37°C or by heating with a 120 watt diathermy unit. Diathermy was by far the most successful method.

5. SOLUTE MODERATORS.

The principal problem in freezing and thawing organs is presented by their size and density, which produce great temperature differentials during cooling and warming. Even an organ as small as a rat heart (1.5 cm. in diameter) cannot be quickly and uniformly frozen and thawed (Karow and Webb, 1961; Smith, 1957). The use of slow freezing and a protective chemical agent appears to be necessary.

It is difficult to attain solute moderator concentrations of sufficiently high levels in the organ before freezing, and many chemical substances have been tried. Glycerol, the solute moderator traditionally used in freezing, has its own toxic effects on the living system - although not as toxic as many other solute moderators (Lovelock, 1953; 1955; Lochte et al, 1958). Chloroform offers some promise as a protective agent in low temperature organ preservation (Connaughton and Lewis, 1961). Another promising solute moderator, dimethyl sulfoxide, was reported by Lovelock and Bishop (1959); Deshpande and Jacob (1963) used this as an anti-freeze agent in kidney storage with some success.

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No single solute moderator can be used with equal success for all tissues, since tissues vary in their sensitivity to a particular agent and it must be matched to the organ being preserved. This relationship has been little investigated.

6. THE RELATIONSHIP OF PRESSURE TO THAWING.

Pertinent to the problems of thawing or re-warming is the observation of Taylor in 1960, to the effect that a pressure of 35,000 lbs. per square inch applied in thawing, when the temperature reaches about -5°C, will induce the temperature to rise rapidly past 0°C without any plateaux. However, such pressures killed 80% of the in vitro human conjunctiva cells with which he was working.

Fortunately, a pressure of 15,000 lbs per square inch has the same effect on thawing and kills less than 5% of the cells.

These experiments prompted the use of hyperbaric oxygenation in sub-zero kidney storage in our experiments.

METHODS OF COOLING.

Although abundant literature is available concerning generalised hypothermia, it is only relatively recently that any attention has been given to the effects of hypothermia on renal function (Moyer et al, 1957; Terblanche et al, 1962, 1961; Isaacson et al, 1964).

Since Swan et al (1953) and other authors observed that generalised hypothermia significantly reduces renal damage resulting from periods of ischaemia, considerable effort has been directed toward the total preservation of renal function using various types of hypothermia. These methods include simple regional immersion and hypothermic perfusion techniques, both in vivo and in vitro, irrigation of the renal pelvis with cool saline (Jones and Politano, 1963), and generalised body hypothermia (Birkland et al, 1959; Semb, 1956; Bahnson, 1953; Ellis et al, 1955; Morales et al, 1957; Harsing et al, 1956).

THE IMPORTANCE OF RAPID AND UNIFORM REDUCTION IN RENAL CORE TEMPERATURE:

Extensive study is in progress on the problems of the uniform cooling and warming of organs. Theoretically, either can be accomplished by conduction or by radiation. Conduction occurs between two contiguous objects of different temperature; the faster-moving molecules of the warmer impart their energy to the colder object, until the temperatures equilibrate. Although radiation (e.g. diathermy) is very successful in warming, it is impractical for cooling, whereas conduction can be used for both warming and cooling.

Uniform heat distribution is desirable to minimise the effects of differential biochemical reaction rates in cooling and warming (Meryman, 1960). Meryman showed that cooling an organ to 0°C

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creates no real problem, but that the achievement of uniform heat distribution during cooling by conduction, in the temperature range of -5°C to -40°C is far more hazardous. In the kidney, cellular damage (particularly to the vascular endothelial and vascular system) is certain within the critical temperature zone.

Dempster et al (1964) regard as absurd any attempt to store kidneys below a temperature range of 4°C to 10°C. This is considered to be a safe range, although some success with storage above and below this range has been described.

The rapidity with which the renal core temperature is lowered is a vital factor in hypothermic protection of the kidney. Metabolic processes and oxygen consumption undergo depression with falling temperature. It is thus obvious that rapid, efficient and uniform lowering of the renal core temperature must be instituted immediately after the procurement of the kidney, or if possible even before it is resected.

There are three basic methods to lower the renal temperature:

1. Surface cooling by immersion of the organ in a cold solution,
2. Perfusion with cold solutions,
3. A combination of surface cooling and perfusion.

1. SURFACE COOLING AND ITS DISADVANTAGES.

Calne et al (1963) and Schloerb et al (1959) have reported successful short term canine renal graft preservation utilising surface cooling. However, certain problems arise when attempting to transpose the technique and data obtained for surface cooling in experimental animals to a clinical situation.

The time required to cool a canine kidney, weighing 50 gm., to a core temperature of 5°C is reported to be approximately 30 minutes (Cleveland et al, 1964). A human kidney is approximately four times that mass, and the time required to achieve a similar

temperature ....

temperature would be much prolonged and the protective effect greatly reduced.

Calne et al (1963) have reported 5 clinical instances where surface cooling was used as a method of kidney preservation, the anoxic period ranging from 78 minutes to 2½ hours. Acute tubular necrosis developed in all 5 patients. These results point to the need for the rapid production of low renal core temperature if the ill effects of anoxia are to be successfully combated by renal hypothermia.

2. PERFUSION COOLING.

In 1908, Alexis Carrel first proposed perfusion cooling as a means of renal preservation. Knight and his associates (1963) obtained very encouraging results in dogs with short term preservation, using cold perfusion under 140 mm.Hg pressure to decrease the renal core temperature to 5°C - 7°C.

With a similar perfusion technique combining surface cooling and perfusion, a renal core temperature of 4°C to 5°C can be achieved in dogs within 1 minute (Cleveland et al, 1964).

Given a choice between surface cooling and perfusion cooling, the latter is to be preferred for certain reasons:

(a) When surface cooling is carried out, the kidney must be enveloped in a plastic bag (or similar) through which the cooling fluid flows. There are technical disadvantages resultant upon this, with a real danger of renal parenchymatous damage.

(b) Kerr et al (1960), among others, have shown that in order to lower renal core temperature to 25°C - 30°C within 5 to 10 minutes, the temperature of the cooling fluid must be as low as 0°C - 1°C. They, and others (Mitchell and Woodruff, 1957), observe that such low immersate temperatures give

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the kidney a frosty appearance, caused by surface necrosis.

Though surface cooling may achieve an adequate renal core temperature drop sufficiently rapidly, it involves a significant risk of damage to the outer cortex of the kidney and might lead to fibrotic scarring. Using perfusion cooling, it is possible to lower the temperature to 20°C within 2 to 3 minutes without requiring an excessively low temperature of perfusate, and the decrease in temperature appears to be simultaneous in the cortex and the medulla (Dottori et al, 1962).

At its point of entry into the renal circulation, the temperature of the perfusion fluid should be kept as low as 5°C to 10°C (Dotteri et al, 1962). By further lowering the perfusate temperature, injuries might develop similar to those noted with surface cooling. The temperature of the fluid should thus not be lower than 5°C.

If an organ is cooled merely by immersion in a low temperature bath, the surface temperature drops first and the interior is unaltered. Thus there will be marked temperature gradients throughout the organ until the whole reaches thermal equilibrium with the low temperature bath. On warming in this way, the phenomenon occurs in reverse: the surface temperature rises sharply to the maximum while the internal environment slowly equilibrates. Because of the relatively low thermal diffusion properties of water, however, rewarming by immersion is an even more difficult procedure than cooling, and the temperature gradient is greater than during cooling.

It has been shown already that a temperature gradient per se is conducive to cellular destruction. Furthermore, it is obviously impossible to produce either a uniform or rapid diminution of renal core temperature by simple immersion. Immediate hypothermic

perfusion / ....

satisfies these criteria and, while immersion cooling may be sufficient to maintain hypothermia, it is an unsatisfactory method for initial cooling.

3. HYPOTHERMIC PERFUSION COOLING.

Many workers have proven experimentally that immediate hypothermic perfusion of the kidney is important in arresting metabolic processes (Manax et al, 1965; Dottori et al, 1962; Cleveland et al, 1964; Eadie et al, 1965; Kiser et al, 1960; Markland and Parsons, 1963; Knight et al, 1963; Dempster et al, 1964). The knowledge derived from such research has been applied successfully to the homotransplantation of both cadaver and live donor kidneys (Calne, 1964; Woodruff et al, 1963; Shackman et al, 1963; Starzl et al, 1964; Murray and Harrison, 1963; Calne et al, 1962; Couch et al, 1964).

Without doubt, the problem is more complex with the cadaver graft in that much of the tolerable ischaemic time has been lost during a period of low organ perfusion before death. Currently, immediate hypothermic perfusion is the best method to halt metabolic activities in the organ to be stored, without producing irreversible damage as a result of ischaemia.

The question of optimal renal core temperature has already been discussed and to achieve this the perfusion fluid must be pre-cooled to $1^{\circ}C - 5^{\circ}C$ (Kiser et al, 1960; Markland and Parsons, 1963).

Two further variables exist: perfusate and perfusion pressure.

Taking into account that the perfusate provides the medium for producing hypothermia, as long as the constituents are non-toxic to the kidney any suitable physiological solution may be used. In fact, Knight et al (1963) made use of various perfusates and concluded that neither the removal of blood by perfusion, nor

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the composition of the perfusate are of great importance. The rate of cooling, however, is vital. The more general attitude is that perfusion can accomplish more than just the cooling of the organ; this is discussed more fully later.

Perfusion pressure is not well documented. In most cases, no measurement is recorded and the perfusion is simply continued until the effluent from the renal vein is clear (Markland and Parsons, 1963; Cleveland et al, 1964; Dottori et al, 1962). However, in a carefully constituted series of experiments, Kiser et al (1961) concluded that the perfusion pressure should not exceed 180 mm.Hg and should be within the range of 100-150 mm.Hg. Matloff and Glowen (1962) and Manax et al (1965) also reported this to be the range of greatest safety, and in clinical reports of homotransplantation too this pressure range is adhered to (Starzl et al, 1964; Woodruff et al, 1963).

Although the amount of perfusion fluid used is not considered to be of much importance, two criteria must be respected. Firstly, sufficient perfusate must be used to adequately lower the renal core temperature and, secondly, all residual blood and its products should be cleared by this perfusion. Both experimentally and clinically, between 150 cc. and 400 cc. has been demonstrated to satisfy these two considerations (Kiser et al, 1960, 1961; Manax et al, 1965; Calne, 1964; Starzl et al, 1964).

METHODS OF PROLONGED ORGAN STORAGE.

SURFACE HYPOTHERMIA.

The benefits, the vital importance of, and the reasons for the use of hypothermia in tissue storage have been fully discussed in the preceding sections. It has been shown that immediate hypothermic perfusion is the most efficient method to satisfactorily lower renal core temperature in the kidney graft. The dangers of sub-zero preservation have been mentioned and the few documented attempts described.

For prolonged hypothermic storage, the same methods apply as for the initial cooling of the organ: surface (immersion) cooling and/or hypothermic perfusion cooling. Prolonged hypothermic perfusion will be discussed in the review of perfusion techniques.

In the past, surface cooling has been commonly used to protect the kidney from the harmful effects of prolonged continuous ischaemia (Bogardus and Schlosser, 1956; Crockett, 1961; Kerr et al, 1960; Mitchell and Woodruff, 1957; Graves, 1963; Semb, 1956; Stueber et al, 1958; Schloerb et al, 1957). This technique creates a number of problems though: it is cumbersome a potential cause of infection and may delay normal healing (Mitchell, 1959). In 1963, Newman commented that, should capsular injury occur and surface cooling be applied, there is a danger of permanent parenchymal damage due to freezing.

These complications apply particularly to prolonged storage of over 12 hours, and in many respects do not warrant anxiety in "Short term" storage of up to about 6 hours. There are reports of short term immersion cooling of cadaver grafts for immediate homotransplantation (Kenyon, 1965; Dempster et al, 1964; Calne et al, 1963).

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The success of simple immersion cooling for 6 to 8 hours is well documented (Cleveland et al, 1964; Kiser et al, 1960; Dempster et al, 1964; Stueber et al, 1958). However, very few attempts have been made at storage for over 12 hours' duration.

Berman (1958, 1960) reported that investigators in Russia have perfected a technique for viable storage of whole organs at +2°C for 27 to 48 hours, but no details have been forthcoming as regards the actual method used, or the results. In 1951, Lefebvre attempted 24 hour storage but "recovery" was only 4 hours. In 1960, Lapchinsky also stored kidneys "successfully" for 28 hours, but no experimental details are given and no other workers have been able to corroborate these reports.

Others have reported that 24 hour simple hypothermic storage in unsuccessful (Calne et al, 1963; Schloerb et al, 1959). Schloerb was able to restore the kidney to normal function only after 8 hours of ischaemia. Calne found that, after preservation for periods up to 12 hours using surface cooling with ice, the kidneys withstood the severe test of an immediate contralateral nephrectomy. Between 12 and 17 hours' storage, damage was sometimes partially reversible but in one experiment of 17 hours and in both of 24 hours' storage, severe irreversible damage resulted. Similar results were reported by Manax et al (1964) after extremely well conducted experimentation.

PERFUSION.

The idea of maintaining, alive, portion of the body in order to study its functions is not a new one. Le Gallois, in 1812, stated that if some means of substituting the heart with a perfusion system could be evolved "one could succeed in maintaining alive indefinitely any part of the body whatsoever."

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About 14 years later, Kay used an artificial circulation to restore the irritability of dying muscles. In 1838, Brown-Sequard published the results of experiments to perfuse the vessels of the head.

Over the next 75 years, various modifications were introduced to improve the technique of perfusion. First another animal was used as the pump oxygenator. Later a pump-lung system was introduced. In 1910, Hooker carried the concept of perfusion to its ultimate refinement by introducing a system which could both aerate the blood and pump it at a variable pulse pressure through any organ with a vascular pedicle.

Attempts to study the physiology of the so-prepared kidney were first made by Starling and Verney in 1925. The work was continued by Bayliss and Ogden (1933) and by Bickford and Winton (1937). The isolated preparation showed itself a useful vehicle for studies in renal physiology. Valid conclusions were derived regarding the role of glomerular filtration, tubular reabsorption and secretion, the effects of hypothermia, and even the specific sites within the tubules where different ions are handled were identified.

After World War II, interest in perfusion shifted to total body perfusion and the development of a pump-oxygenator system to substitute the heart and lungs in cardiovascular surgery.

More recently, regional perfusion has been used as a means to deliver anti-cancer drugs to a particular part of the body.

With the current surge of interest in kidney transplantation, there was renewed interest in kidney perfusion. Cough and his associates (1958) re-introduced the technique - not for physiological study but as a means of storage to maintain viability while the kidney is in transit from donor to recipient.

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For the storage of organs, perfusion has been performed by means of various techniques with different experimental aims.

Normothermic Perfusion: The in vitro normothermic perfusion of an isolated kidney, for storage, is aimed at translating normal body haemodynamics to the in vitro environment. It is obvious that perfusate oxygenation is necessary.

Hypothermic Perfusion: The importance of initial hypothermic perfusion to lower renal core temperature has been discussed. In prolonged organ storage, too, this technique has proved to be successful but oxygenation of the perfusate is also necessary.

Many variables exist in any perfusion set up, related to the constitution of the perfusate, the perfusion pressure and rate of flow. In addition, there are technical problems, mainly depending on the methods of oxygenation, cooling and pumping.

The background of the two broad categories - normo- and hypothermic perfusion - will be discussed separately.

NORMOTHERMIC PERFUSION.

Isolated normothermic kidney perfusions have been attempted for more than 100 years, and have been utilised by many in studies of the physiological processes of the kidney, as well as in transplantation studies. The duration of these perfusions has been limited to approximately 7 hours, with sub-normal blood flow and significant impairment of function. Attempts to re-implant the isolated perfused kidney have been unsuccessful.

In 1849, Loebell initiated isolated kidney perfusions and used defibrinated blood for the study of urine formation. Other early workers (Bedder, 1862; Beinge and Schmiedeberg, 1876) succeeded in obtaining renal blood flows of 20 to 100 cc. per minute, using oxygenated, defibrinated blood.

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Bainbridge and Evans (1914) increased renal blood flow by incorporating an animal lung into their perfusion circuit and postulated that the improved flow rates thus achieved were the result of "de-oxifying" the blood. Starling and Verney (1925) also used the animal lung to improve renal blood flow, perfusing isolated kidneys which actively excreted urine for 5 hours. While carrying out a variety of studies with organ perfusions in the 1920's, Carrel and Lindbergh (1938) had limited success with kidney perfusions.

Winton and his associates (1937) extensively studied the physiology of the isolated perfused kidney, including inulin and creatinine excretion and the influence of the temperature.

Brull and Louis-Bar (1957) demonstrated that heparinised, as well as defibrinated, blood is vasoconstrictive when kidneys are perfused in an artificial preparation. They noted that blood coming in contact with glass, rubber and plastic, shows vaso-constrictive effects, and that increased concentrations of heparin do not decrease it. They were able to abolish or reduce this effect with cyanide.

Couch et al (1958) made use of the isolated kidney in perfusion studies designed to evaluate its usefulness in organ storage prior to transplantation. They further studied such preparations for donor antigen transformation, and as a means to evaluate homograft rejection responses. They maintained urine production in the perfused kidney for up to 7 hours, but subsequent attempts at re-implantation were unsuccessful.

Shatkin et al (1962) more recently added a dialyser to the apparatus and perfused kidneys for 5 hours in this manner. The perfusion flow rates through the kidney, however, were only 1 - 2 cc. per gm. per minute.

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Preliminary studies, and a review of the work of others, suggest that the limit for normothermic perfusion of the isolated kidney is approximately 7 hours. With a perfusate containing a balanced salt solution and low molecular weight dextran, using improved dissection techniques and with a generous amount of procaine, it has been shown that the kidney may retain its original functional capacity with at least 7 hours of isolated normothermic perfusion. However, in none of these animals was immediate contralateral nephrectomy possible and the success rate certainly did not approach 100%, even after only 7 hours' perfusion storage. There was no success after 24 hours.

Considerable importance has been attached to the length of time for which a normothermic perfused kidney will evidence normal renal function. Barkin et al (1963) noted, over a 3 hour observation period, close correlation of blood flow rates to in vivo levels of function, with a constant oxygen consumption. Urea and creatinine concentration was adequate, despite the absence of antidiuretic hormone, and sodium and chloride were conserved with simultaneous potassium excretion. The pH of the perfusate blood was maintained at normal levels for at least 60 minutes.

In preliminary studies by Telander (1962, 1964), fresh, heparinised blood diluted with a balanced salt solution, proved unsuccessful for prolonged perfusion. Extensive tubular damage and oedema of the kidneys were found on histological examination. However, with the addition of low molecular weight dextran, a significant improvement was achieved in the ability of the organ to retain its original functional capacity. The low haematocrit, due to dilution with the balanced salt solution and low molecular weight dextran, was also believed to be of some importance, since it decreases viscosity and resistance, thereby increasing blood flow. Normal renal function was evidenced with up to 7 hours of isolated normothermic perfusion.

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HYPOTHERMIC PERFUSION.

The Advantages of Hypothermic Perfusion.

For practical purposes, the benefits of hypothermia in organ storage are undisputable. Perfusion with a cooled solution has been shown to be important in the rapid lowering of the intra-renal temperature. The exact role played by perfusion in relation to hypothermia in prolonged storage is difficult to assess. Without doubt, the results of hypothermic perfusion are superior, certainly to normothermic perfusion, and appear to be better than those of hypothermia alone (Eadie and Ritchie, 1965; Jones and Politano, 1963). For the very reason that so little protection is afforded the ischaemic kidney by normothermic perfusion, Matloff and Glowen (1962) concluded that the protection provided by hypothermic perfusion stems primarily from the cooling effect. They felt that perfusion itself has neither a beneficial nor a deleterious effect upon the ischaemic kidney.

By combining perfusion with hypothermia, there are these possible advantages:-

(a) Perfusion is a better and safer method to maintain the kidney at a low temperature;

(b) Although, as has been described, metabolic processes and oxygen requirements are remarkably reduced at low temperatures, they are not abolished. With oxygenation of the perfusate, this small - but nonetheless vital - amount of oxygen is supplied, maintaining functional viability until such time as the kidney is rewarmed and re-implanted.

Though both points are valid, the latter appears to have more practical importance.

In 1961, Smith succinctly showed that cooling causes slowing of the biochemical processes involved in respiration, metabolism and all other inter-actions between the cytoplasm of the cells

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and their environment. The important point is that these processes are slowed, not halted.

Markland and Parsons (1963), recognising the irreversible changes consequent to renal anoxia, realised that super-cooling to well below freezing point would be necessary to prevent them.

It is as well to understand that anoxia initially disturbs enzyme actions, probably causing a temporary period of anaerobic glycolysis prior to cell death. Intracellular pH falls with accumulation of lactic acid. Similarly, vessel spasm occurs whilst in fine capillaries cell aggregation and thrombosis cause further damage. Even at extremely low temperatures, some metabolic activity has been demonstrated by Karow and Webb (1964).

Although simple hypothermia has often been employed for organ storage, the shortcomings of the results have been generally recognised (Kiser et al, 1961). Dempster et al (1964) confirmed the results of Lefebvre (1951) and of Lefebvre and Nizet (1952), reporting and illustrating the total medullary damage so consistently found in these experiments with simple hypothermia. The criticisms levelled against the use of perfusion concerned the technical problems presented by the use of a blood perfusion apparatus, and the dangers of liberated vasoconstrictor substances.

Oxygenation and Pumping.

It is almost universally accepted that oxygenation of the perfusate for prolonged organ storage is essential. Nizet and Cuypers (1962) stated that, unless oxygenated fluid is used, perfusion is harmful because the perfused organ becomes oedematous.

In recent times, Matloff and Glowen (1962) are the only workers who feel that oxygenation of the perfusate beyond exposure to room air is unnecessary, and that hypothermia alone sufficiently protects the ischaemic perfused kidney.

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With the establishment of cardiopulmonary bypass procedures, pump-oxygenator techniques were refined and are in wide successful clinical use. Without much difficulty in most published reports, similar circuits (with slight modification) have been used for isolated kidney perfusion, though it would be tedious and out of place to describe these techniques here. Furthermore, hyperbaric oxygenation has also been introduced, both of perfusates and of immersates for surface cooling. This innovation will be discussed later.

The Perfusate and its Constitution.

Much work has been done - largely by trial and error methods - on the use of various perfusates. Attempts have been made to exclude blood from the mixture in that the aggregation of red blood cells has been implicated as a major cause for lowering the flow rate, necessitating an increase in perfusion pressure to maintain an adequate flow.

For immediate post-nephrectomy perfusion, the important point is to lower renal core temperature rapidly. To achieve this it is obviously unnecessary to perfuse a blood solution. In fact, Manax et al (1965) have greatly stressed the importance of clearing the renal vasculature of any residual blood or its products. The constitution of the perfusion fluid for this purpose, both clinically and experimentally, is simple. Because of rapid hypothermia, oxygenation is unnecessary and, by the use of low molecular weight dextran, hepatin and an antispasmodic, the vascular aggregation, thrombosis and vessel spasm can be avoided (Knight et al, 1963; Kiser et al, 1960).

Manax et al (1965) examined an important factor required to maintain cell life outside the body during a period of ischaemia: the prevention of lethal changes in cellular hydration.

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The influence of fluid shifts on tissue viability was recognised by Ringer as early as 1900, in studies on isolated skeletal muscle and the heart. These fluid shifts are equally important in the kidney.

Pilot studies have shown that when a kidney is removed and replaced within an hour, perfusion in vitro is probably unnecessary. Any disturbed mechanism quickly corrects itself upon restoration of the circulation. In contrast, when a kidney is held in vitro for 2 hours or longer, and then is replaced without intervening perfusion, it functions poorly and may not remain viable. Stagnant, hypoxic blood retained in the kidney is apparently responsible for the in vitro damage sustained.

There are three criteria for the ideal fluid for organ perfusion. The perfusate should (Humphries et al, 1963, 1964; Manax et al, 1965).

(1) buffer adequately in the absence of a haemoglobin-oxygen system,
(2) be isotonic with respect to critical anions and cations,
(3) be isosmotic with the intracellular fluid.

Moreover, in addition to removing red blood cells, free haemoglobin and other cellular debris, the solution should contain nutrients which are protective during the in vitro preservation period.

Manax et al (1965) demonstrated quite conclusively that blood left in the canine kidney causes damage. Hypoxic blood buffers poorly, tends to haemolyse readily and produces haemoglobin casts and blood urine upon revascularisation. Ischaemic, unperfused dog kidneys left for periods greater than one hour at room temperature before replacement do not survive (Calne et al, 1963; Hamilton et al, 1948). Perfusion of the kidney with fresh, unoxygenated blood produces no better results, in terms of post-transplant function and survival.

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If a non-toxic, non-damaging perfusate is used, organ perfusion has several advantages. Residual blood is effectively removed, minimising the possibility of damage from sludging, haemolysis and platelet adhesion. A buffer may be readily added to the perfusate fluid. Perfusion allows heparinisation of the kidney vasculature. Low molecular weight dextran, furthermore, appears to improve the blood flow after revascularisation.

These and others (Calne et al, 1963; Hamilton et al, 1948; Humphries et al, 1963) have shown that the least damaging fluid for perfusion of the canine kidney is a balanced salt solution which is buffered, isotonic with respect to all major electrolytes, effectively isosmotic, and which contains heparin and procaine. The total measurable osmolality of this combination is higher than that of intracellular fluid (Barnard, 1965) but its effective osmotic pressure is close to that of intracellular fluid.

Long (1961) and others have described red blood cells aggregation with prolonged extracorporeal perfusion, and the associated plugging of small vessels and the resultant decreased blood flow with poor tissue perfusion. The anti-sludging effect of low molecular weight dextran and its ability to decrease red blood cell aggregation, with improved micro-circulation, are now generally recognised (Judd et al, 1964; Hendren and Hardin, 1964; Hitchcock et al, 1964; Winfrey and Foster, 1964; Powley, 1964; Chisholm, 1965).

There is a vast store of literature regarding the diversity of advantages offered by low molecular weight dextran or Rheomacrodex. Many of these are of singular importance to kidney storage:

1. It improves peripheral blood flow (Gelin and Shoemaker, 1961; Gelin and Thoren, 1961; Atik et al, 1963; Dempster et al, 1964;

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2. It is isosmotic (Hint, 1963);
3. It counteracts the tendency to intravascular aggregation resultant upon hypothermia (Löfström, 1959; Rush et al, 1961);
4. It prevents intravascular aggregation or sludging (Pierce and Law, 1962; Pories, 1962);
5. It improves local organ perfusion (Kiser et al, 1960, 1961; Dottori et al, 1962; Telander, 1962);
6. It enhances oxygen tension and consumption (Löfström, 1959; Pierce and Law, 1962);
7. It prevents thrombosis in small artery surgery (Winfrey and Foster, 1964).

A number of different perfusates have been investigated for in vitro kidney storage, including:

(a) Whole blood (Macksood et al, 1961; Humphries et al, 1964; Hitchcock et al, 1964);
(b) Half blood (Humphries et al, 1964; Barkin et al, 1963; Kiser et al, 1960);
(c) Plasma and serum (Manax et al, 1965; Humphries et al, 1963);
(d) A buffered balanced physiological solution including low molecular weight dextran (Markland and Parsons, 1963; Hitchcock et al, 1964; Kiser et al, 1960, 1961; Humphries et al, 1962; Telander, 1962; Dempster, 1963; Simso et al, 1963; Dempster et al 1964).

On reviewing the results of prolonged storage (over 12 hours) there can be no doubt that low molecular weight dextran has been responsible for a remarkable improvement in function and survival after re-implantation. The exclusion of blood entirely from the perfusate has not proved to be advisable and the addition to it of a balanced physiological solution has had definite advantages.

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Theoretically, it would seem that perfusion with blood diluted with both low molecular weight dextran and a balanced physiological solution should be most successful. Humphries et al (1964) made use of such a perfusate and, of all reports, have had the greatest measure of success with prolonged storage.

Perfusion Pressure and Flow Rate.

Markland and Parsons (1963) found the most satisfactory fluid for short-term perfusion of the kidney to be 5% dextrose water, papaverine and heparin, at a temperature of 8°C. Dottori et al (1962), investigating various perfusates, found the necessary pressure for adequate flow to be above physiological range when using whole blood. When less viscous fluids were tested, however, satisfactory perfusion was possible at pressures of 40-80 mm.Hg.

Most investigators have noticed a marked decrease in renal blood flow in the dog kidney soon after the onset of perfusion (Belzer et al, 1964). By diluting the perfusate, using a low renal blood flow, it has been possible to perfuse a kidney for up to 24 hours with successful re-implantation (Humphries et al, 1964).

In 1965, Steyn and Mobley reviewed factors influencing hypothermic perfusion, including the haemodynamics of cold solutions, the effect of cold on oxygen utilisation, release of vasospastic substances, and cold-induced vasospasm. They reported that:-

(a) The degree of hypothermia influences the renal resistance,
(b) Vasospasm is abolished by rewarming,
(c) Carbon dioxide is essential for prolonged perfusion,
(d) Papaverine does not improve renal blood flow,
(e) Low molecular weight dextran produces a marked improvement in blood flow.

Humphries et al (1963), using various perfusates including both whole blood and blood-free solutions, demonstrated that the flow rate gradually decreases during perfusion regardless of the perfusate used. The increasing resistance may be the result of

vasoconstrictors ,

vasoconstrictors, mechanical blockage of small vessels by particulate matter, or as a result of kidney swelling.

The observations of Nizet and Cuypers (1962) indicate that the vasoconstrictors released when the kidney is perfused with blood come from the red cells. When filtered serum is used, this source of vasoconstriction is absent and all significant particulate matter is removed. From this it would appear that kidney swelling is the important cause for the diminution of flow rate during perfusion.

This decrease in renal blood flow during perfusion has been noticed by many. Using whole blood as the perfusate and incorporating an oxygenator at flow rates of 120-160 cc. per minute, Belzer et al (1964) studied the factors influencing renal blood flow during isolated perfusion. They report two blocks to renal blood perfusion and conclude that

(i) Preliminary filtration of whole blood through glass-wool markedly improves renal blood flow and eliminates the early block phenomenon; the addition of unfiltered blood to the perfusate immediately reverses the position;

(ii) A second block, occuring later in perfusion, can be temporarily reversed by the addition of procaine or papaverine to the perfusate; it can be prevented by the addition of the antiserotonin agent UML-491 and benadryl to the perfusate;

(iii) The greater the dilution of whole blood with acqueous solutions for perfusion, the less apparent are the outflow blocks.

Using these observations constructively, they perfused dog kidneys successfully with whole blood for up to 22 hours, with a renal blood flow of 120-160 cc. per minute. However, flow rates such as these, approximating normal renal flow rates, are only possible with normothermia.

/ ....

Hypothermic perfusion necessitates quite different rates of flow. In the first place, with a reduction in metabolism, normal perfusion rates are not only unnecessary but may be dangerous. Secondly, a reduction in flow consequent to hypothermia per se becomes necessary.

Humphries et al (1963) emphasised the importance of kidney swelling in relation to flow decrease, and affirm (1963, 1964) that there is less kidney swelling with low perfusion pressures. For this reason, higher flow rates were obtained at low perfusion pressures over a 24 hour period.

Gollan (1959) also favours the use of low perfusion pressures with hypothermia.

It is assumed that continuous perfusion is preferable. However, Lapchinsky (1960) used perfusion at the beginning and end of the storage period only and many workers, including Hitchcock (1964) perfuse for just long enough to "core cool" the kidney. Lyons and Clarke (in a personal communication to Humphries et al, 1963) reported that dogs perfused with blood (free of oxygen but normal in pH) were more easily resuscitated than dogs not perfused at all. The only advantage, however, may have been the improved myocardial contractability resulting from the removal and neutralisation of excess lactates.

HYPERBARIC OXYGEN.

PRESSURE TERMINOLOGY.

As a rule, the degree of pressurisation is referred to in terms of "atmospheres". A vacuum - as in Space - is spoken of as having a pressure of zero atmospheres absolute. At sea level the weight of air pressing on each square inch of the earth's surface is 14.7 lbs. (760 mm.Hg), which is referred to as one atmosphere absolute. However, confusion is possible because at one atmosphere absolute the gauges used to measure pressure show a zero reading; hence, one atmosphere absolute is equal to zero atmosphere gauge pressure.

Most basic research concerned with man's ability to survive under increased atmospheric pressures has been related to underwater diving (a dive of 33 feet is equivalent to a gauge pressure of one atmosphere - or two atmospheres absolute).

PHYSIOLOGICAL BASIS OF HYPERBARIC OXYGENATION.

Oxygen is transported in the circulation, both chemically bound to haemoglobin and also physically dissolved in the blood, but normally only the former is important for oxygen transfer. However, the amount of physically dissolved oxygen is directly proportional to the percentage of oxygen inspired and to atmospheric pressure.

Breathing air at normal atmospheric pressure, the partial pressure of oxygen (pO_2) in arterial blood is 100 mm.Hg. When breathing 100% oxygen at the same pressure, the pO_2 becomes 675 mm.Hg - 76^0 mm.Hg minus the partial pressures of carbon dioxide (40 mm.Hg) and water vapour (45 mm.Hg). When breathing 100% oxygen at an ambient pressure of 3 atmospheres absolute, the arterial pO_2 becomes 2195 mm.Hg (3 x 760 minus 40 mm.Hg CO_2 minus 45 mm.Hg H_2O), which is approximately twenty-fold that breathing air at normal pressure.

/ ...

In the healthy subject this elevation of oxygen tension is approximated but is not quite achieved because of the blood that does not reach the alveolar capillary bed in its passage from pulmonary artery to pulmonary vein (venous admixture). In the patient who is aerating poorly, or who has a right to left cardiac shunt, this factor may reach major proportions. Knowledge of ambient pressure and percentage of inspired oxygen is simply not enough, therefore: the arterial pO_2 must be known in order to evaluate the role of this mode of therapy - hyperbaric oxygenation.

At normal body temperatures the absorption co-efficient for physical solution in blood is 0.022. When 100% oxygen is administered in a hyperbaric chamber pressurised to three atmospheres absolute (3 ATA), the amount of physically dissolved oxygen will thus be raised to approximately 6 volumes %. Since the arteriovenous difference in oxygen content is approximately 6 volumes % for most organs, theoretically it is possible to dispense with haemoglobin as a means of oxygen transport. This was demonstrated by Boerema et al (1960) in his paper "Life Without Blood", describing experiments where the blood of pigs was replaced by plasma or dextran solutions while the animals breathed oxygen at 3 ATA.

This thesis is not concerned with the recognised clinical hazards of high oxygen tensions, related largely to the central nervous and respiratory systems. On the other hand, the fact that these clinical syndromes may result from either tissue enzyme interference or from circulatory interference (or both) is one which is vital to the proper understanding of isolated organ storage.

(a) Circulatory Effects of Hyperbaric Oxygen:

The slowing of the heart rate under high pressure oxygen was first described by Paul Bert (1878), who observed this phenomenon directly in young, transparent eels. Dautrebande and Haldane (1921) also reported bradycardia at increased oxygen tensions, and assumed

that / ...

that there was a fall in cardiac output, which would protect the body. Bean and Rottschafer (1938) showed that this bradycardia was caused in dogs by vagal action rather than by a direct effect on the heart muscle, or its rhythm control mechanism.

It is obvious that the cerebral circulation is critically important under high pressure oxygen conditions, as the brain is the most vulnerable organ. Tinel (1927) first commented on visible cerebral vasoconstriction, as judged by increased brain pallor in trefined subjects with the administration of pure oxygen. Since then, cerebral vasoconstriction under high oxygen pressure has been amply confirmed by more precise techniques. Lambertsen and his colleagues (1953), using a nitrous oxide technique, showed that at 3.5 atmospheres absolute oxygen-breathing causes an increase of cerebral vascular resistance and a decrease in cerebral blood flow.

There are no documented reports of similar experiments on renal blood flow, either in vivo or in vitro. Although brain tissue is much more sensitive than other tissues, similar thought less pronounced changes must occur in the kidney.

As a result of this cerebral vasoconstriction, Lambertsen et al (1953) demonstrated that the jugular venous (and presumably the central nervous system) pO_2 rose, with a fall in arterial pO_2. They also confirmed that the convulsant effect of high oxygen pressure was greatly enhanced by relatively low tensions of carbon dioxide. By the administration of a 2% carbon dioxide, the jugular venous pO_2 could be raised from below 100 mm.Hg to 1,000 mm.Hg in a subject breathing oxygen at 3.5 ATA.

The importance of these observations to isolated kidney storage perfusion is obvious. In the absence of an adequate perfusate pO_2, intrarenal vasoconstriction may be expected, possibly producing inadequate perfusion of the organ. If the perfusate pO_2 is maintained at adequate levels, however, blood flow should improve with less cellular damage.

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Presumably cellular dysfunction is responsible for the convulsant effect of high pressure oxygen in association with low tensions of carbon dioxide.

It can be argued that similar mechanisms apply to an isolated organ perfused under high pressure oxygen, through vasoconstriction and inadequate perfusion, and this apparently is rectifiable by the addition of carbon dioxide to the hyperbaric oxygenation chamber environment.

(b) The Effects of Hyperbaric Oxygenation on Tissue Metabolism:

Bert first noted, in 1878, that a pressure of oxygen greater than 1 atmosphere absolute is toxic to "every living thing". Since then, considerable discussion has taken place as to the fundamental nature of this phenomenon, and it is believed to be largely due to the inactivation of certain tissue enzyme systems (Bean, 1945; Stadie et al, 1945).

There is little doubt that the exposure of tissues to high pressures of oxygen will result in an alteration in their metabolism. Possibly this alteration has some place in therapeutics, in the same way as has that produced by hypothermia on ionising radiations.

It has been shown that the respiration of brain slices is irreversibly poisoned by high pressure oxygenation. Brain tissue is much more sensitive than the other tissues which have been studied (testis, kidney, lung, muscle) with the exception of liver, which is only slightly less sensitive.

Many enzymes and co-enzymes concerned with carbohydrate metabolism are involved. Those of the sulphydryl group are particularly vulnerable. The brain tissue of rats convulsed in high pressure oxygen shows no apparent impairment of respiration. The tensions of oxygen and time of exposure necessary to cause impaired tissue respiration and enzyme damage in vitro are far in excess of those that cause convulsions in vivo. The intact

animal / ...

animal or human appears to recover completely from severe convulsions, whereas the effect in vitro is irreversible. The difficulty of reconstituting the sulphydril group in vitro is well known. If, as it appears, convulsions appear in a very early stage of enzyme damage, reactivation of these enzymes or co-enzymes in the intact organism is feasible (Donald, 1945).

Although in mammals convulsions may be the most dramatic manifestation of oxygen toxicity, the toxic action of oxygen takes many forms and has been found to occur in all (or almost all) species of animal or plant life studied under elevated pressures of oxygen. Thus it is improbable that there is only one mechanism of oxygen poisoning; it is much more reasonable to assume that oxygen in toxic concentrations has many actions on metabolism and function, varying in importance in different conditions and in different species.

Finally, it should be emphasised that changes in tissue oxygen concentration may produce metabolic effects which are physiological rather than toxic, and which may be of great import in the regulation of cell function. One example of a possible reaction of this type is the rapid loss of tonus observed by Bean and Bohr (1944) in smooth muscle exposed to elevated pressures of oxygen.

(c) <u>The Effect of Hyperbaric Oxygenation on Temperature</u>:

The depression of oxygen consumption found at normothermic temperatures under high pressure oxygen is substantiated by the results of Jacobson et al (1963), who reported that the oxygen consumption of canine brain in vivo was reduced by 40% when the pressure of oxygen breathed by the dog was raised from 150 mm.Hg to 1520 mm.Hg. Dickens (1946) has shown that liver is less susceptible to the depressant effect of hyperbaric oxygen than is brain.

/ ...

These findings are in keeping with the observation of Smith et al (1963) that the safe period of time for which the circulation may be totally arrested in dogs can be increased from 4 minutes to 8 minutes if the animal breathes oxygen at 2 ATA instead of at 1 ATA. In this case, more oxygen is supplied and less is required.

In 1965, Norman suggested that, at reduced temperatures, an increase in oxygen tension causes an increase in oxygen consumption until a critical oxygen tension is reached (around 500 mm.Hg at 28°C and 1,000 mm.Hg at 15°C) and thereafter the expected fall occurs. This theory is supported by Grossman and Penrod (1949) and Campbell (1937), who observed that hypothermia prolongs the period for which rats can be exposed to high pressures of oxygen before signs of oxygen poisoning occur.

Stimulation of oxygen consumption at hypothermic levels, in response to moderate increases in pressure, suggests that the reduction in oxygen consumption brought about by hypothermia is not only due to decreased oxygen requirements by the tissues, but also to a progressive inability of the tissues to utilise the oxygen supplied at low temperatures. In this way, a high tension of oxygen may be necessary to ensure adequate oxygenation at low tissue temperatures.

Norman (1965) also indicated that preliminary saturation of the tissues with hyperbaric oxygen, at normothermic temperatures, prior to a period of circulatory arrest, increases the safety of this procedure by increasing the amount of oxygen available and by reducing the tissue oxygen requirements. At hypothermic temperatures it may be necessary to deliver oxygen to the tissues at higher pressure, to ensure adequate oxygenation, and it may be necessary to raise the pressure of oxygen progressively with the reduction of the temperature.

/ ...

(d) The Effect of Hyperbaric Oxygen on Freezing and Thawing:

In 1960, Taylor demonstrated that by applying very high pressures (in the order of 35,000 lbs. per square inch) during thawing, he was able to obtain a rapid temperature rise above $0^{\circ}C$ without any plateau, and thus without any cellular destruction.

HYPERBARIC OXYGEN AS AN OXYGENATOR SYSTEM.

Almost all of the oxygenators in current use inflict damage to the red blood cells. Lee et al (1961) noted that the membrane lung type caused less damage to blood than any other oxygenator. Humphries et al (1964), using such an oxygenator, attempting 24 hour kidney storage, found that blood damage was not excessive as indicated by plasma haemoglobin levels, which did not exceed 130 mgm.% at the end of the storage period. A factor which may have helped to minimise haemolysis was the use of formalin instead of detergents to sterilize the plastic oxygenator (Hall et al, 1962).

The probable importance of carbon dioxide during hyperbaric oxygenation has already been mentioned. Humphries et al (1964) felt that the presence of carbon dioxide in oxygen may provide a more physiological perfusate than would pure oxygen. A normal pCO_2 helped to prevent "arterial bed block" in the perfusion of canine livers (Kestens and McDermott, 1961). By using 2.5% carbon dioxide in oxygen at $10^{\circ}C$ and measuring the pCO_2 at $37^{\circ}C$, pCO_2 ranged between 29 mm.Hg and 42 mm.Hg, and pH between 7.11 and 7.35 Since the actual pH at $10^{\circ}C$ was probably somewhat higher (Pierce, 1962; Rewin et al, 1955) the use of a higher concentration of carbon dioxide may have been better, to avoid the possibility of alkalosis.

/ ...

Many investigators have depended on dissolved oxygen for satisfactory oxygenation. More oxygen may not be necessary with hypothermia and, in association with higher tensions (e.g. 3 ATA pressure) may actually be harmful, causing enzyme damage (Bean, 1945). Negovski (1962) warns against the supply of excess oxygen (to the brain at least) during resuscitation after hypoxia. Nevertheless, a greater supply of oxygen as borne by red cells may be of benefit with hyperbaric oxygenation.

THE RESULTS OF HYPERBARIC OXYGENATION FOR KIDNEY STORAGE.

The recorded instances of the use of hyperbaric oxygenation in attempted organ storage are exceedingly rare. One of the few reports came from Manax et al (1964) who performed an extremely well conducted series of experiments. They concluded that the combination of hyperbaric oxygen at 3 ATA with hypothermia at between 4°C and 10°C was successful for 24 hour kidney preservation. They were unable to provide a satisfactory explanation for the apparent advantage afforded by the use of oxygen under pressure, but implied that it might be of importance during cooling and because at low temperatures oxygen is still necessary for cellular viability.

Manax and his associates (1964) have also attempted to store other organs using hyperbaric oxygen, and have successfully preserved small bowel in vitro.

Kenyon and Mearles (1965) demonstrated that, at 3 ATA, the duration of cardio-respiratory arrest may be increased by 20% in hamsters with hyperbaric oxygen used in conjunction with hypothermia.

More recently, Manax et al (1965) successfully stored organs for 48 hours with hypothermia under hyperbaric conditions but, as was the case in their previous experiments (Manax et al, 1964), immediate contralateral nephrectomy was not possible after storage.

A REVIEW OF THE RESULTS OF PROLONGED KIDNEY STORAGE.

In the foregoing sections, where the various techniques used for in vitro renal preservation have been discussed, the results achieved by some workers have been mentioned incidentally. In by far the majority of published reports the storage period has been shorter than 12 hours. In fact, reports of attempted storage for between 12 and 24 hours are few and far between.

The results of attempted prolonged kidney storage have been gathered and are presented here in table form, only the broad principles of each experimental series being noted.

One of the most important aspects is the staging of contralateral nephrectomy after storage. It can be seen that very few attempts have been made to assess function of the stored organ in otherwise bilaterally nephrectomised animals. The only successful results have been obtained after late contralateral nephrectomy.

NAME AND YEAR		STORAGE TIME (hrs)	PERFUSION.	HYPOTHERMIA
Telander	1962	7	(Blood, LMWD, Salts)	-
		24	(100-140 mm.Hg.)	-
Humphries	1962	24	Yes	4°C
Matloff & Gowen	1962	3	Yes	4°C
Calne	1963	12	-	4°C
		17	-	"
		24	-	"
Humphries	1963	24	(Plasma, serum and)	4°C
			(blood)	"
			"	"
			"	"
Hoffman	1964	12	-	4°C
		24	-	"
Telander	1964	7	Yes	-
		24	Yes	-
Manax	1964	24	-	4°C
			-	37°C
			-	4°C
Hitchcock	1964	8	-	4°C
		20	-	"
		5	Yes	37°C
Humphries	1964	24	(40-80 mm.Hg)	4-10°C
			(10 - 28 cc./minute)	
			(Blood and salts)	

OXYGEN	OPPOSITE NEPHRECTOMY	SURVIVORS
Yes	5 - 10 days	7 of 7
"	14 - 21 days	0 of 2
Yes	-	2 of 41
-	-	2 of 2
-	(Late or not at)	1 of 2
-	(all)	0 of 4
-	( )	0 of 3
-	-	0 of 6
Yes	3 weeks	2 of 9
"	"	2 of 11
"	"	0 of 3
-	2 - 4 weeks	8 of 14
-	"	2 of 16
Yes	2 - 3 weeks	8 of 11
"	"	0 of 1
-	-	0 of 10
3 ATA	-	0 of 10
3 ATA	2 - 4 weeks	6 of 30
-	1 - 3 weeks	4 of 6
-	1 - 3 weeks	2 of 6
Yes	5 - 10 weeks	7 of 7
Clowes type	3 weeks	5 of 7

THE PATHOLOGY OF KIDNEY STORAGE.

The vital significance in this thesis of the functional ability of the kidneys after storage has been mentioned previously. Though both the microscopic and macroscopic pathology of kidneys stored in different circumstances will be described throughout, the greatest stress is laid on the functional ability of the stored organ to sustain life.

Any attempt to gauge the relative merits of different storage procedures on histological grounds alone is arbitrary and 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. It is by no means uncommon to find the relationship of functional capacity to be inconsistent with the pathology. However, obviously the pathological picture depends upon the post-storage functional ability of the kidney: the more successful the storage procedure, the better will be function and, equally, the less will be the evidence of pathological change. Perfect post-storage function undoubtedly would be confirmed by a normal histological picture.

The pathological spectrum of renal ischaemia or anoxia is a particularly wide one. The aim of any storage procedure is naturally to sustain viability and to prevent these anoxic changes. Clinically, the term "acute tubular necrosis" denotes the changes which result from inadequate blood supply - and therefore inadequate oxygenation of the renal parenchyma. In that it is the failure of the storage technique that renders the kidney susceptible to an oxygen lack, all the variations on the theme "acute tubular necrosis" are invariably evident both clinically and pathologically.

/ ...

In some instances the actual storage techniques have resulted in cellular damage. Cells are killed during the processes of freezing and thawing. Perfusion at too high a flow rate, or at too high a perfusion pressure in relation to the temperature, causes oedema, disruption of small blood vessels and intra-renal areas of haemorrhage. It is interesting to note that there is no record in the literature of any renal pathological changes resulting from the use of hyperbaric oxygen.

The pathology of renal ischaemia or anoxia has been well recognised for many years. In recent times, with higher power magnification and the use of electron-microscopy, more detailed and precise information has been obtained which is of special significance to early renal tubular abnormality. It is important to understand that tubular degeneration can also result from ingested or inhaled toxins; under these conditions, necrosis of the epithelium of the renal tubules is uniform but does not include destruction of the basement membrane. With ischaemia alone, however, the basement membrane may be fragmented and ruptured with complete disintegration of the tubular structure, and leakage of the tubular contents into the renal interstitium. This latter lesion has been called "tubulorrhexis" (Oliver, 1951). Lucke (1946), who described the ischaemic lesion earlier, believed that it occurred largely in the distal portion of the nephron, and therefore named the syndrome "lower nephron nephrosis". The studies of Oliver showed this appellation to be inappropriate since the lesion could and does occur in any part of the nephron.

Oliver (1951) and others have emphasised the patchy nature of the ischaemic lesion, pointing out that damaged areas are interspersed with areas in which tubular epithelium appears to be quite normal.

On macroscopic examination the kidneys are enlarged and swollen. The capsule is tense and the parenchyma bulges through

the cut / ...

cut surface of the capsule. The cortex appears pale and the medullary area congested, suggesting marked interstitial oedema.

The presence (or absence) of interstitial oedema is a critical point. Upon this hinge many arguments concerning the aetiology of the oliguria which is observed in acute renal failure. Oliver's studies in 1951 revealed that healing of the lesion occurs with re-absorption of the interstitial oedema and repair of the tubular epithelium. In the regenerative process, epithelial cells grow along the intact basement membrane to re-establish tubular integrity. Where disruption of the basement membrane has occurred though, the absence of a continuous supporting structure results in failure of the tubular lining re-epithelialisation. In some instances, growth of connective tissue into the tubular lumen - through the rupture in the basement membrane - also prevents the restoration of tubular integrity.

Although the glomeruli were typically uninvolved in Oliver's studies, other authors (Price and Palmer, 1960; Sevitt, 1959) who studied both post mortem material and biopsy specimens found glomerular involvement, varying from hyalinisation of glomeruli to pre-glomerular fibrosis. These changes were noted by Price and Palmer (1960) in both the ischaemic and the nephrotoxic lesion.

Since the renal tubules' blood supply comes by way of the glomerulus, it seems reasonable that any decrease in renal blood flow would first affect the post-glomerular tissue, i.e. the tubules. Because the areas involved may alternate with uninvolved areas, recovery of a large degree of renal function may be possible, even with total disruption of some affected nephrons. Involvement of the glomeruli to the extent of hyalinisation or pre-glomerular fibrosis is quite possible with severe and prolonged disruption of blood supply to the nephron.

Occlusion of non-ischaemic tubules by interstitial oedema may also produce some glomerular damage. Where thrombosis of the afferent arteriole occurs, the glomerulus will become infarcted and will be

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destroyed, as is the entire nephron. When the vascular lesion is severe enough to affect all the glomeruli, the renal cortices are involved in a diffuse or symmetrical type of lesion in which the entire cortex is infarcted. This is "symmetrical cortical necrosis". The ultimate phase of renal ischaemia, of course, is reached with obstruction of the renal artery, when the entire kidney is infarcted.

In this possibly over-simplified view it is attempted to relate the whole pathological spectrum of acute renal failure - from minor degrees of ischaemia to total infarction of the kidney - to the extent and duration of renal ischaemia.

IN VITRO KIDNEY STORAGE.

EXPERIMENTS.

MATERIALS AND METHODS.

Six experiments were performed to study the feasibility of successful 24 hour kidney storage. A standard surgical procedure was used throughout for the initial nephrectomy and capsulotomy of the organ, and for re-implantation of the kidney after storage. The storage method varied in each of the six experiments. The post-operative treatment and general care of the animal was similar in each case.

To permit fair comparison of the storage techniques, every effort was made to ensure that the only variation in these experiments constituted the method of storage.

Ischaemic Time.

In these experiments, "ischaemic time" denotes the period when the kidney is deprived of a blood supply, and excludes the 24 hour in vitro storage period. Comprising the 3 - 5 minutes delay between initial nephrectomy and the commencement of storage, plus 20 - 30 minutes while the vascular anastomoses are completed during re-implantation, the ischaemic time averaged between 30 and 40 minutes.

In any method of in vitro storage the organ is without a proper blood supply, and is thus inadequately oxygenated during storage. In these experiments, however, this fact is disregarded when using the term "ischaemic time".

EXPERIMENTAL GROUPS.

There were six experimental groups in this study of in vitro kidney storage, using various techniques. Storage time was 24 hours in each group, and the techniques used were as follows:-

/ ...

GROUP I Hypothermia (2° - 10°C) - 9 dogs

GROUP II Freezing (-5° to -10°C) - 4 dogs

GROUP III Freezing with Hyperbaric Oxygenation (3 ATA) - 4 dogs.

GROUP IV Hyperbaric Oxygenation at Room Temperature (25° - 27°C) - 5 dogs.

GROUP V Hypothermia with Hyperbaric Oxygenation - 10 dogs.

GROUP VI Hypothermia (2° - 5°C) with Hyperbaric Oxygenation (3 ATA) and Kidney Perfusion at various flow rates and pressures, using three different perfusates - 39dogs:

Group I Haemodilution - 29 dogs
Perfusion flow: 1000 ml/hr. - 5 dogs
400 ml/hr. - 5 dogs
200 ml/hr. - 7 dogs
150 ml/hr. - 12 dogs

Group II Balanced Physiological Fluid - perfusion flow 150 ml/hr. - 6 dogs.

Group III Whole Blood - perfusion flow 150 ml/hr. - 4 dogs.

Adult mongrel dogs weighing between 35 lb. and 50 lb. were used for all these experiments.

SURGICAL TECHNIQUES.

The dog was anaesthetised with intravenous pentobarbitone sodium, intubated, and was manually ventilated using a mixture of nitrous oxide and oxygen.

(a) NEPHRECTOMY. (Fig. 1)

The most important consideration in performing a nephrectomy for purposes of transplantation is to cause minimal trauma to the kidney (Ackermann and Barnard, 1965 b).

For experimental purposes, the right kidney is preferred as there is a much lower incidence of anomalous renal arteries on that side. A right, subcostal muscle-splitting incision affords easy access and wide exposure. The hepato-renal peritoneal attachments are incised first and the kidney is separated from its anterior peritoneal covering. Non-touch technique is used to free and strip the kidney.

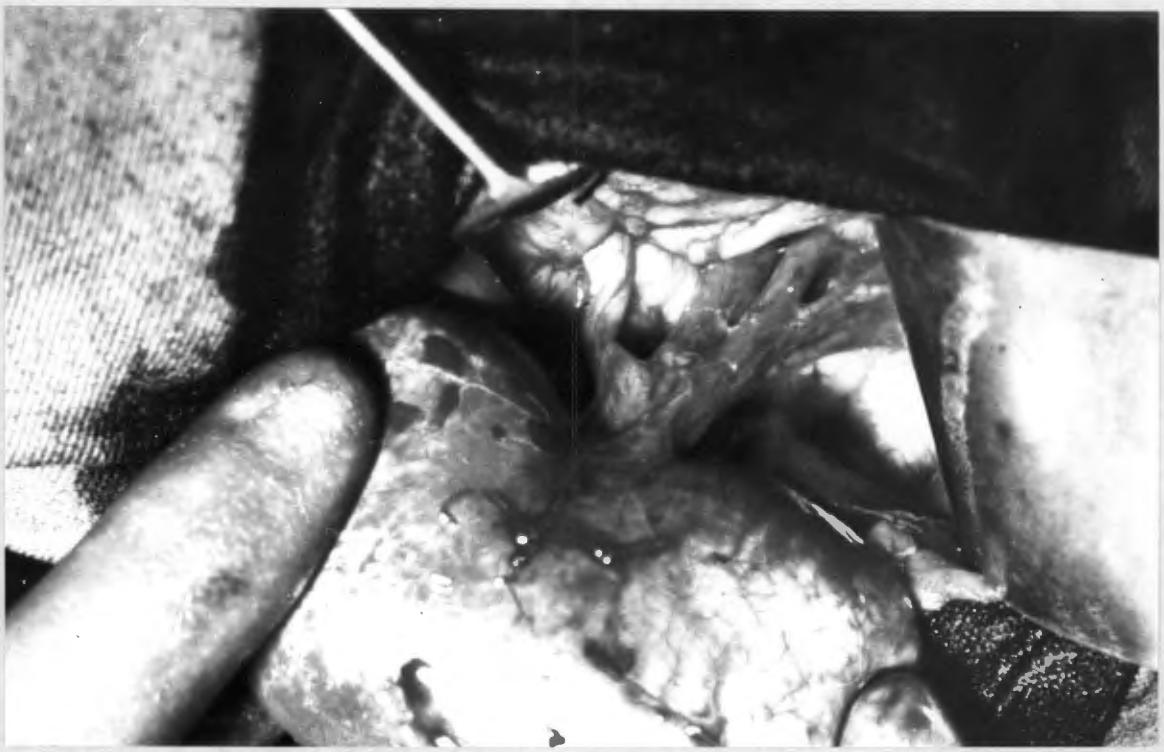
During dissection of the vascular pedicle and ureter, traction on the kidney (or its compression) is carefully avoided. Fastidious dissection of the blood vessels and the ureter commences at the hilum and continues distally as far as the inferior vena cava. As great a length of renal artery as possible is exposed. The kidney is allowed to lie free during this manoeuvre, to ensure venous drainage and to prevent compression trauma to the renal parenchyma. Great caution is exercised in ligating hilar vessels, particularly arterial branches.

The renal artery and vein are ligated simultaneously if possible. Alternatively the artery is ligated immediately before the vein. Venous occlusion may not take place before arterial ligation.

The cuff of the inferior vena cava is often removed with the renal vein.

/ ...

Fig. 1.



This photograph illustrates two dangerous practices during dissection of the vascular pedicle in the initial nephrectomy: (1) finger compression of the kidney, and (2) traction on the pedicle.

(b) IMMEDIATE HYPOTHERMIC PERFUSION OF KIDNEY (Fig. 2).

Immediately following nephrectomy, prior to capsulotomy and stripping of the renal artery adventitia, the kidney is perfused with a specially prepared, cold solution (Starzl et al, 1964; Murray and Harrison, 1963).

Pre-cooled to a temperature of between 5°C and 10°C, this solution is made up with:-

Rheomacrodex in saline	500 cc.
Heparin	15 mgm.
Procaine 1%	50 cc./50 mgm.

The technique used is simple. The renal artery is cannulated with a blunted, wide-bore needle and the perfusion solution is thus injected. The kidney is perfused at a pressure of between 100 and 150 mm.Hg until the return from the renal vein is clear.

The total volume of fluid usually required is less than 250 cc. for each kidney perfused.

(c) CAPSULOTOMY. (Fig. 3).

Once the kidney has been excised, following immediate perfusion cooling but before stripping the renal artery adventitia, the capsule is incised and stripped along its entire length (Ackermann et al, 1965 c). It is important not to incise the renal parenchyma.

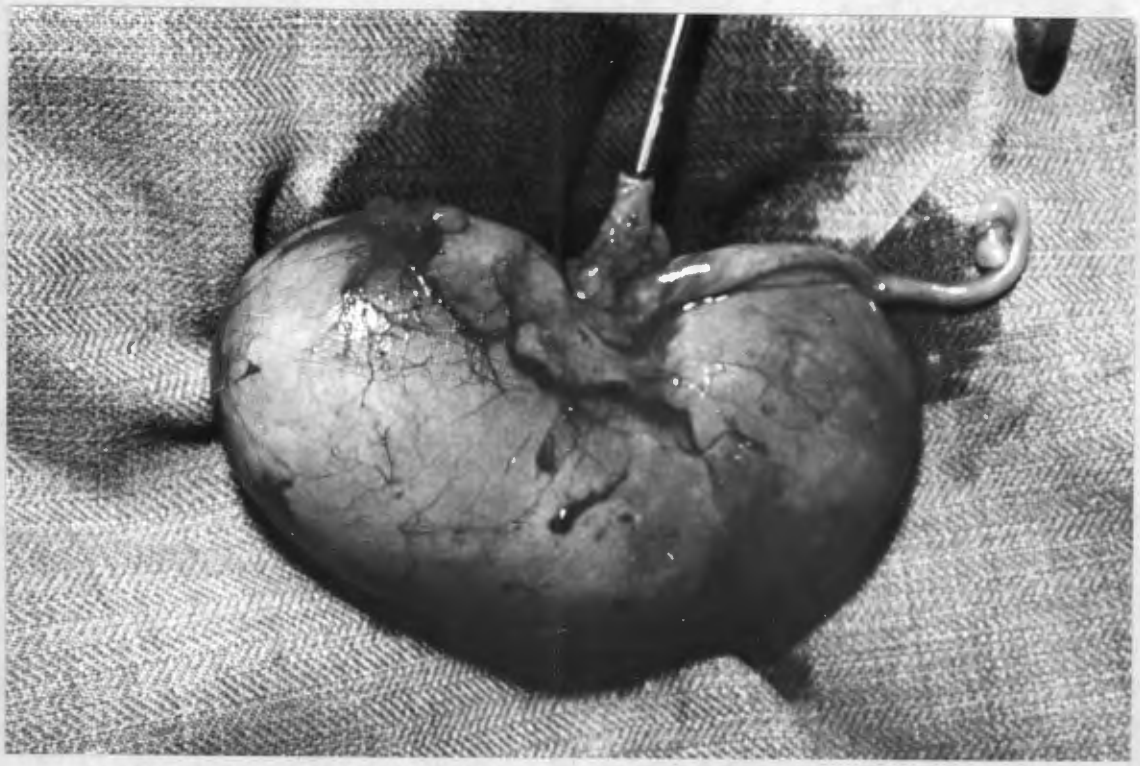
(d) TRANSPLANTATION

It is of utmost importance to bear in mind that, in these experiments, the stored kidney is always re-implanted in the original donor - i.e. autotransplantation. Consequently there are no biological problems of incompatibility.

In all these experiments the stored kidney is re-implanted in the dog's neck. The renal artery and vein are anastomosed to common carotid artery and external jugular vein respectively: the carotid-jugular complex (Pierce and Varco, 1964).

/ ...

Fig. 2.



Photograph illustrating immediate hypothermic kidney perfusion after initial nephrectomy, using a blunted, wide-bore needle inserted into the renal artery. Note the blood-stained effluent from the renal vein.

Fig. 3.

This photograph shows the extent of the wide capsulotomy which is carried out on the kidney after nephrectomy.

Fig. 4.

At the stage when this photograph was taken, the external jugular vein and common carotid artery have been carefully dissected free, in preparation for re-implantation of the stored kidney into the dog's neck.

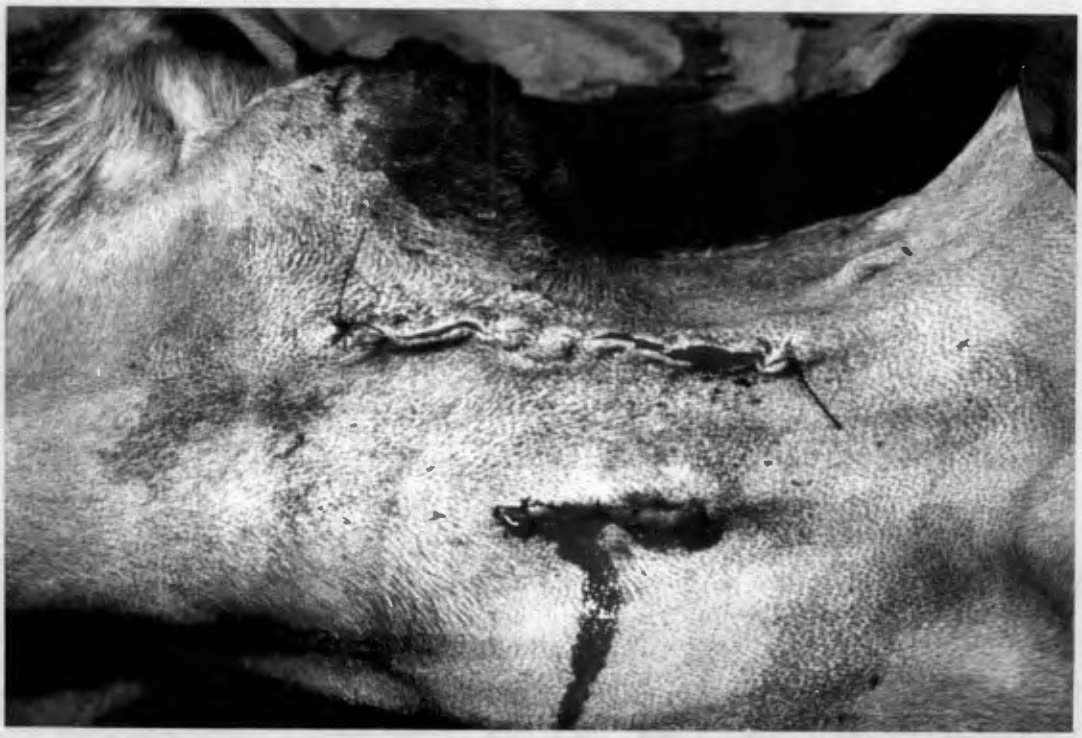
Fig. 5.

This photograph was taken at the stage of re-implantation when the anastomosis of external jugular vein to renal vein, and of common carotid artery to renal artery, has been completed.

Fig. 6.

At the time when this photograph was taken, the transplanted kidney has been placed in the specially-prepared sac deep to the panniculus carnosus. Note the haemostasis at the suture lines and the immediate flow of urine from the ureter, lying free on the gauze swab.

Fig. 7.



Autotransplantation of the kidney has been completed. The photograph illustrates the "fish-mouth" muco-cutaneous ureterostomy. Note blood-stained flow of urine (from unligatured ureteric artery).

A 10 cm. incision is made to the left of the midline in the mid-cervical region. The external jugular vein, lying superficially, is dissected free. The common carotid artery is approached either posterior to the strap muscles, or by blunt dissection through the muscles themselves. Care is taken to preserve undamaged the vagus nerve in freeing the artery of its attendent structures (Fig. 4).

"Bulldog" arterial clamps are applied to the vessels proximally and they are ligated distally. Adventitia is carefully removed from the incised end of the artery. The renal artery and vein are anastomosed end-to-end to the common carotid artery and external jugular vein, respectively, using either five '0' or six '0' arterial silk (Fig. 5). Adhesives were never used.

By blunt dissection, an adequate cavity is constructed for the kidney in the dog's neck, deep to the panniculus carnosus and lateral to the skin incision, (Fig. 6). A small rectangle of skin is excised in the midline, caudad to the main incision. The tissue between this opening and the site of re-implantation is tunnelled and the ureter is delivered. A "fish mouth" opening is made in the ureter and a muco-cutaneous ureterostomy is created by careful suture of ureteric mucous membrane to the skin (Fig. 7).

(e) CONTRALATERAL (LEFT) NEPHRECTOMY.

Contralateral nephrectomy was performed at the time of re-implantation in some animals, and from two to three weeks later in others. In a few cases, contralateral nephrectomy was not performed.

When this research was begun it was not realised that life-sustaining function of the stored kidney might not be possible until after a two to three week regenerative period. In the early experiments, consequently, contralateral nephrectomy and re-implantation were performed simultaneously without any set indication. Once it was realised that a delay is sometimes

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imperative to enable the kidney to regain adequate function, the following criteria were established for removal of the remaining kidney:-

1. An adequate urinary output, assessed both before and after an intravenous water-load;
2. The absence of any marked abnormality of urinary sediment (particularly of casts);
3. A proteinuria of ++ or less;
4. Normal size, shape and consistency of the re-implanted graft in the dog's neck;
5. A urinary urea approximating that observed in the urine secreted by the dog's undisturbed left kidney.

Left (contralateral) nephrectomy is performed through a left muscle-splitting incision. The renal artery, vein and ureter are individually ligated but no attempt is made to conserve any length of these structures.

STORAGE.

In all these experiments the duration of storage was 24 hours. The method of storage varied in each of the six experimental groups but the apparatus was standardised and, in each case, the technique was unaltered.

Four methods of storage were employed: hypothermia (2° - 10°C), freezing (minus 5° - minus 10°C), hyperbaric oxygenation (at 3 atmospheres absolute) and continuous kidney perfusion. These methods were used either singly or in various combinations, as follows:

Group I	-	Hypothermia only,
Group II	-	Freezing only,
Group III	-	Freezing with hyperbaric oxygenation,
Group IV	-	Hyperbaric oxygenation only,
Group V	-	Hypothermia with hyperbaric oxygenation,
Group VI	-	Hypothermia, hyperbaric oxygenation and perfusion.

(A) HYPOTHERMIA (2° - 10°C)

Apparatus:

During hypothermic storage the kidney is immersed in a pre-cooled solution which is made up from the following:-

Rheomacrodex in saline 0.92 %	100 cc.
Dextrose water 5%	10 cc.
Potassium chloride 10%	0.7 cc.
Calcium chloride 10%	1.4 cc.
Sodium bicarbonate 4%	15 cc.
Procaine 1%	25 cc.
Heparin	15 mgm.

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A standard easily regulated refrigerator is used to attain the desired temperature.

A sterile, glass beaker is used as the container for the kidney and immersate during storage.

Technique:

The immersate is pre-cooled in the beaker, in the refrigerator. After resection of the kidney for transplantation, following perfusion cooling, the kidney is submerged in the immersate and placed in the refrigerator for 24 hours. The temperature range of 2° - 10°C is maintained for this period. (Fig. 8).

At the end of storage the kidney is again perfused, at between 2° and 5°C, and is re-implanted in the dog's neck. Here the kidney is exposed to body warmth, and it is gradually rewarmed in this way during the 20 to 30 minutes period while the vascular anastomoses are completed.

(B) FREEZING (minus 5° - minus 10°C).

Apparatus:

The solution used for immersion of the kidney during storage is similar to that employed for hypothermic storage, as already detailed.

A sterile glass beaker is the container for the kidney and immersate.

A deep-freeze unit is used to attain sub-zero temperatures.

Technique:

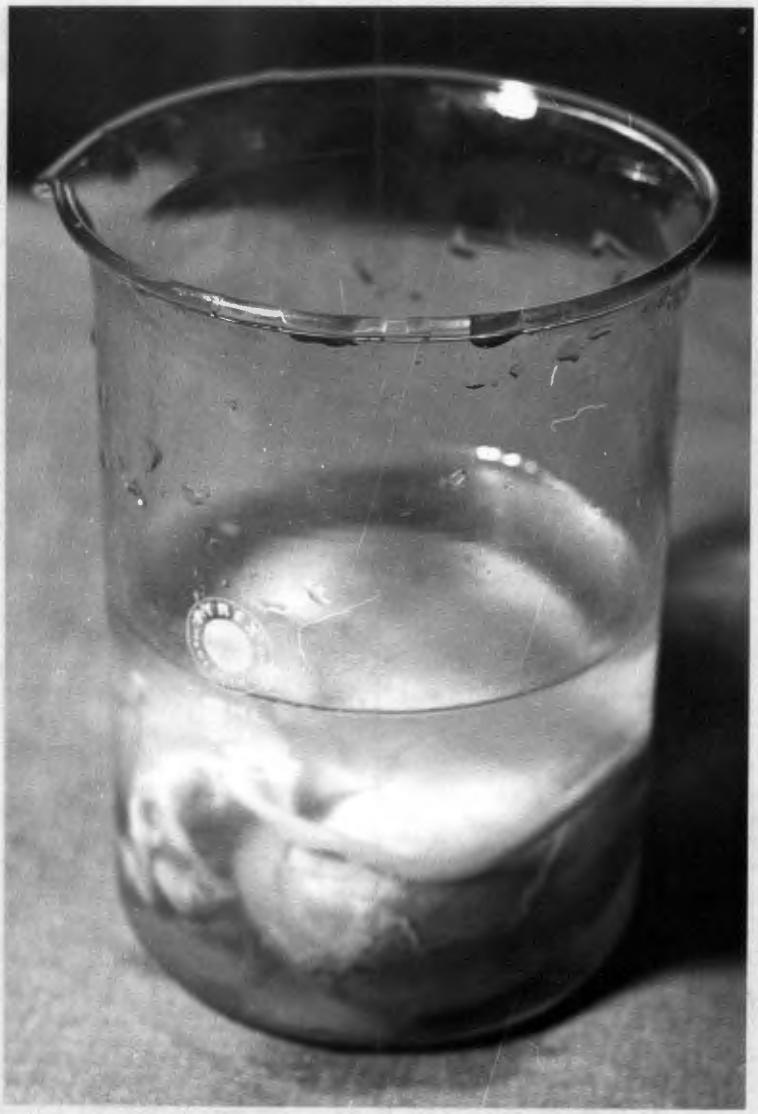
No attempt is made to freeze the immersate before storage; the solution is cooled to just above 0°C. in the beaker.

After nephrectomy and immediate perfusion cooling, the kidney is submerged in the immersate and placed in the deep-freeze unit, where the required temperature is maintained throughout storage.

At the end of storage the kidney is again perfused at between

/ ...

Fig. 8.



Photograph of the kidney to be stored immersed in the specially prepared solution, in the beaker.

2° and 5°C. It is placed in the dog's neck and rewarming is effected by body warmth in this way.

(C) HYPERBARIC OXYGENATION

Apparatus:-

A hyperbaric oxygen chamber was manufactured locally, to specifications provided, following the A.S.M.E. code of construction and in conformance with Factories Act requirements (Fig.9a). Argon-welding was employed and the joints were smoothly ground and polished, inside and out.

The chamber is cylindrical, 12 inches high, with an internal diameter of 9 inches, and made from high grade stainless steel (grade 316). The dished lid is secured with four swing bolts. A dished head base completes the shell of the chamber, which stands on a funnel-shaped pedestal for stability. Working pressure is 50 lbs./square inch and the chamber was tested to 75 lbs./square inch pressure. Within the chamber is a perforated, removable bottom plate. The thermometer projects below this plate. The fixtures comprise:-

Pressure gauge reading up to 60 lbs./sq.inch

Thermometer (40°F - 110°F)

Two perfusion nipples, 2 mm. internal diamter,
(which provide access to the chamber and are easily closed off)

Quick coupling oxygen connector for compression,

Exhaust valve,

Safety valve operating at 52 lbs./sq.inch.

A standard oxygen cylinder of 2,000 lbs./sq.inch is used for compression, by way of a reducing valve (Fig.9b).

During storage, the kidney is placed in a sterile glass beaker containing the previously described immersate solution.

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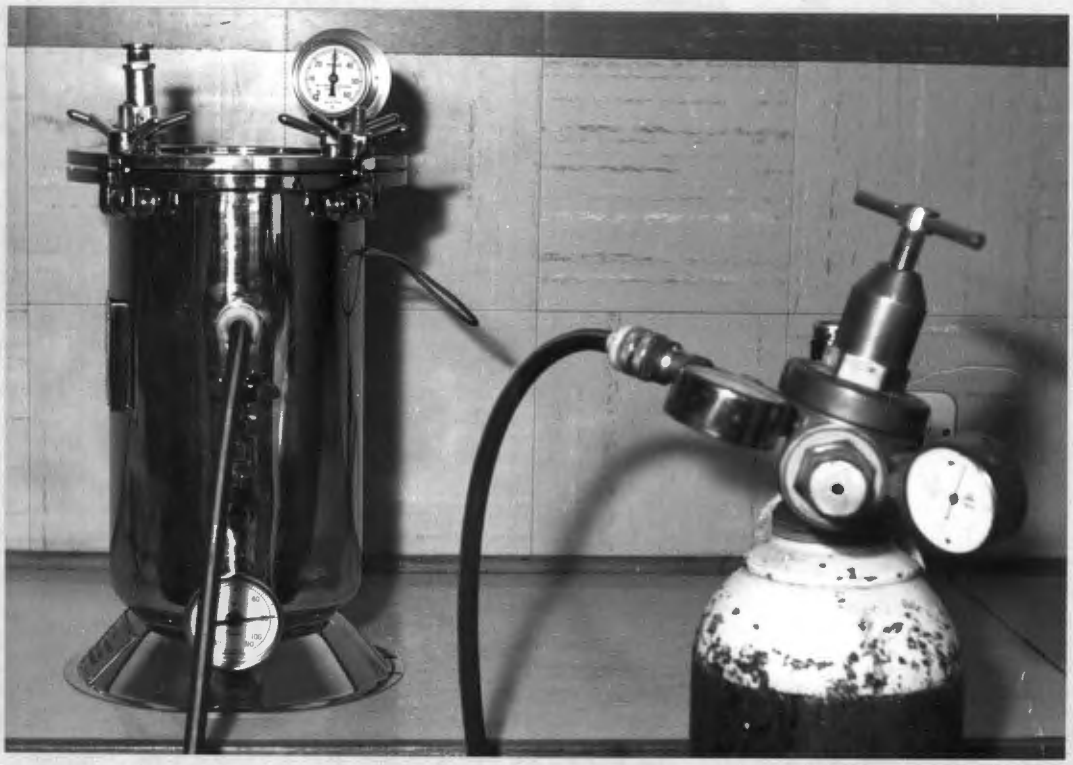
Fig. 9 a.

Photograph of the hyperbaric oxygen chamber which was used in these experiments. Note the pressure gauge, safety valve, perfusion nipples, quick-coupling connector, exhaust valve and low-reading thermometer.

Fig. 9 b.



This photograph was taken during compression of the hyperbaric chamber to 3 atmospheres absolute of oxygen. The perfusion nipples are blocked off with tubing. In the foreground is the oxygen cylinder with reducing valve.

Fig. 10.

In this photograph, the immersed kidney has been placed inside the hyperbaric chamber. Once the chamber has been sealed, compression will commence immediately.

Fig. 11.

The hyperbaric chamber, compressed to 3 ATA, is shown inside the refrigerator used to maintain hypothermia.

Technique:-

The chamber and its components are sterilised with the lid off. The chamber is then resealed immediately. Hyperbaric oxygenation is used in two ways during these experiments: (1) alone, and (2) in combination with cooling.

(1) Hyperbaric oxygenation alone: the kidney is not cooled at any stage after nephrectomy; even the initial perfusion cooling is omitted. The kidney is placed in the immersate within the chamber at room temperature. The chamber is compressed to 3 ATA for the storage period. (Fig. 10)

(2) Hyperbaric oxygenation combined with cooling: the chamber is pre-cooled to the desired temperature. After nephrectomy and initial perfusion cooling, the kidney is placed in the immersate and into the chamber. The chamber is compressed to 3 ATA and is placed in the refrigerator (Fig. 11) where constant pressure and temperature are maintained throughout storage (except for brief periods of decompression when samples are taken - Group VI experiments).

At the end of storage, decompression of the chamber is effected by the exhaust valve and takes 5 to 10 minutes. The chamber is opened and the beaker and kidney removed. When hypothermia is used with hyperbaric oxygenation, the kidney is immediately perfused at from 5° - 10°C. The kidney is then rewarmed by body heat in the dog's neck, on re-implantation.

D. KIDNEY PERFUSION DURING STORAGE WITH HYPOTHERMIA AND HYPERBARIC OXYGENATION.

1. Apparatus:

Most of the equipment used for hypothermia and hyperbaric oxygenation is as already described. The additional equipment, used within the hyperbaric chamber, is as follows (Fig. 12).

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(a) Reservoir: 1½ litre pyrex flask with a specially blown effluent to accommodate the Tygon and rubber tubing used for perfusion during storage. This run-off is flush with the bottom of the flask. No immersate or beaker is used. The perfusate collects in this reservoir, leaves the chamber to return to the pump for recirculation during storage.

(b) Stand: made from stainless steel, this was designed to bear the kidney during storage, on a shelf resting on the brim of the reservoir. It incorporates a corrugated, steeply sloped run-off which rests on the floor of the reservoir.

(c) Cushion: a compressed ivalon sponge cushion was provided for the kidney during storage.

The following apparatus is used for kidney perfusion during storage:-

(a) Pump:

A compact, low-flow, kinetic, clamp-tubing pump (Fig.13a) with a capacity of 1.5 - 1,000 cc./hour (Model AC 4/E Sigmamotor). This operates on the principle of positive displacement.

A loop of tubing is held between two clamping positions, one at right angles to the shaft and the other eccentric. As the shaft rotates, the clamping position moves around the loop of tubing, forcing its contents along (Fig.13b). With this peristaltic action the liquid/gas being pumped is completely contained within the tubing. As the clamp position moves around the circle, the tubing behind this point returns to its normal concentric form, creating a vacuum which again fills the tubing. Each complete cycle displaces a unit volume of liquid. Flow rate is determined by the number of cycles per minute multiplied by the displacement.

A slotted tube retainer prevents tubing creepage. Steel, annular ball bearings ensure silence and accuracy. The pump has a

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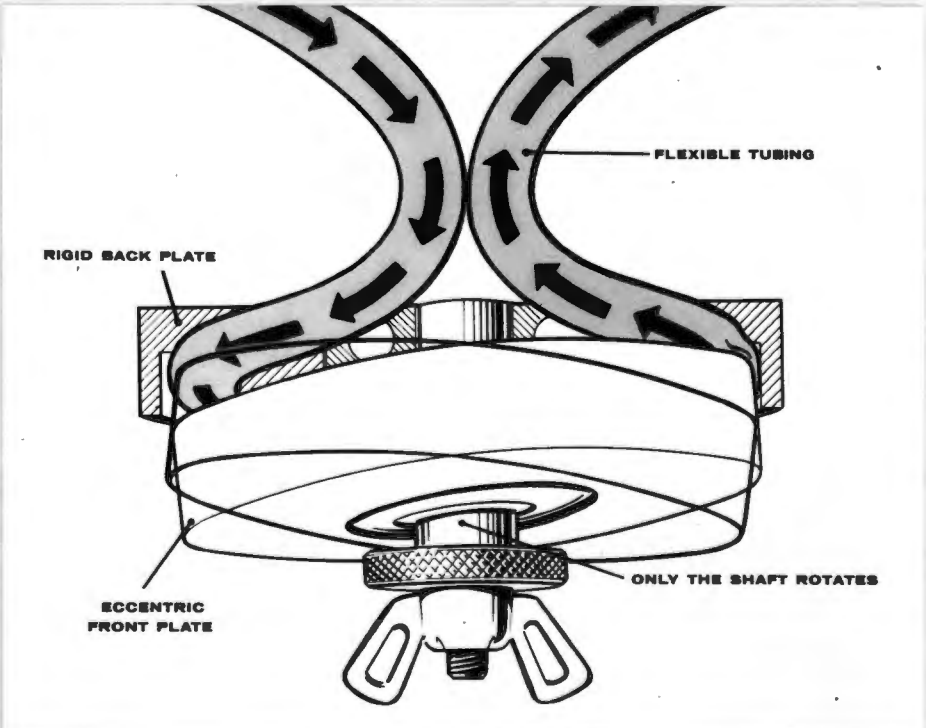
Fig. 13 a.

Low-flow, clamp tubing pump used for perfusion storage, showing the pump head, clearly calibrated flow-rate gauge, switch and speed regulator.

Fig. 13 b.

Diagram of the pump head action: as the shaft turns the clamping position moves around the loop of tubing and forces the contents along.



direct drive motor which operates continuously without overheating, and is suitable for moving liquids or gases. This model's speeds range from 1/3 r.p.m. to approximately 300 r.p.m. Flow rate is controllable up to 1,000 cc./hour by means of an EC gear head motor and solid set convertor operating on regular A.C. supply. The percentage of immediate volume and speed is indicated on a large precision gauge, allowing simple calibration of flow rates.

The entire circuit is protected by means of two fuses, on the front panel. This panel also incorporates a neon pilot light. Ample ventilation is provided to maintain temperatures slightly above ambient for extended use. The overall noise level is minimised by a sponge-like insulation on the anterior casing. The pump unit weighs 10 pounds and measures 8 x 6½ x 8 inches.

(b) Tubing:

Tygon tubing 1/8" x 1/16" is used for the pump head, and high pressure type rubber tubing 1/8" x 1/16" for the remainder of the circuit. A 3 way tap is incorporated in the circuit, connecting the Tygon and rubber tubing and facilitating the taking of specimens.

2. Perfusates.

Three perfusates were used in these experiments:-

(1) Haemodilution (Group I)

Autologous whole blood	125 cc.
Rheomacrodex in saline 0.92 G%	100 cc.
Dextrose in water 5%	10 cc.
Potassium chloride 10%	0.7 cc.
Calcium chloride 10%	1.4 cc.
Sodium bicarbonate 4%	15 cc.
Procaine 1%	25 cc.
Heparin	15 mgm.

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(2) Balanced Physiological Solution (Group II)

Rheomacrodex in saline 0.92 G%	200 cc.
Dextrose in water 5%	10 cc.
Potassium chloride 10%	0.7 cc.
Calcium chloride 10%	1.4 cc.
Sodium bicarbonate 4%	15 cc.
Procaine 1%	25 cc.
Heparin	15 mgm.

(3) Whole Blood Perfusion (Group III)

Autologous whole blood	250 cc.
Procaine 1%	25 cc.
Heparin	15 mgm.

Whichever of these perfusates was used, 250 cc. was required for each kidney stored.

Perfusion of the kidney was only used in Group VI experiments, and these were subdivided into three groups depending on the perfusate used.

3. Technique.

The tubing is assembled with the 3-way tap connector before sterilisation. All the tubing is then immersed in an alcohol/formalin solution for 24 hours. No detergents are used.

The hyperbaric chamber is autoclaved, as described previously. The ivalon cushion, stainless steel kidney stand and pyrex reservoir are boiled for sterilisation.

Once sterilisation is complete, the Tygon tubing is fitted to the pump head and the rubber ends to the inflow and outflow nipples on the outside of the hyperbaric chamber, under sterile conditions (Fig. 14). The reservoir, stand and ivalon cushion are placed within the chamber. The reservoir effluent is connected to the outflow nipple on the interior of the chamber.

Balanced, buffered physiological solution is placed in the reservoir. In Groups I and II the correct amount of fluid to be

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Fig. 14.

Perfusion storage with hypothermia and hyperbaric oxygenation: the assembled set-up is shown, the perfusion tubing being slotted into the pump head and the ends attached to the appropriate nipples on the chamber wall.

used during storage is placed in the reservoir at this time; for the Group III experiments the same solution is used but will be removed before storage. Approximately 50 cc. of the solution is run through the circuit, to ensure absolute cleanliness. The chamber is then closed and placed in the refrigerator, to be cooled to the required temperature.

In experiments where autologous whole blood is required (Groups I and III), a cut-down is made over either femoral artery of the anaesthetised dog. By blunt dissection, the artery is exposed, ligated distally and controlled proximally with a "bulldog" clamp. An incision is made and a polythene cannula inserted and secured in the artery with a single silk tie suture. The proximal clamp is released, allowing the required blood to flow into a heparinised vacolitre. The artery is ligated proximally, after removal of the cannula, and the wound is closed in layers in the usual way. This procedure is performed immediately before the dog is positioned for right nephrectomy.

The chamber is removed from the refrigerator and opened. In Group I experiments the heparinised blood is added to the pre-cooled contents of the reservoir. For Group III experiments, the reservoir and circuit are carefully emptied and the heparinised blood only is placed in the reservoir. In the Group II experiments the contents of the reservoir are not disturbed.

A sterile thermometer is placed in the reservoir at this stage.

Recirculation of the perfusate is commenced at the maximum flow rate of 1,000 cc./hour. The chamber is closed and compressed with oxygen to 3 atmospheres absolute. It is returned to the refrigerator where recirculation of the perfusate continues while the nephrectomy is being performed.

Perfusion:

Immediately following nephrectomy and capsulotomy, the kidney is perfused for initial cooling, as in previous experiments. The flow pressure is between 100-150 mm.Hg and the usual pre-cooled

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physiological solution is perfused until the effluent from the renal vein is clear. A blunted No. 15 needle is inserted and attached as close as possible to the cut end of the renal artery for perfusion, and tubing is connected to the needle base (Fig. 15).

Before the chamber is decompressed, the required flow rate for perfusion is established. The chamber is decompressed and opened. The kidney is placed on the ivalon cushion, onthe metal stand on the reservoir. The tubing attached to the renal artery is connected to the inflow nipple on the inside of the chamber. Care is taken to ensure the patency of the artery distal to the needle point.

Perfusion of the kidney is commenced. The chamber is closed securely and recompressed to 3 ATA, and is returned to the refrigerator for the duration of the 24 hours' storage (Fig. 16).

Procedure Post-Storage:

At the end of the storage period the chamber is removed from the refrigerator and decompressed. Perfusion is discontinued and the kidney is carefully removed, with its inflow attachments, to the operating table. The kidney is then perfused at low pressure with a pre-cooled (15°C) solution of Rheomacrodex, heparin and procaine 1%. The silk tie suture holding the needle in place in the renal artery is cut and the needle is removed. The traumatised end of the renal artery is resected.

Rewarming is effected in the usual way. The kidney is re-implanted into the neck of the anaesthetised dog and is warmed by body heat in this position.

4. Histology:

Where histology is the stored kidney is required, the organ is photographed, incised and the representative sections taken. The kidney is then discarded.

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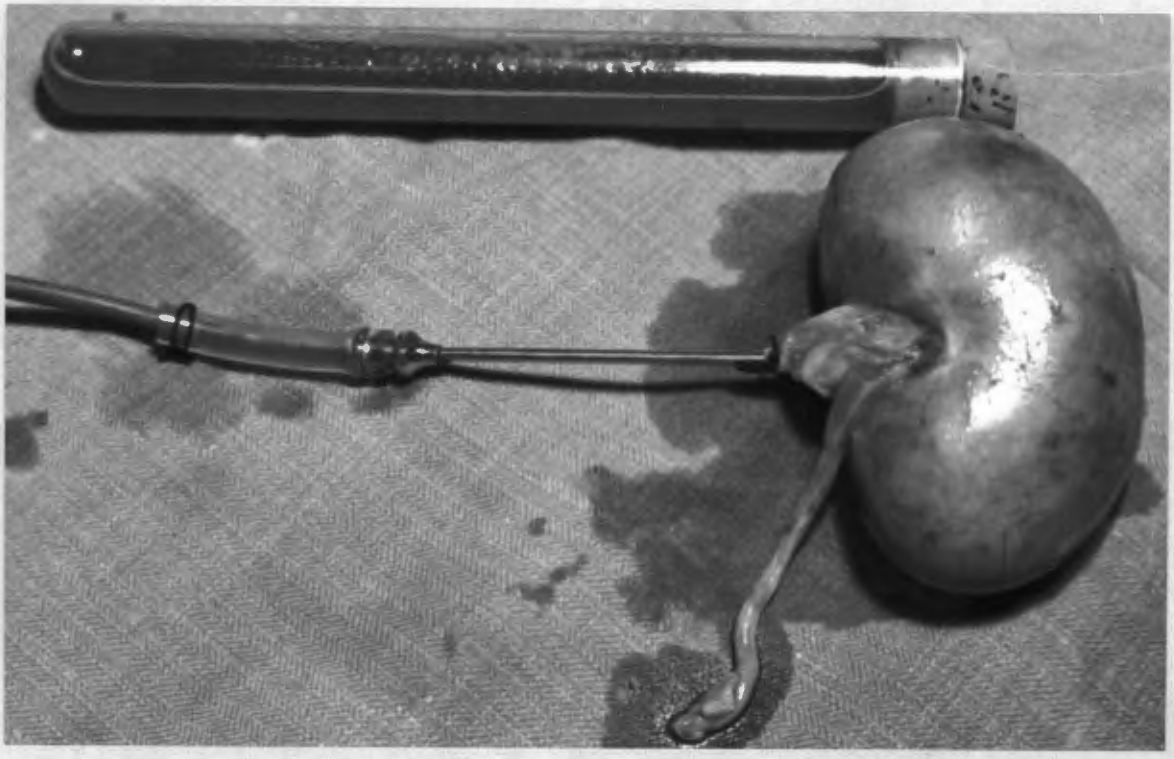
Fig. 15.



In this photograph, the cannula used for perfusion has been secured in the renal artery and is attached to the perfusion line. The test tube shown contains a specimen of the perfusate, for electrolyte and urea estimations.

Fig. 16.

Photograph showing the assembled apparatus for perfusion storage with hypothermia and hyperbaric oxygenation (Group VI). The chamber has been placed in the refrigerator, after compression to 3 ATA, and perfusion of the kidney is in progress.

INVESTIGATIONS.

These investigations were performed daily in these experiments, except where otherwise indicated. They commenced on the day on which contralateral nephrectomy was performed (i.e. when the animal was rendered dependent on the stored organ) and continued for the 14 days constituting "survival".

The purpose of the investigations was to assess renal function of the stored kidney, and to exclude any indication of threatened renal homograft rejection (Fig. 17) (Ackermann et al, 1965 c).

1. BLOOD.

(a) Haemoglobin (Hb.): This was measured with a standard colour-matching instrument.

(b) White blood cell count (WBC): A special pipette was used for dilution and the white blood cells counted on a Thoma-Zeiss counting chamber with Neubauer rulings.

(c) Stained blood film (differential count): The film is stained with Leishman's stain, the method being a simplification of that first introduced by Romanowsky. All blood elements are examined with special attention to the white cell series; a differential count of the latter is done.

(d) Blood urea nitrogen (BUN) is estimated by a micro-analytical method.

2. SERUM.

Sodium (Na^+), potassium (K^+), chloride (Cl^-) are estimated by micro-analytical methods carried out in the chemical pathology laboratory. Creatinine clearance is estimated by micro-analysis and from the urine (see below).

/ ...

Fig. 17.

DIAGNOSTIC CRITERIA OF RENAL HOMOGRAFT REJECTION.

1. ABNORMAL URINARY SEDIMENT STAIN.
2. DECREASED TOTAL URINE OUTPUT.
3. UNEXPLAINED TEMPERATURE ELEVATION.
4. INCREASE IN SIZE OF HOMOGRAFT WITH PRONOUNCED TENDERNESS.
5. INCREASE IN PROTEINURIA.
6. RISE IN B.U.N.
7. DECREASE IN RENAL CLEARANCE VALUES.
8. POSITIVE URINARY CATALASE.
9. INCREASED TOTAL WHITE COUNT WITH MARKED POLYMORPH PREPONDERANCE.

3. URINE. (Fig. 18).

(a) Protein: A qualitative and rough quantitative guide to proteinuria was possible, using "Albustix" reagent strips.

(b) Sodium, potassium, chloride, urea and creatinine are all evaluated by micro-analysis performed in the chemical pathology laboratory.

(c) Urinary catalase estimation (Kenah, 1964): Catalases are enzymes which catalyse the decomposition of hydrogen peroxide, with liberation of molecular oxygen, and may be detected by the disappearance of hydrogen peroxide from the substrate.
Method (Feigl, 1946) - The titanium salt reaction is used for the peroxide test. (The test using lead sulphide paper also serves).

1. $TiOSO_4 + H_2O_2 + H_2SO_4 = H_2(TiO_2(SO_4)) + H_2O$

2. $PbS + 4\ H_2O_2 = PbSO_4 + 4\ H_2O$

A drop of the solution to be tested for catalase is mixed with a drop of 3% solution of H_2O_2 and left for 1 hour. In the presence of catalase, the addition of a titanium salt will give no colour (or only a slight yellow) while the blank test develops an orange-yellow colour.
A drop of the solution treated with hydrogen peroxide may be placed on lead sulphide paper, as an alternative test, when the positive or negative hydrogen peroxide reaction may be noted.

(d) Stained urinary sediment smears (Calne, 1964; Ackermann and Barnard, 1965 a): At 12 hourly intervals, a fresh specimen of urine, 5-10 cc., is collected from the cutaneous ureterostomy. Contamination by the surrounding hair or skin is carefully precluded,

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Fig. 18.

The kidney is resting on its ivalon cushion above the reservoir, within the hyperbaric chamber. The photograph shows the finger-stall, secured to the cut end of the ureter, used for urinary collection. Any overflow will fall into the reservoir, not being lost.

facilitated by the previously constructed muco-cutaneous junction. The specimen is centrifuged for 2 minutes at 1,500 r.p.m. and all except approximately 1 cc. of the supernatent clear urine is discarded. By gentle agitation the sediment is re-suspended. A drop is placed on a slide and as thin a smear as possible is made and allowed to dry. Three different stains were used, the third being most commonly employed in these experiments: (1) 3parts Gentian Violet with 97 parts Safranin, (2) a combined Wright's /Giemsa stain, and (3) standard Leishman's stain. The third method is a simplification of that introduced by Romanowsky. The dry film is well covered with the stain. After 1 minute, double the quantity of distilled water is added and thoroughly mixed. After 7 minutes, this mixture is decanted and the film covered with distilled water for another 2 minutes. This water is washed off with fresh distilled water, and the smear is blotted dry. Examination of the smears is performed under both low and high power magnification, at least 15 minutes being given to each. The points to be noted are:-

(1) Presence (or absence) of lymphocytes,
(2) The number of these cells relative to other types of white and red blood cells,
(3) a rough estimate of the actual number of lymphocytes,
(4) whether these cells are free, clumped or in casts,
(5) haematuria,
(6) polymorphs (index of infection),
(7) tubular casts, ureteric or tubular epithelial cells, hyaline casts, haemoglobin casts, etc.

/ ...

(e) Creatinine Clearance estimation was infrequently performed in Groups I to V experiments because of the technical problems in urinary collection. In Group VI experiments, intravenous infusions of 1 litre normal saline (as a water load) were given prior to creatinine clearance estimation. The estimate was made by micro-analysis in the chemical pathology laboratory.

4. In addition to the above, in Group VI experiments the following additional investigations were performed during the 24 hour storage period:

1. Perfusion pressure: The pressure line was attached to a Siemens Cardirex 6-channel recorder. Recordings could be made only after decompression of the hyperbaric chamber. As soon as the reading was standardised, recompression was carried out. The duration of decompression never exceeded 5 minutes.

2. Full acid-base determinations (Astrup method), electrolyte study and urea estimation of the perfusate. Specimens were taken at full compression by way of the 3-way tap connector incorporated in the perfusion line.

3. Urinary electrolyte studies, urea, protein estimation and approximate volume of urine. For these studies, decompression and opening of the chamber were necessary, although perfusion was not discontinued. These determinations were performed in some experiments only, selected at random, in each of the three sub-groups in Group VI. The total decompression time was 5 minutes and coincided with pressure recording episodes.

5. GENERAL CONDITION OF THE DOG.

The experimental animal was examined daily for any signs of infection or poor condition.

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6. LOCAL EXAMINATION.

In each experiment, the condition of the re-implanted kidney was carefully evaluated daily, special attention being paid to the size and consistency of the graft.

SURVIVAL.

The only true indication of the success of a storage procedure is the survival of the bilaterally nephrectomised animal with a single, previously stored, re-implanted kidney.

Survival time in these experiments is 14 days, commencing with re-implantation. During this fortnight, intensive investigations were performed to assess the function of the re-implanted organ. Where late contra-lateral nephrectomy was performed, the investigations were begun at that stage and survival time denotes the 14 day period following contralateral nephrectomy.

GENERAL CARE OF ANIMALS.

DRUG THERAPY.

No chemotherapeutic drugs were used in these experiments.

Antibiotics were administered only when specifically indicated, and then for a period of 6 to 8 days. The antibiotics used in these experiments were streptomycin (1 G. daily) in combination with penicillin (1 million units) daily, and intravenous tetracycline (275 mgm. daily).

INTRAVENOUS FLUIDS.

A slow infusion of 5% dextrose in water was administered during each operation.

Post-transplantation, there are two indications for intravenous fluid administration:

1. In an attempt to prevent infection, by the flushing effect of increased urinary output, and
2. To produce a water load, maintaining an adequate urinary output, to facilitate the assessment of clearance values (Dowdle and Eales, 1964).

In both instances, either 500 cc. of 50% normal saline, or the same quantity of a balanced physiological solution, was used.

DAILY CARE OF ANIMALS.

The dogs receive a standard diet and are exercised daily.

R E S U L T S.

R E S U L T S.

In the first five experimental groups it should be borne in mind that much of the work described has been performed previously by others (see the Review of the Literature). The experiments were repeated firstly to confirm the inadequacy of these methods as storage procedures in these experimental conditions, and secondly, to establish the standard techniques. Thus not many experimental animals were used in the first five experimental groups; the methods used were so obviously ineffective for 24 hour kidney storage that no purpose would have been served in further experimentation.

In discussing the results in each group, the post-storage findings will be described under the following headings:

(a) Before transplantation of the stored kidney,
(b) After re-implantation, during the 30 minute observation period,
(c) Contralateral nephrectomy,
(d) Course,
(e) Pathology,
(f) Discussion,
(g) Conclusions.

RESULTS.

GROUP I.

KIDNEY STORAGE USING HYPOTHERMIA ONLY.

There were 9 experiments in Group I, the kidney being stored for 24 hours at a temperature of between 2°C and 5°C.

(a) Before transplantation:

The kidney was soft and white after storage but in consistency was tense to the point of rubbery toughness. Darkish blood invariably oozed from the areas denuded by capsulotomy.

(b) Following re-implantation:

On restoration of the blood supply the kidney developed a mottled pink but relatively normal appearance. It increased in size by one-third to one-half. During the 30 minute observation period there was usually a conspicuous colour alteration from pink to a darker more congested hue; consistency and size remained unchanged. A few drops of clear urine were excreted during this period.

(c) Contralateral nephrectomy:

The immediate urinary output prompted simultaneous contralateral nephrectomy in the first 4 animals. In the other 5, left nephrectomy was performed between 20 and 28 days after re-implantation.

No criteria for this procedure had been established and, had these been available, contralateral nephrectomy would have been performed in 1 dog only 20 to 28 days following re-implantation; this dog was the sole survivor in Group I.

(d) Course:

None of the 4 dogs which underwent immediate left nephrectomy survived more than 3 days, all dying from uraemia despite the fact that small amounts of urine were still being passed at the time of death.

Of the other 5, 4 succumbed from uraemia within 4 days of left nephrectomy ~~due to uraemia~~. There had been some urinary output but this was

insignificant in amount when compared with that excreted in the sole survivor after left nephrectomy.

At the end of the 14 day survival period, renal function in the only survivor in Group I was still abnormal (Fig. 19) and renal parenchymatous damage was assumed to be irreversible at this stage. This dog died on the 29th day after left nephrectomy as a result of intercurrent infection.

(e) Pathology:

In the 4 dogs which had simultaneous re-implantation and left nephrectomy, post mortem examination showed swelling and congestion of the stored kidney. The organ was blue-black in colour with apparent areas of massive subcapsular haemorrhage. Microscopy showed the glomeruli to be essentially normal, predominant damage being tubular, with massive small vessel thrombi.

In four dogs with late contralateral nephrectomy, the re-implanted kidney was shrunken and largely fibrotic on post mortem examination. However, there was some evidence of tubular regeneration with organisation of the small vessel thrombi. The glomeruli appeared relatively normal.

In the fifth dog (the only survivor in Group I), which died on the 29th day following late left nephrectomy, there was less scarring in the stored kidney than had been evident in the others of this group. Tubular regeneration was more noticeable and the glomeruli were essentially normal. Although the destruction was considerably less extensive than in the others, however, insufficient renal tissue had survived for life-sustaining function of the stored kidney.

(f) Discussion:

The benefits of hypothermia to ischaemic and anoxic tissue have been discussed at some length in the Review of the Literature. Immediate hypothermic perfusion of the kidney to be stored has been

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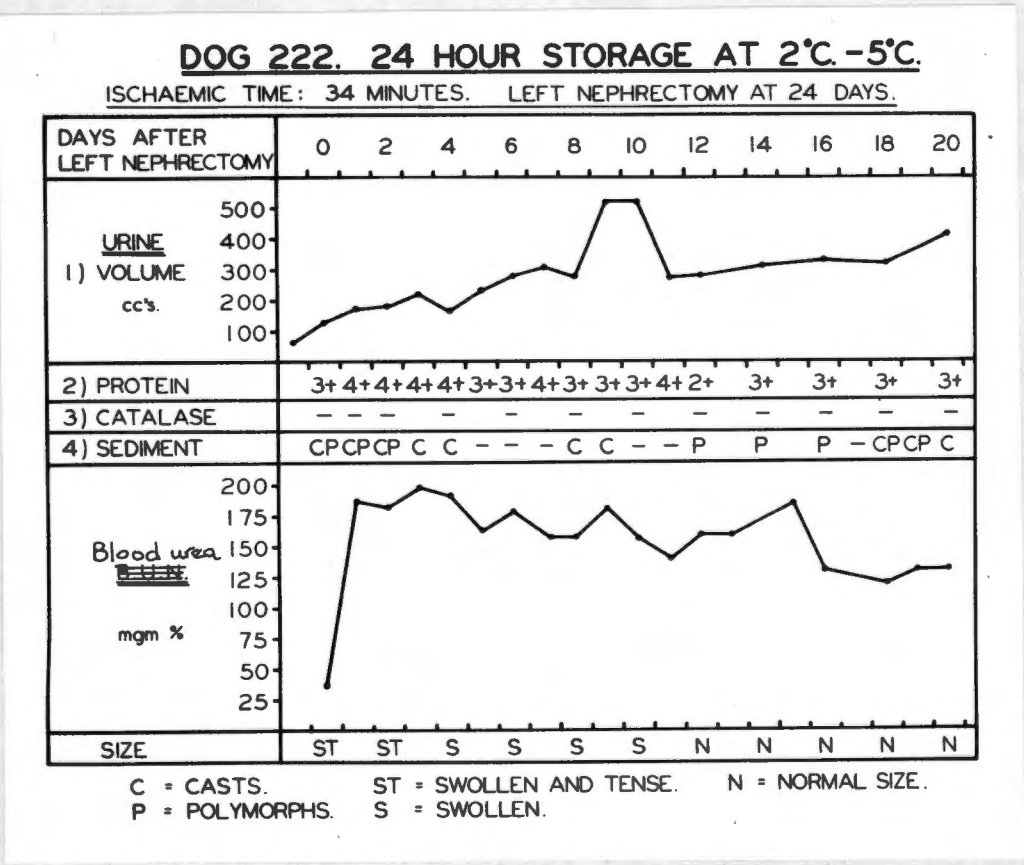
Fig. 19.



Group I - Composite chart of the findings on investigation in the sole survivor after late contralateral nephrectomy. Twenty days after late nephrectomy of the normal left kidney abnormal function of the re-implanted, stored kidney still persists.

shown to be the most efficient and safe way of lowering the renal core temperature. The great importance of the uniformly rapid production of renal hypothermia by this method has been stressed by many workers. By comparison, immersion or surface cooling of the organ is inefficient and, in fact, by producing temperature gradients in this way the parenchymatous cells may be actively damaged. For these reasons, surface cooling was used in Group I only to maintain the hypothermic levels already achieved by the initial perfusion cooling of the kidney.

From the results obtained in Group I it is obvious that hypothermia alone cannot arrest or depress cellular metabolic activity sufficiently to preserve reasonable renal function after 24 hours' storage.

So severe was ischaemic necrosis in these organs that (with one exception) even during two to three weeks' delay before late contralateral nephrectomy, there was insufficient regeneration to restore function.

The survival of one animal for 14 days after left nephrectomy, indicating a lesser extent of renal damage, is difficult to explain. The experimental procedure was exactly similar in all 9 cases. It is suspected this was a remarkable individual animal with less susceptibility to the effects of ischaemia.

(g) CONCLUSION:

Kidneys cannot be successfully stored for 24 hours using hypothermia at 2°C-5°C alone. However, this technique does reduce the ill-effects of total ischaemia and anoxia somewhat as complete necrosis of the stored organ was never seen.

RESULTS.
GROUP II.

KIDNEY STORAGE BY FREEZING.

There were 4 experiments in Group II and the kidney was stored for 24 hours at a temperature of between minus 5°C to minus 10°C.

(a) Before transplantation:

The kidney had invariably been frozen solid during storage, in the immersate, and ice had to be carefully removed from the renal artery and vein. As the kidney warmed it became soft. In appearance it was deathly pale.

(b) After re-implantation:

The stored kidney became tensely swollen and blue-black in colour. During the 30 minute observation period no urine was excreted and there was no noticeable colour change.

(c) Contralateral nephrectomy:

This procedure was not performed in any of the Group II dogs. The criteria for opposite nephrectomy had been established at this stage and were not present in any of these animals.

(d) Course:

There was no urinary output at any stage from these kidneys and, within 3 to 5 days of re-implantation, no recognisable kidney was palpable in the dog's neck.

(e) Pathology:

Examination of the stored kidney showed the whole organ to be necrotic, both macroscopically and on histology. Destruction involved both cortex and medulla. Thrombosis of the renal artery and vein was an invariable finding.

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(f) DISCUSSION:

Thermal injury is inevitable at the temperature levels at which these kidneys were stored. Furthermore, although the customary initial rapid reduction in renal core temperature by perfusion cooling was ensured, no technique was used to speed up the further temperature drop through or to the range critical for thermal injury, nor was any attempt made to achieve rapid rewarming post-storage, when further thermal damage is known to occur. Thus it is not surprising that 24 hour storage by simple freezing was unsuccessful in Group II.

The extent of damage caused by relatively slow freezing and thawing can be gauged by simple comparison of these results with those obtained in Group I. The storage temperature difference is only about $10^{\circ}C$ and yet, functionally and pathologically, the kidneys stored by freezing were destroyed. The damage to these kidney was so much worse than that observed in Group I that only thermal injury can account for the difference.

(g) CONCLUSION:

Freezing and thawing are lethal to kidneys stored for 24 hours in these experimental conditions.

RESULTS.

GROUP III.

KIDNEY STORAGE BY FREEZING WITH HYPERBARIC OXYGEN.

Kidneys from 4 dogs were stored for 24 hours at minus 5°C to minus 10°C in 3 atmospheres absolute of oxygen.

Exactly similar findings to those described in Group II experiments were noted in these experiments. Contralateral nephrectomy was never performed, not being indicated. Pathological examination of these stored kidneys showed total cortical and medullary necrosis, as in the previous group.

DISCUSSION:

As found using freezing alone, this method of kidney storage was totally unsuccessful.

In the Review of the Literature it was shown that tissues subjected to very high pressures are protected against the effects of freezing and thawing, in experiments conducted at 15,000 lbs. per square inch atmospheric pressure, using simple tissues. In Group III experiments, subjecting a whole organ to a pressure of 3 ATA, no protective effect was apparent. Any other benefit derived from hyperbaric oxygenation was impossible to gauge in the presence of the gross destruction produced by freezing and crystallisation in these stored kidneys.

CONCLUSION:

Using a pressure of 3 ATA of oxygen, there was no apparent amelioration of the renal damage produced by freezing and thawing, and this method for 24 hour kidney storage was totally unsuccessful.

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RESULTS.

GROUP IV.

KIDNEY STORAGE USING HYPERBARIC OXYGEN ALONE.

Kidneys from 5 dogs were stored for 24 hours under 3 atmospheres absolute of oxygen, at room temperature (approximately 25°C).

(a) Before transplantation:

After storage the kidney was pale pink in colour and comparatively normal in appearance.

(b) After re-implantation:

There was considerable swelling after restoration of the blood supply. The kidney was duskily mottled, the core being dark red in colour. During the 30 minute observation period a small but significant amount of urine was excreted.

(c) Contralateral nephrectomy:

This was performed in only 1 of the 5 dogs in Group IV, 26 days after re-implantation, urine excretion having continued until this time in this animal.

(d) Course:

Urinary excretion was not long maintained in 4 dogs and the stored kidney was obviously clinically necrotic within 5 days of re-implantation, and was removed.

In the fifth dog the urinary output continued and left nephrectomy was performed on the 26th day after re-implantation. Although the output of urine continued, the dog died within 72 hours in renal failure.

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(e) Pathology:

Histology of the 4 kidneys removed within 5 days of transplantation showed both cortex and medulla to be necrotic. Cortical necrosis was of patchy distribution and in some areas the glomeruli appeared normal. The medulla was uniformly necrotic, grossly so in the region of the cortico-medullary junction.

In the fifth dog, which died within 72 hours of left nephrectomy, 29 days after re-implantation, the histological findings were similar but were less severe and of lesser distribution. Here again maximal damage was in the area of the cortico-medullary junction, the proximal tubules being totally destroyed.

(f) Discussion:

In this group, hyperbaric oxygenation was only preservative used for 24 hour storage. From the results it is clear that, at room temperature, considerably more than high pressure oxygen is required for successful kidney storage.

It is interesting to compare the results of storage using hypothermia alone with storage using hyperbaric oxygen alone. Low temperature afforded the kidney greater protection, both functionally and pathologically, and it seems fair to conclude that this method singly is of greater practical benefit.

In the circumstances of this experiment, diffused oxygenation of the stored organ was inadequate. Oxygen dissolves readily and the immersate must have contained ample oxygen during storage. The problem lies in the slow diffusion of the immersate through the renal parenchyma. It was hoped that hyperoxygenation of the immersate would permit slow diffusion without acute tissue oxygen shortage. However, oxygen requirements at room temperature probably far exceed the supply possible by these means; the presence of haemoglobin may be essential for oxygen transport to tissue cells (this possibility will be discussed later) and this would be the missing factor in Group IV experiments.

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(g) CONCLUSION:

The storage of kidneys for 24 hours using hyperbaric oxygenation at 3 ATA, at room temperature, does not preserve adequate renal function. Hypothermia alone better minimises the deleterious effects of organ anoxia than does hyperbaric oxygen.

RESULTS.

GROUP V.

KIDNEY STORAGE USING HYPOTHERMIA WITH HYPERBARIC OXYGENATION.

There were 10 experiments in this group, kidneys being stored for 24 hours at from 2°C to 5°C under 3 atmospheres absolute of oxygen.

(a) Before transplantation:

As noted in the Group I experiments, after storage, the kidney was soft and white in colour and tensely tough in consistency.

(b) After re-implantation:

The kidney became pink on restoration of the blood supply and was still comparatively normal in appearance after 30 minutes. A moderate size increase occurred but was less pronounced than in other Groups. Urinary output was prompt and the urine was clear.

(c) Contralateral nephrectomy:

Simultaneous left nephrectomy was performed in 4 dogs.

The criteria for contralateral nephrectomy were satisfied within 20 to 28 days after re-implantation in the other 6 dogs.

(d) Course:

All 4 dogs which underwent immediate left nephrectomy died from renal failure within 6 days of this procedure.

In the remaining 6 dogs, renal function was far superior to the others, before left nephrectomy. Two of these 6 animals died within 14 days of late contralateral nephrectomy (i.e. non-survivors), kidney sepsis being uncontrollable in each case. In the other 4 dogs renal function was impaired for a few days following late left nephrectomy, but in 3 of these 4 survivors there was apparent complete return of renal function (Fig. 20). These 3 animals then

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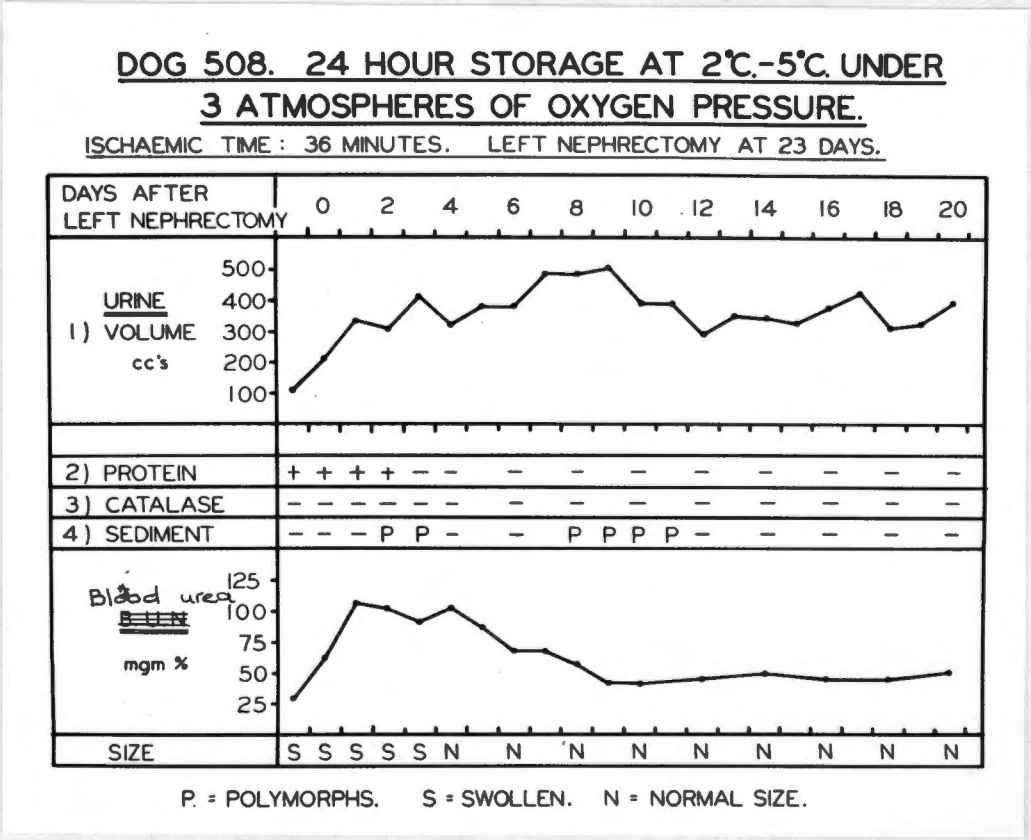
Fig. 20.



Group V - Composite chart of the findings on investigation in a typical survivor after late contralateral nephrectomy. Note the return of renal function towithin normal limits ten to 12 days following left nephrectomy.

continued uneventfully, as though autotransplantation had been carried out without any intervening storage of the kidney. In the fourth survivor, renal function was persistently sub-normal 40 days after contralateral nephrectomy, though the dog was alive and otherwise well, and renal damage must be presumed to have been irreversible in this dog.

(e) Pathology:

To facilitate the description of the pathological findings in Group V, the animals are divided into three categories: (1) those 4 with simultaneous left nephrectomy, (2) the 2 dogs which died within 14 days of late left nephrectomy, and (3) the 4 survivors after late nephrectomy of the opposite kidney.

(1) All 4 dogs undergoing simultaneous left nephrectomy died within 6 days. There was nothing of note on macroscopic examination of the stored kidney (Fig. 21) but microscopy revealed definite evidence of ischaemia. Focal areas of tubular destruction predominantly affected proximal convoluted tubules. There was interstitial oedema and there was evidence of small vessel thrombosis. A suggestion of tubular cell vacuolation was also noted. The glomeruli were normal, as was the general renal architecture.

(2) In the 2 non-survivors which died within 14 days of late left nephrectomy, sepsis precluded conclusive examination of the stored kidney.

(3) One of the 4 survivors after late left nephrectomy was sacrificed 35 days later especially for histology. Renal function as defined in these experiments was within normal limits at this stage and, though there was still evidence of tubular regeneration, the kidney was pathologically normal in appearance (Fig. 22).

(f) Discussion:

The combined use of profound hypothermia and hyperbaric oxygenation for kidney storage resulted in the survival of 4 of the 6 animals in whom contralateral nephrectomy had been deferred for

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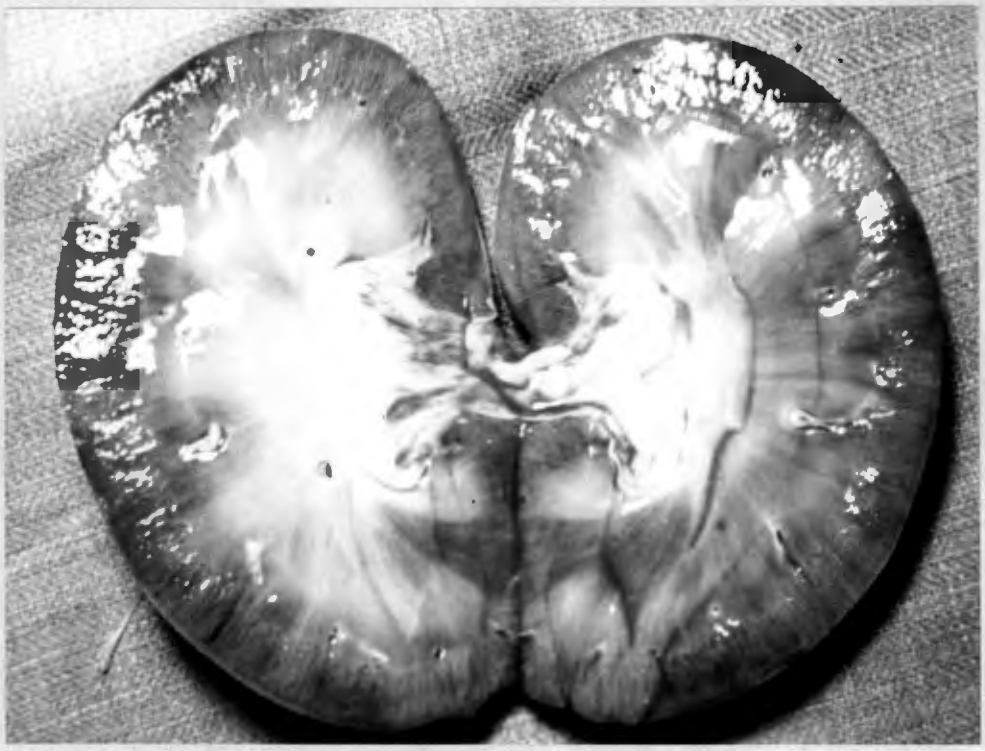
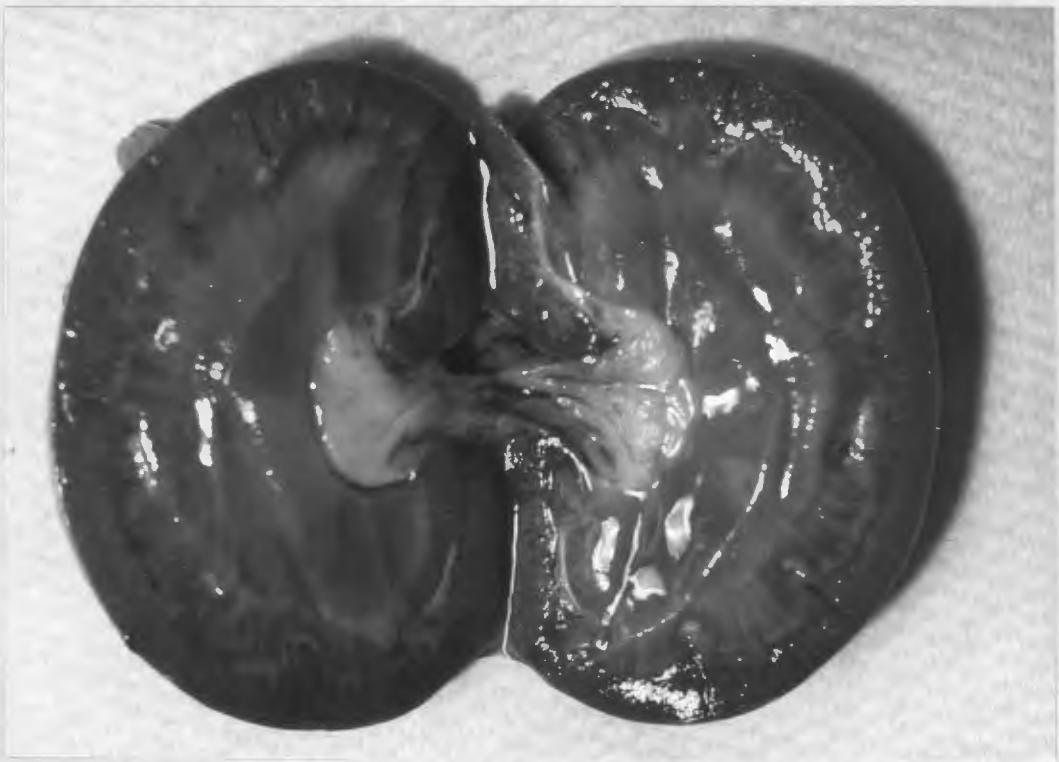


Fig. 21.

Group V - post mortem appearanc of kidney. The dog died 5 days after transplant and contralatera nephrectomy. Medullary suffus ion and slight evidence of cortico-medullar haemorrhage is present.

Post mortem appearance of kidney. The dog was sacrificed on the 29th day after delayed contralateral nephrectomy and the cut surface of the kidney is indistinguishable from normal (Group V).

Fig. 22.



two to three weeks after re-implantation. All 4 dogs in which immediate contralaterial nephrectomy was performed simultaneously with re-implantation died in renal failure within three to six days. Using this storage technique, organ viability was preserved to some extent but was insufficient to withstand the assault of immediate total dependance on the stored kidney.

To gauge the relative value of hypothermia and hyperbaric oxygenation the results of Groups I and IV must be compared and, functionally, there was little to choose between these two methods in the final outcome. Hypothermia alone produced one survivor (an inexplicable survivor) but the development of gross renal dysfunction was more protracted using hypothermia alone than using hyperbaric oxygenation alone. The pathological differences were more pronounced. Tubular and cortical necrosis was far more marked in the kidneys stored at room temperature with hyperbaric oxygenation than in those stored at low temperature. Cortical necrosis was never seen after hypothermic storage and the glomeruli were normal.

CONCLUSIONS:

The combination of hypothermia with hyperbaric oxygenation was only of benefit for 24 hour kidney storage when delayed contralateral nephrectomy was performed. Insufficient renal function was preserved to sustain life immediately after storage and autotransplantation.

KIDNEY STORAGE USING HYPOTHERMIA, FREEZING AND HYPERBARIC OXYGEN: A BRIEF SURVEY OF THE RESULTS ACHIEVED IN THE GROUPS I - V.

As an introduction to the experimental Group VI, it seems fitting at this juncture to discuss briefly the results obtained in the first five experimental groups. (Table I).

Although the animal's survival with a single stored kidney is the outstanding gauge of successful storage, other parameters of renal function were also taken into account in these experiments. The rejection phenomenon was never a complicating factor in these experiments, in assessing function, autotransplantation being featured. However, renal function of the stored organ must be sufficient clinically to make the diagnosis of threatened rejection possible. In other words, in addition to assessing ordinary kidney function in these experiments, the possibility of the exclusion by storage of any of the important criteria of threatened graft rejection (Ackermann and Barnard, 1965, c) also had to be ascertained.

Storage produced no definite signs which could possibly confuse the diagnosis of graft repudiation.

Hypothermia.

The success of hypothermia for certain surgical procedures led to the investigation of its effects upon the entire organism as well as on individual organs (Shields and Lewis, 1959; Sealy et al, 1960; Terblanche et al, 1961). In operations involving prolonged renal ischaemia hypothermia tends to preserve kidney function, by depressing tubular mechanisms (Ariel et al, 1943; Isaacson et al, 1964).

The reduction in oxidative metabolism during hypothermia is a manifestation of the slowing of chemical reactions which occurs with temperature reduction, (Karow and Webb, 1964). Hypothermia depresses oxidative metabolism in the kidney far more than in the body as a whole (Karow and Webb, 1964; Schloerb et al, 1959). The

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TABLE I.

GROUP.	NUMBER OF DOGS	STORAGE TEMPERATURE	OXYGEN AVAILABILITY	STAGING OF CONTRALATERAL NEPHRECTOMY.	SURVIVORS
1	9	2°C. TO 5°C.	—	4 IMMEDIATELY AFTER RE-IMPLANTATION 5 AT 20-28 DAYS	— 1
2	4	-5°C. TO -10°C.	—	THESE KIDNEYS WERE FUNCTION-LESS AND LEFT NEPHRECTOMY WAS NEVER POSSIBLE.	—
3	4	-5°C. TO -10°C.	3 ATA.	AS IN GROUP 2.	—
4	5	±37°C	3 ATA	1 AT 26 DAYS POST-REIMPLANTATION	—
5	10	2°C TO 5°C.	3 ATA.	4 IMMEDIATELY 6 AT 20-28 DAYS.	— 4

Groups I to V: Results.

An over-all picture of the results of in vitro kidney storage using hypothermia, freezing and hyperbaric oxygenation (singly or in various combinations). The only survivors were obtained in Group V (storage with hypothermia and hyperbaric oxygenation) and this only after delayed contralateral nephrectomy.

relative changes in renal oxygen consumption are greater than those in the body as a whole (Kameya et al, 1960; Kerr et al, 1960). The oxygen requirement of the kidney at 5°C has been estimated at 5% of normal, an almost incredible diminution (Levy, 1959).

There are many reports of successful hypothermic kidney storage for short periods, up to about 6 or 8 hours (Bogardus and Schlosser, 1956; Stueber et al, 1958; Kiser et al, 1960). There are isolated reports of hypothermic storage of kidneys for 24 hours (Slapak et al, 1964).

We were unable to achieve successful 24 hour storage using hypothermia alone, except in one dog (a peculiar and inconclusive result) after delayed contralateral nephrectomy and in this single case, though the dog was still alive 60 days later, renal function was markedly impaired.

Especially in combination with other techniques, however, hypothermia is the most promising method for the preservation of viable material (Slapak et al, 1964; Humphries et al, 1964).

Freezing.

Tissues cannot long survive in the ordinary frozen state (Lovelock, 1953; Walton, 1957; Lovelock and Smith, 1959), but methods have been developed to alleviate the traumatic effects of freezing, (Lovelock and Bishop, 1959; Connaughton and Lewis, 1961; Stulberg et al, 1962) and many tissues - including blood and semen in cell suspensions, skin and glands - can be stored in this way currently (Billingham and Medawar, 1952; Brown and Hardin, 1953; Polge, 1957; Sherman and Lin, 1958; O'Brien and Watkins, 1960; OBrien et al, 1961; Playfair and Davies, 1964). However, as yet, a large organ such as the kidney or the heart has not been frozen and successfully resuscitated.

Supposing that hyperbaric oxygenation might protect the organ during thawing (Manax et al, 1964), temperatures of minus 5°C to minus 10°C were used in our experiments. It is not necessary to

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expound on the theory of injury through crystallisation here (Brown and Hardin, 1953).

In both of the experiments using hypothermia at sub-zero levels (Groups II and III), with or without hyperbaric oxygenation, the renal cells were destroyed. These storage methods failed completely in functional and pathological results, but it is not quite accurate to state that hyperbaric oxygenation provides no protection against damage from thawing. The only deduction possible is that, with these methods of freezing and thawing, the use of high pressure oxygen did not appear to ameliorate renal damage.

Hyperbaric Oxygenation.

The use of 3 atmospheres absolute of oxygen in these experiments was empirical. Most experimental and clinical workers have used this level (Humphries et al, 1963; Meijne et al, 1964; Manax et al, 1964; Hyperbaric Oxygenation Symposium, 1965).

Increased tensions of oxygen are supplied to the stored organ to maintain satisfactory oxygenation in the presence of total ischaemia. The actual delivery of oxygen to the kidney depends on gaseous diffusion into the immersate and thus to the organ, but whether the oxygen actually reaches renal cells is unknown. It has been shown that, at 5°C, the kidney only requires 5% of its normal oxygen supply (Levy, 1959) and, although there is no haemoglobin in the immersate, it was felt that 3 ATA pressure of oxygen would supply this. The dissolved fraction of oxygen in the blood is used first (Hyperbaric Oxygenation Symposium, 1965) but this does not imply that it is used in preference to the haemoglobin part. Perhaps only haemoglobinated oxygen can be absorbed rapidly by the tissues and the function of the dissolved fraction is to maintain fully saturated haemoglobin. If this is so, hyperbaric oxygenation cannot benefit the stored organ as used in these experiments.

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The second reason for supplying high pressure oxygen to the kidney during storage was, as mentioned previously, in an attempt to minimise damage resulting from thawing - although this theory has not been substantiated experimentally. No improvement was noted in either the pathological or the functional results.

It seems fair to conclude that, in these conditions, hypothermia was singly of more value than hyperbaric oxygenation, based on the results. However, high pressure oxygen did provide some benefit - apparent when combined with hypothermia. Once the kidney was frozen during storage, however, the injury to the cells proved lethal, whether hyperbaric oxygenation was used or not.

Pathology.

The correlation of pathology with the functional result lies in the histological differences between the kidneys that evinced a transient diuresis and those which were totally anuric. Studies by Oliver (Oliver et al, 1951) indicate that the renal tubules sustain the major damage in the ischaemic kidney. The regenerative process involves the restoration of morphological and functional integrity of the tubules. Transient diuresis is interpreted (and confirmed histologically) as evidence of tubular damage, whereas glomerular damage is also shown in anuric kidneys (Schloerb et al, 1959).

Functional Results.

The importance of immediate renal function post-storage has been stressed already. In clinical practice it is essential not only to diagnose threatened graft rejection with certainty, but that the patient derives maximal benefit immediately from the transplanted organ. In none of these experiments would either of these conditions have been satisfied.

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The most encouraging results were achieved initially by the use of immediate post-nephrectomy perfusion cooling (for uniformly rapid reduction in renal core temperature) followed by 24 hours' storage using simple immersion cooling, at 2°C to 10°C. By combining this technique with high pressure oxygenation of the immersate during storage, a striking improvement in post-storage viability and functional capacity was achieved. Although these kidneys could not sustain life immediately after storage, the anoxic or ischaemic damage present was reversible. Two to three weeks after storage and autotransplantation, contralateral nephrectomy was attended only by slightly disordered function of the previously stored organ, and as a rule this returned to within normal limits eight to ten days later. The damage sustained during storage thus was reversed, and these kidneys could withstand the assault of having to function unaided on removal of the left kidney.

However, the experimental aim was to achieve immediate function of the kidney post-storage sufficient to sustain life unaided by the normally placed, opposite organ. It seemed logical to assume that some additional preservative measure was essential and continuous perfusion of the kidney during storage was the obvious first choice. It was realised that many technical problems would arise from the incorporation of this technique with hypothermia and hyperbaric oxygenation, but these were overcome and the results are discussed in the following pages (Group VI).

RESULTS.

GROUP VI.

KIDNEY STORAGE USING HYPOTHERMIA, HYPERBARIC OXYGENATION AND PERFUSION.

In all, 39 experiments constituted Group VI, the kidneys being stored for 24 hours using hypothermia ($2^{\circ}C$ - $10^{\circ}C$), hyperbaric oxygenation (3 ATA) and continuous perfusion of the organ . Three perfusates were used, at different flow rates, as follows:-

Group I Haemodilution (50%)
- (a) 5 dogs - 1,000 ml./hr. flow rate
- (b) 5 dogs - 400 ml/hr. flow rate
- (c) 7 dogs - 200 ml/hr. flow rate
- (d) 12 dogs - 150 ml/hr. flow rate

Group II Balanced Physiological Solution
- 6 dogs - 150 ml/hr. flow rate.

Group III Whole Blood
- 4 dogs - 150 ml/hr. flow rate.

An overall picture of the results achieved is shown in Table II. There was complete success in Group I (d) only. Some survivors were obtained in Group I (c) as a result of delayed contralateral nephrectomy but there was no immediate success. The results of these experiments are therefore discussed in relation to the wholly successful Group I (d), where the right kidneys from 12 dogs were stored for 24 hours using hypothermia, high pressure oxygen and perfusion with a 50% solution of autologous whole blood and a balanced physiological fluid. Each of these 12 kidneys was re-implanted after storage, with simultaneous left nephrectomy, with immediate satisfactory renal function and one hundred per cent survival.

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TABLE II.

RESULTS OF KIDNEY STORAGE USING HYPOTHERMIA (5°C. - 10°C.), HYPERBARIC OXYGEN (3 A.T.A.) AND PERFUSION WITH IMMEDIATE CONTRALATERAL NEPHRECTOMY.

GROUP	PERFUSATE	FLOW RATE (ml./hr.)	NUMBER OF DOGS	INITIAL PRESSURE - 24 hr. PRESSURE (mm. hg.)	MACROSCOPY	SURVIVORS
1	50% BLOOD 50% PHYSIOL SOLUTION.	a) 1,000	5	110 - 200	RUPTURED.	0
		b) 400	5	60 - 120	OEDEMA +++ MEDULLARY HAEM.	0
		c) 200	7	50 - 80	OEDEMA + MEDULLA SUFFUSED	4
		d) 150	12	40 - 65	NORMAL.	12
2	100% BLOOD	150	4	60 - 150	MEDULLARY HAEM.	0
3	100% PHYSIOL SOLUTION.	150	6	40 - 50	PALE, OTHERWISE NORMAL.	0

GROUP VI: RESULTS.

An over-all picture of the results achieved with in vitro kidney storage using organ perfusion, hypothermia and hyperbaric oxygen. Using 50% haemodilution, low flow and low pressure perfusion, there was 100% survival after 24 hour storage, and re-implantation with immediate left nephrectomy.

Using a similar storage technique, but at a higher flow rate and perfusion pressure, there was a certain amount of success, but only after re-implantation with delayed left nephrectomy.

The variations from the findings in the successful group noted in the other groups of Group VI are considered as possible reasons for the failure of the other experiments, and are compared with those observed in group I (d). These differences occurred either during the storage period and/or after storage and re-implantation of the kidney. The findings during storage will be discussed first, then the results of pathological examination after the storage period, and finally the differences noted after storage and re-implantation will be detailed.

A. DURING THE STORAGE PERIOD.

1. URINE.

(a) Volume:

Because of the conditions of the Group VI experiments, it was not possible to measure the total urinary output. Four-hourly specimens revealed no variation associated with storage time.

Although not measured, appreciably less urine was passed by the kidneys in groups II and III and in the faster flow perfusion experiments of group I, than in the successful experiment group I (d).

(b) Protein:

There was never a proteinuria of more than + in the successful group during storage. In the others there was invariably a proteinuria of 3+ to 4+.

(c) Electrolytes and Urinary Urea:

In all groups, after four hours' storage, urinary sodium, potassium, chloride and urea values approximated those of the perfusate.

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(d) Catalase:

Never positive in the successful experiments or in those where haemodilution or bloodless perfusion had been used, the findings were positive in group II kidneys, where whole blood had been perfused, the urine being heavily blood-stained.

(e) Stained Urinary Sediment:

There was no abnormality detected in the successfully stored organs. Red cell and tubular casts were evident where whole blood perfusion was used (group II), and in the other groups hyaline, granular and tubular casts were noted. In the high flow rate perfusion experiments in group I, greater abnormality was associated with the higher flow rates.

2. PERFUSATE ACID-BASE AND ELECTROLYTE BALANCE.

Successfully stored kidneys:

Details of the results of these investigations in 5 typical representatives of the 12 successful experiments are set out in Table III.

There was a marked increase of pO_2 during the first 8 hours of storage, with a constant gradual further rise. There was 100% oxygen saturation within 30 minutes of commencing storage. A gradual but significant drop in pH occurred over the 24 hour period but pH levels were never far from normal limits. Very low pCO_2 values were noted, with a definite increase proportional to the length of storage. A marked base deficit and very low standard bicarbonate value remained constant during storage. Serum sodium and chloride estimates were high, whereas serum potassium was normal, any signaificant variation being explicable by laboratory error. BUN remained low but there was a constant tendency to rise. Plasma haemoglobin levels were never remarkably raised.

Unsuccessfully stored kidneys:

There were no survivors in group I (a). The findings in a typical representative are detailed in Table IV. The pO_2 values

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TABLE III.

ACID-BASE AND ELECTROLYTE BALANCE WITH 50% HAEMODILUTION AND PERFUSION FLOW OF 150 ml / hour

Hours	Temp °C	pO2 mm.Hg.	pCO2 mm.Hg.	pH	Std.B m.eq./l	B.E. m.eq./l	Na m.eq./l	K m.eq./l.	Cl m.eq./l	Urea mgm %	Plasma Hb. mgm/100 ml.
½	15	185	8.0	7.46	11.4	-11.6	146	4.2	118	21	6.1
8	10	796	9.5	7.42	10.4	-12.2	144	4.0	118	22	22.0
24	8	1040	13.2	7.34	10.4	-12.2	148	3.9	117	30	37.2
½	12	206	7.6	7.42	10.6	-16.2	144	4.2	121	12	5.7
8	10	680	9.2	7.40	11.0	-15.4	146	4.3	123	13	26.2
24	5	1170	14.6	7.38	10.7	-15.6	146	4.8	121	16	34.5
½	10	168	6.4	7.42	10.8	- 9.8	148	4.0	122	14	4.2
8	5	840	7.0	7.41	11.1	-10.8	148	4.4	122	13	24.6
24	7	+1200	14.6	7.31	10.9	- 9.4	146	4.2	120	18	34.6
½	12	179	10.2	7.44	9.9	-15.6	147	4.1	119	19	8.1
8	8	724	11.4	7.39	10.4	-18.4	149	4.4	121	22	19.9
24	5	1180	11.8	7.36	10.3	-15.8	146	4.4	121	32	28.9
½	14	211	8.6	7.41	10.7	-12.6	144	3.9	118	14	5.5
8	5	826	8.8	7.40	10.8	-11.8	144	4.4	119	18	22.8
24	5	+1200	10.6	7.31	10.8	-12.0	146	4.1	122	18	36.4

Group VI - group I(d): 24 hour storage with hypothermia, hyperbaric oxygenation and organ perfusion with 50% haemodilution at 150 ml./hr. flow rate and low pressure. There is a small increase in pCO_2 and BUN with constant serum potassium and pH.

TABLE IV.

ACID-BASE AND ELECTROLYTE BALANCE

GROUP 1 (a) 50 % Haemodilution and Perfusion Flow of 1000 ml / hour

HOURS	Temp. °C.	pO2 mm.Hg.	pCO2 mm.Hg.	pH	Std.B. m.eq./l	B.E. m.eq./l	Na. m.eq./l	K. m.eq./l	Cl. m.eq./l	Urea mgm %	Plasma Hb mgm/100 ml
½	18	360	6.4	7.41	6.4	-14.4	150	4.1	122	12	7.4
8	8	1080	10.2	7.38	7.2	-17.6	152	5.8	120	14	50.2
24	6	+1200	10.6	7.24	7.0	-24.0	151	8.2	122	14	98.6

GROUP II Whole Blood and Perfusion Flow of 150 ml / hour

½	20	140	27.1	7.49	22.0	- 2.3	138	3.8	101	32	18.6
8	6	425	22.0	7.46	20.1	- 8.6	142	5.2	94	32	68.5
24	5	660	14.1	7.40	14.9	-14.9	139	7.9	99	34	138.4

GROUP III No Blood and Perfusion Flow of 150 ml / hour

½	16	550	0	7.35	0	-30	149	4.9	118	0	0
8	5	+1200	0	7.32	0	-34	146	5.4	116	0	0
24	6	+2500	0	7.24	0	-38	142	8.6	115	0	0

Unsuccessfully stored kidneys - acid-base and electrolyte determinations. There is invariably an increase in serum potassium without a rise in either pCO_2 or in BUN levels.

were higher than in the successful group. The pCO_2 rose during the first 8 hours, subsequently remaining constant or actually falling. A significant fall in pH occurred after 8 hours, reflected in a more marked base deficit. Increased serum potassium was noted, mainly after 8 hours of storage. There was no significant rise in BUN, but a relatively noteworthy rise occurred in the plasma haemoglobin level.

In the other two sub-groups of Group I, using haemodilution perfusion and faster flow rates, the results lay between those noted in group I (a) and the successful group of experiments. However, it should be mentioned that the metabolic and electrolytic values observed in I (c) - using 200 ml/hr. perfusion - were barely distinguishable from those found in the successful group; it was in I (c) of course that the only other survivors were obtained, after delayed contralateral nephrectomy.

In group II, where whole blood was perfused at 150 ml/hr., there were no survivors (Table IV). Lower pO_2 values than in the successful experiments were found. Initial pCO_2 was high and fell over the 24 hour storage period. There was a slight decrease in pH and an increase in base deficit, with a fall in standard bicarbonate. A marked hyperkalaemia became more pronounced towards the end of storage. There was no significant variation in BUN. Gross haemolysis was reflected in high plasma haemoglobin levels.

In group III, using physiological fluid perfused at 150 ml./hr., there were no survivors either (Table IV). The pO_2 levels were very high. A significant drop of pH and an increase in base deficit were noted. At the end of storage, the serum potassium was high. There was no recordable production either of CO_2 or of urea during storage.

3. PERFUSION PRESSURE. (Table II)

A distinct relationship was noted in group I between pressure and flow rate: the faster the flow, the greater was perfusion pressure. In addition, perfusion pressure increased with a constant flow rate over the storage period.

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(a) Successfully Stored Organs:

Using 150 ml./hr. organ perfusion during storage, the pressure was low initially and there was no increase in the first 4 hours. Subsequently the pressure rose steadily but never exceeded 60 mm. mercury in this group (I (d)).

(b) Unsuccessfully Stored Organs:

At faster perfusion rates with haemodilution, the initial pressure was considerably higher than in the successful group and the relative increase during storage was far greater. In lower flow unsuccessful experiments, there was no increase in perfusion pressure during the first 4 hours but the rise which ensued was rapid and continued steadily throughout the storage period.

In group II, using physiological fluid perfused at 150 ml./hr., the perfusion pressures closely followed the pattern observed in the successful experimental group.

In group III, where whole blood was perfused at low flow rate, the initial perfusion pressure was high compared with the successful group, and was also higher than that noted in group II. The rise in pressure occurred early in storage and progressed rapidly.

B. PATHOLOGY AFTER ORGAN STORAGE.

After 24 hours of storage using hypothermia, hyperbaric oxygen and organ perfusion, kidneys were removed for histology.

(a) Successfully Stored Organs:

Immediately after storage the kidneys which functioned well subsequently were indistinguishable from normal on macroscopic examination (Fig. 23). No note having been made of weights pre- and post-storage, it was not possible to detect minimal weight gain (due to fluid accumulation). The cut surface appearance was completely normal (Fig. 23).

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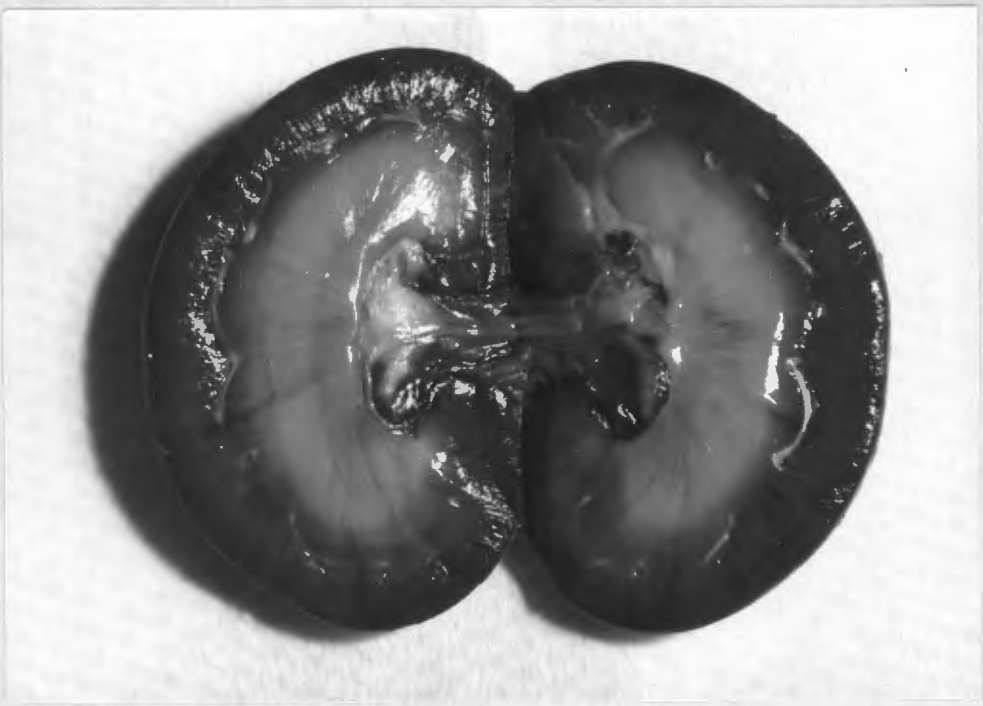

Fig. 23. Group VI - group I(d): cut surface appearance of kidney immediately after storage Normal except for slight oedema.

Fig. 24. Group VI - group I(e): cut surface appearance of kidney immediately following the storage period. There is slight cortico-medullary haemorrhage and oedema, but the kidney appears normal otherwise.

On microscopy, the following deviations from normal were observed in the successful group:-

(i) mild interstitial oedema,
(ii) capsular dilatation, and
(iii) limited evidence of small vessel thrombosis.

(b) Unsuccessfully Stored Organs:

In group I (c), where there was incomplete success, the kidneys were similar macroscopically to the successfully stored organs post-storage (Fig. 24). In the higher flow experiments using haemodilution, oedema was reflected by an increase in kidney size, especially in the area denuded by capsulotomy, and was most marked where the fastest flow rates had been used for perfusion during storage. In fact, in group I (a) gross oedema actually resulted in rupture of the renal parenchyma at the site of capsulotomy. On incising the kidney in this group the parenchyma bulged out glistening, the cortex was noticeably broad and the medulla deeply suffused with obvious haemorrhage at the cortico-medullary junction (Fig. 25). After slower rate perfusion these changes were less marked (Fig. 26).

In group II (blood-free perfusate) there was no evidence of any size increase, nor of the tense and rubbery consistency usually associated with oedema. The cut surface appearance manifested mild oedema in a pale organ which nonetheless showed evidence of medullary suffusion (Fig. 27).

In group III (whole blood perfusate) oedema caused considerable swelling of the organ, but not to the extent of parenchymatous rupture. It was interesting that the changes noted in this group were almost identical to those seen in I (a). Although oedema was not quite so obvious on examination of the cut surface, medullary necrosis was severe (Fig. 28). The cortex was broad but, compared with the medulla, comparatively normal.

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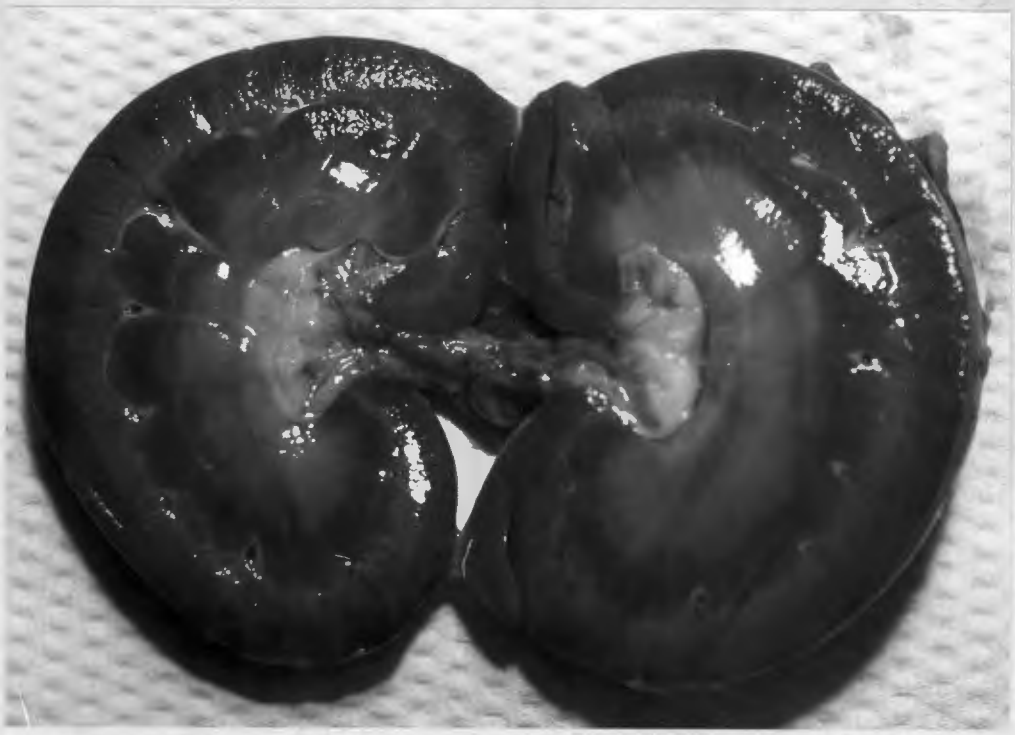
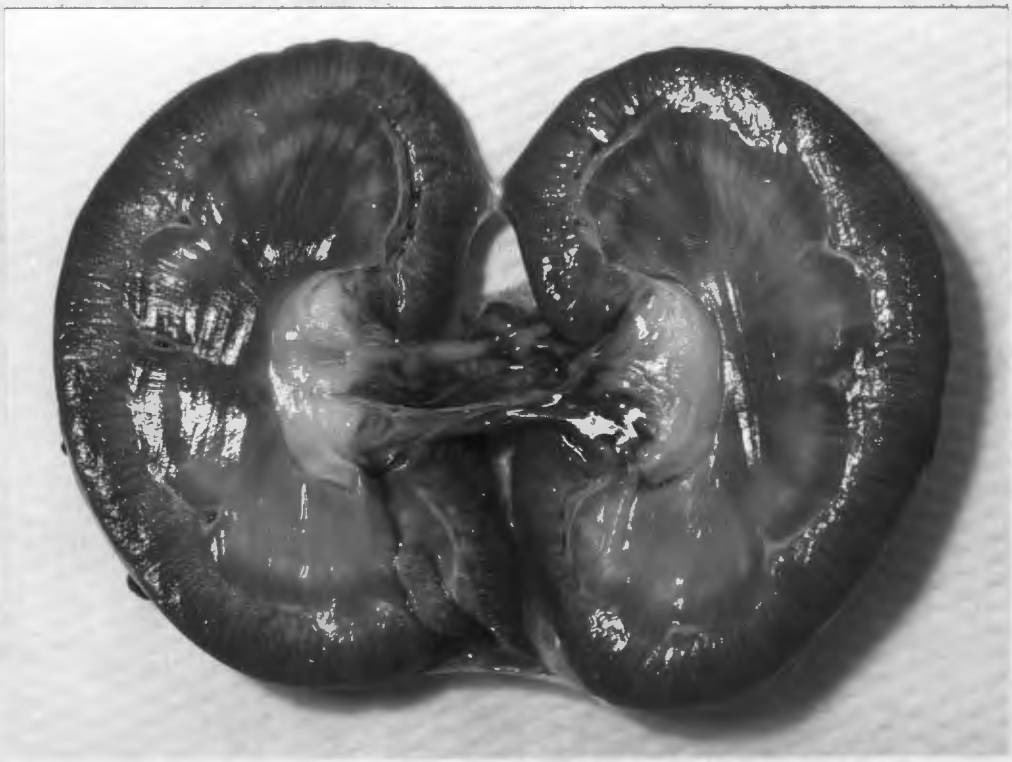


Fig. 25.

Group VI group I (a): Cut surface appearance of kidney immediately following storage period. There is gross oedema, broadening of cortex and medullary necrosis especially at the cortico-medullary junction.

Group VI - group I(b): Cut surface appearance of kidney immediately following the storage period. There is medullary necrosis but oedema is not as marked as in Fig. 25.

Fig. 26.



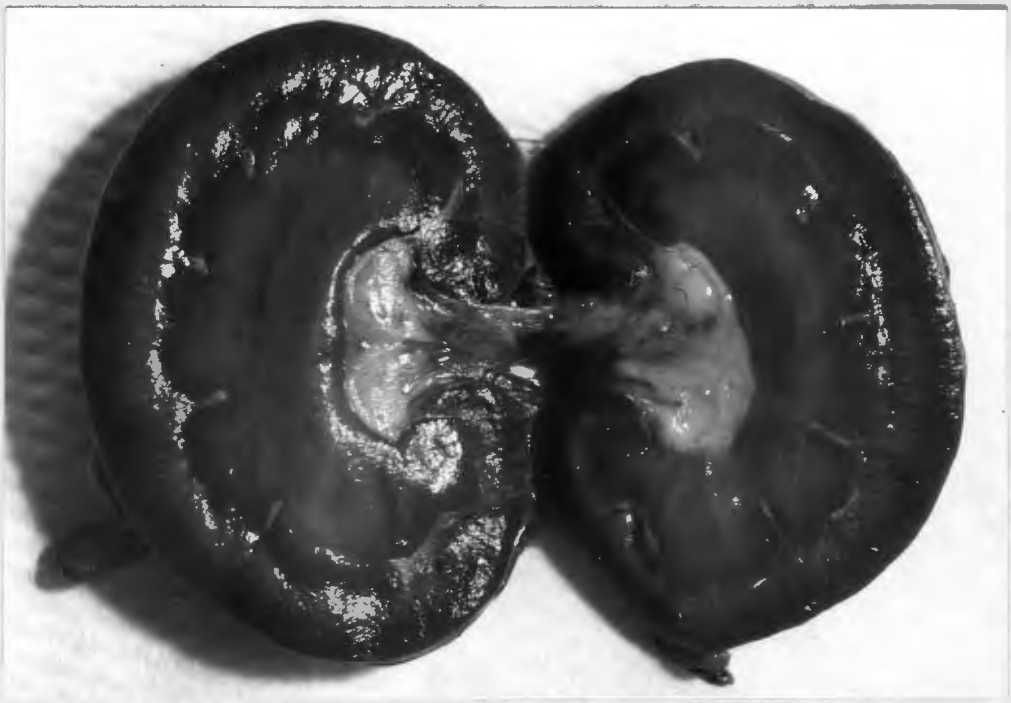
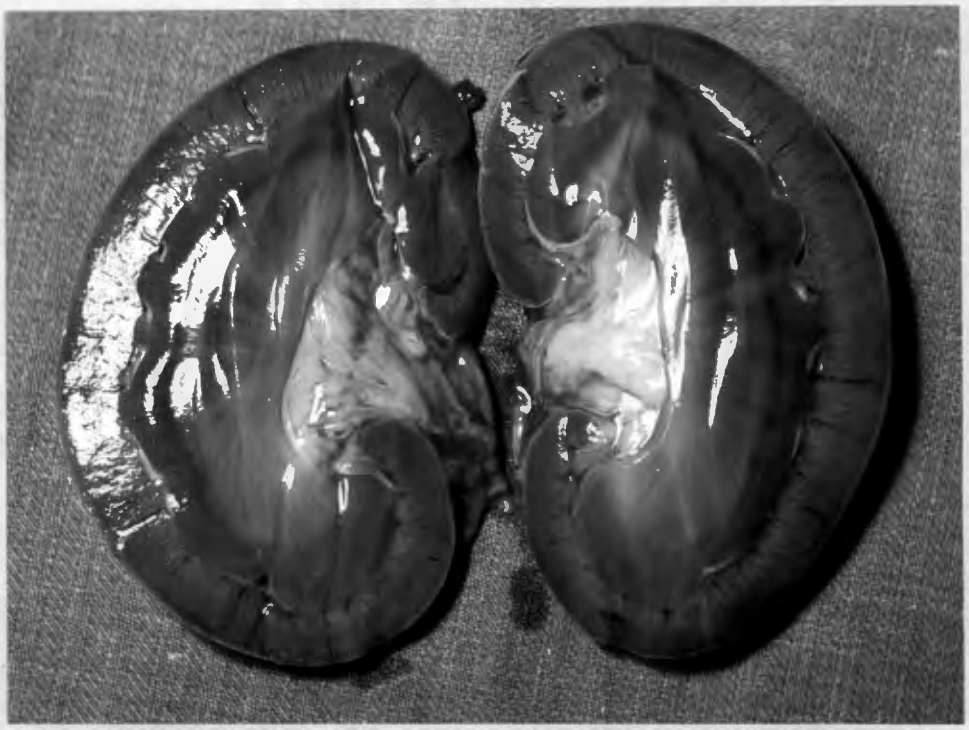


Fig. 27.

Group VI - group II: cut surface appearance of a kidney immediately following storage. There is medullary necrosis and oedema.

Group VI - group III: cut surface appearance of a kidney immediately following the storage period. The organ is pale and slightly oedematous, and there is medullary suffusion.

Fig. 28.



Microscopic examination in the faster flow experiments of group I revealed the damage to be more pronounced, the higher the perfusion flow rate during storage. Oedema was the most striking feature, involving interstitia. In some areas tubular epithelial cells showed signs of hydropic degeneration. Capsular dilatation was present and there were intracapillary thrombi - especially within the glomeruli. In group I (a) there was frank cellular necrosis, particularly in the area of the cortico-medullary junction.

In group II oedema was evident. There was dilatation of small capillaries and lymphatics, interstitial oedema, capsular dilatation and interesting focal areas of interstitial plasma cell collections.

In group III microscopy revealed extensive intra-capillary thrombosis, tubular cell necrosis - predominantly of the proximal tubular epithelium - and more generalised vacuolation of tubular cells. There was capsular dilatation and interstitial oedema. Occasional glomeruli were engorged with blood, with basement membrane thickening.

C. AFTER STORAGE AND AUTOTRANSPLANTATION.

1. Contralateral Nephrectomy:

Except in the successful experiment (group I (d)), whenever contralateral nephrectomy was performed simultaneously with re-implantation of the stored organ, the animal died within 6 days from renal failure.

Contralateral nephrectomy was deferred for from 2 to 3 weeks in some cases in group I (c), which accounted for the survival of 4 of the 7 animals in this group.

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2. Investigations.

All the investigations were directed at (a) the exclusion of any diagnostic criteria of graft rejection which might have resulted from the storage procedure per se, and (b) assessment of renal function in the stored organ.

(a) All the non-survivors succumbed within 7 days of post-storage autotransplantation, from renal failure. At no stage was there any diagnostic evidence suggestive of rejection in the survivors.

(b) Post-storage renal function:

Immediately following transplantation, almost all the investigated parameters of renal function in group I (d) were disordered (Fig. 29). The exception was urinary output, which was prompt, commencing as soon as the vascular anastomoses had been completed. The urine was clear and the volume excreted was within the limits of normal (as previously noted) from the onset.

It is perhaps of interest to describe at this juncture a phenomenon which invariably occurred in other experiments if contralateral nephrectomy was delayed for 24 to 48 hours. In such animals very little (20 to 100 ml.), or no urine, was passed until the remaining kidney had been removed. Within a few minutes of this procedure, copious amounts of urine were excreted and the urinary output was maintained thereafter. In all other respects, however, these kidneys behaved identically to those transplanted with simultaneous contralateral nephrectomy.

Disturbance of renal function was never particularly marked in the successfully stored organs, and returned to normal within 14 days. This is evident by the 100% survival achieved in this group.

(i) Urinary protein:-

A proteinuria of + to ++ (and very rarely +++) was noted. This was maximal during the first few days but was one of the earliest disorders to disappear.

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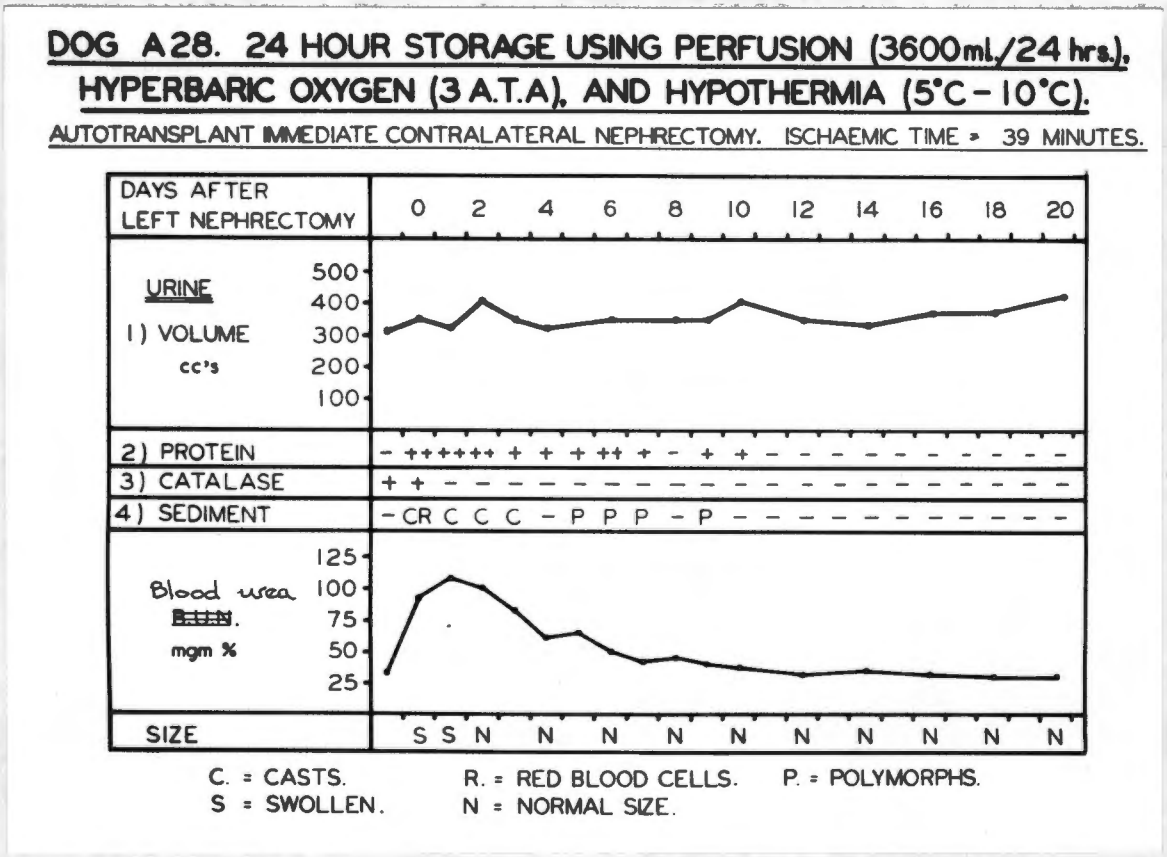
Fig. 29.



Group VI - group I(d): Composite chart showing renal function in a typical survivor in the only completely successful experimental group. Renal function is within normal limits 8 - 10 days following storage, re-implantation and immediate left nephrectomy.

(ii) Urinary sediment:-

Occasional hyaline casts were seen and, for the first six hours following transplantation, red blood cells from the unligated ureteric artery. In the absence of infection there were no other abnormalities.

(iii) Serum and urinary electrolytes:-

No significant variations from normal were ever apparent.

(iv) BUN and urinary urea:-

A raised BUN was always reflected by diminished urinary urea excretion. Neither alteration was ever gross and in no experiment was the BUN in excess of 150 mgm.%. Normal values had returned within 14 days.

(v) Creatinine clearance:-

Although the degree of the disorder was never severe, it was persistently abnormal for much longer than any other parameter of renal function studied. Normal clearance values were seldom obtained in less than 18 days. (It should be noted that this was not a routine investigation in all cases).

(vi) Urinary catalase:-

Positive results to the qualitative test were found routinely for one to three days after transplantation. False positives also manifested, with relatively mild urinary tract infection.

The above findings all relate to the successful group I (d) experiments. The only other group where there was any success was group I (c).

In the animals which survived after deferred contralateral nephrectomy in group I (c), there was similar renal dysfunction to that seen in the successfully stored organs, but of a more severe and protracted nature. In some the deficit was incompatible with life and in others the residual functional abnormality was persistent, even after four weeks; this was especially notable in BUN and creatinine clearance estimates.

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During the two to three week period when one normally situated kidney was left unmolested, urinary output from the stored and re-implanted kidney in group I (c) was minimal. As in the successful group, increased urinary output occurred after contralateral nephrectomy but was not as dramatic in that the volume excreted was lower than normal for at least 24 to 48 hours.

3. Pathology.

Macroscopy:

Some animals were sacrificed expressly for histology in the successful experimental group I (d), after the 14 day survival period during which the investigations were carried out. Both on external examination and on incision these kidneys were normal (Fig. 30).

In the partly successful group I (c), where slightly higher perfusion rates had been used during storage, the kidneys were also ostensibly normal on examination (Fig. 31). In the non-survivors in this group, however, the kidneys were moderately swollen with suffusion of the medulla, most marked at the cortico-medullary junction (Fig. 32).

In all the other groups, where no success whatsoever was achieved, the post mortem changes were gross (Figs. 33, 34, 35 and 36). The kidneys were swollen, engorged, and there were obvious subcapsular areas of discoloration. There was frank necrosis of the medulla and, in some cases, associated cortical necrosis was also present.

Microscopy:

In the successfully stored kidneys, apart from a limited number of tubular casts, the tubules and glomeruli were normal. There was no suggestion of necrosis and only limited evidence of focal areas of tubular regeneration were detectable.

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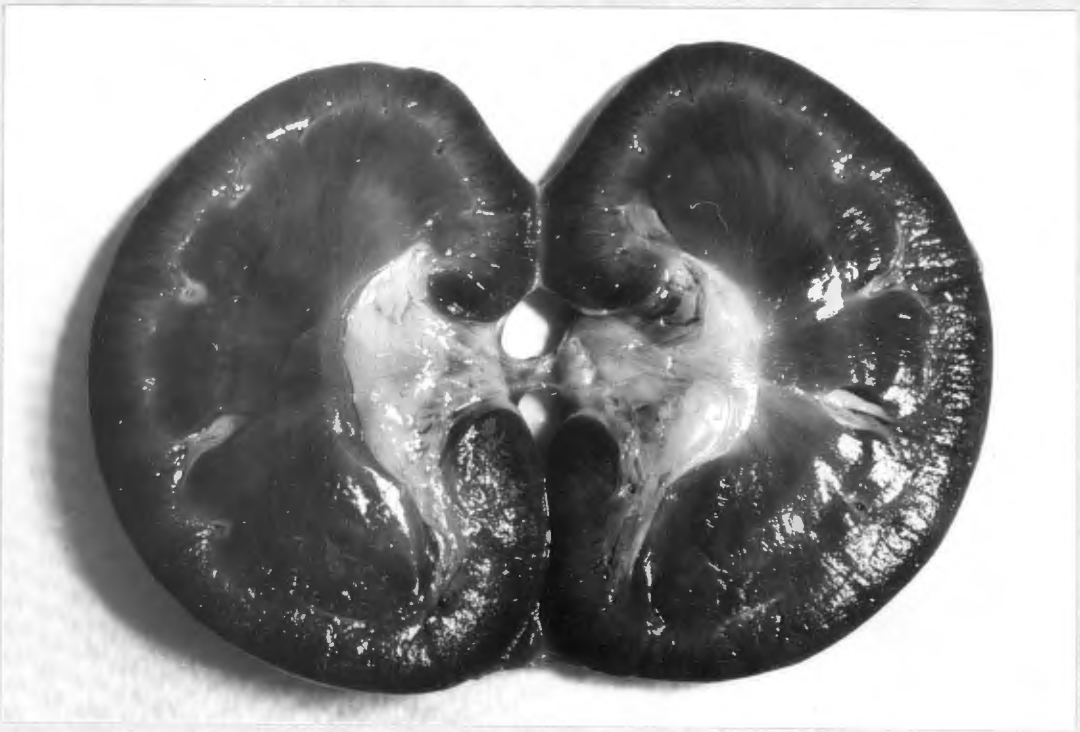
In the survivors in group I (c) there were similar findings on microscopic examination. The only difference was that more extensive areas of tubular regeneration and occasional small infarcts were present.

In all the other groups, histology of the stored organ confirmed the extensive (and in many cases total) necrosis of both cortex and medulla. In most, glomeruli were apparently normal and the cortico-medullary area was maximally necrotic and congested. It is interesting that this "intermediate zone" was uniformly the predominant one destroyed.

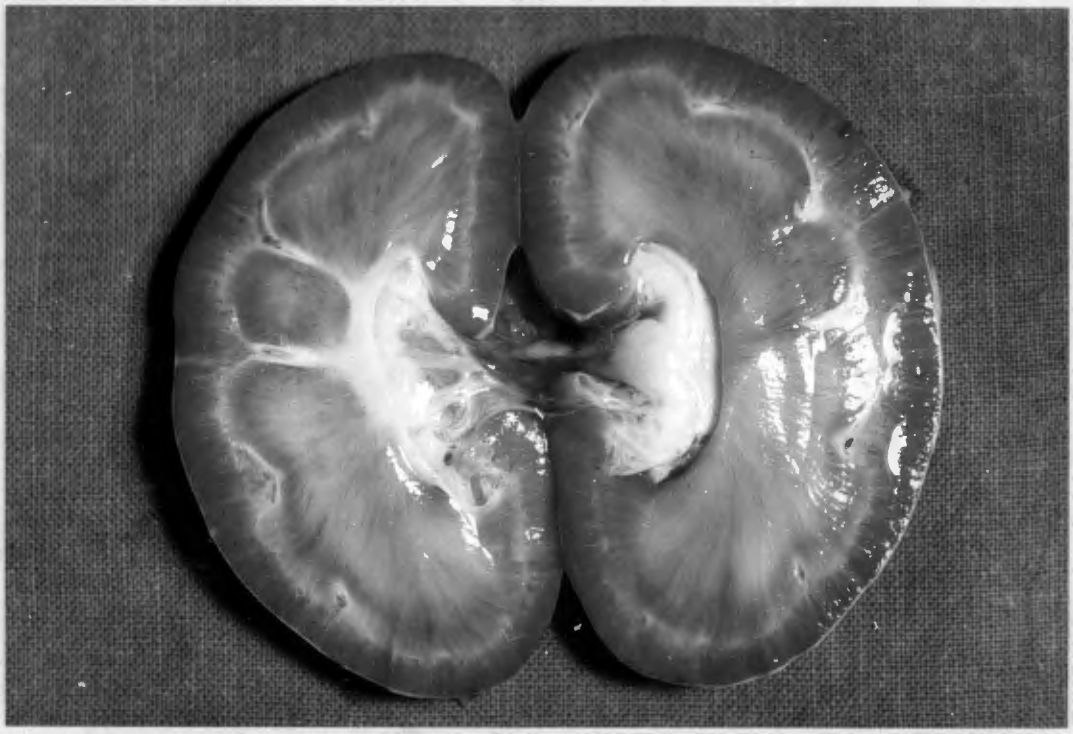
There was no obvious disparity in the findings which could be attributed to the use of whole blood, blood-free or maximal flow rate haemodiluted perfusates.

Fig. 30.



Group VI - group I(d): post mortem cut surface appearance of a kidney in the only completely successful experimental group. The dog was sacrificed 24 days after storage, re-implantation and immediate contralateral nephrectomy. The appearance of the kidney is quite normal.

Fig. 31.



Group VI - group L(c): post mortem specimen. This kidney was removed from a survivor in this group 19 days after contralateral nephrectomy. The appearance of the cut surface is within normal limits.

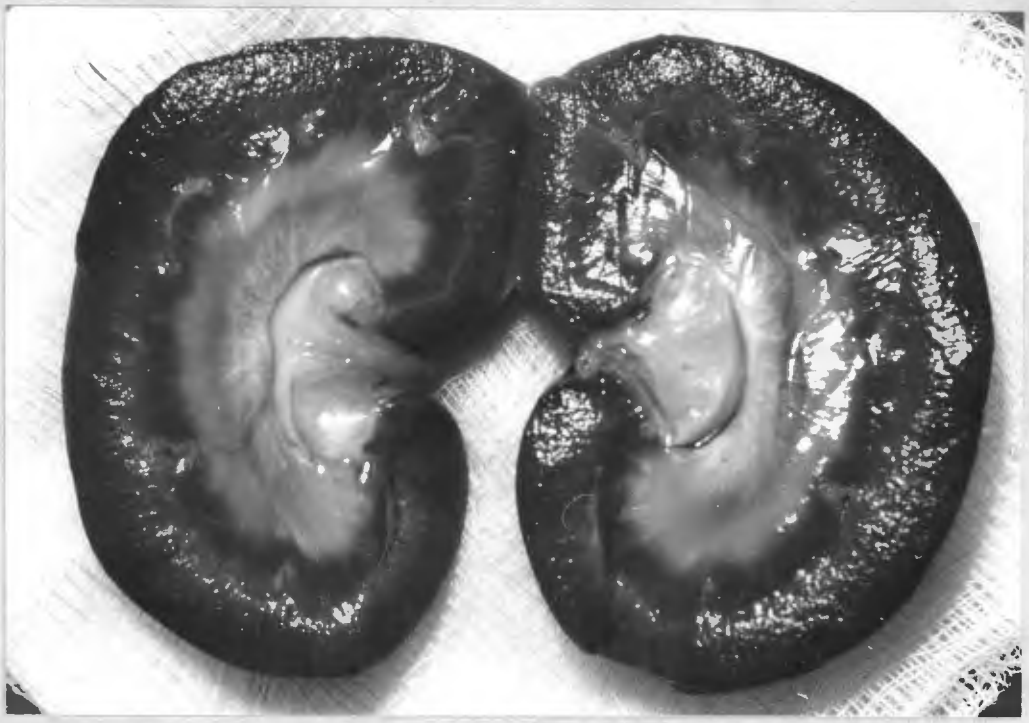


Fig. 32.

Group VI - group I(c): post mortem cut surface appearance of kidney. The dog died in renal failure 4 days after storage, re-implantation and immediate left nephrectomy. There is mild oedema with evidence of medullary necrosis, though the cortex appears normal.

Fig. 33. Group VI - group I(a): post mortem cut surface appearance of kidney, 3 days after storage, re-implantation and immediate left nephrectomy. Medullary and cortical necrosis is present.

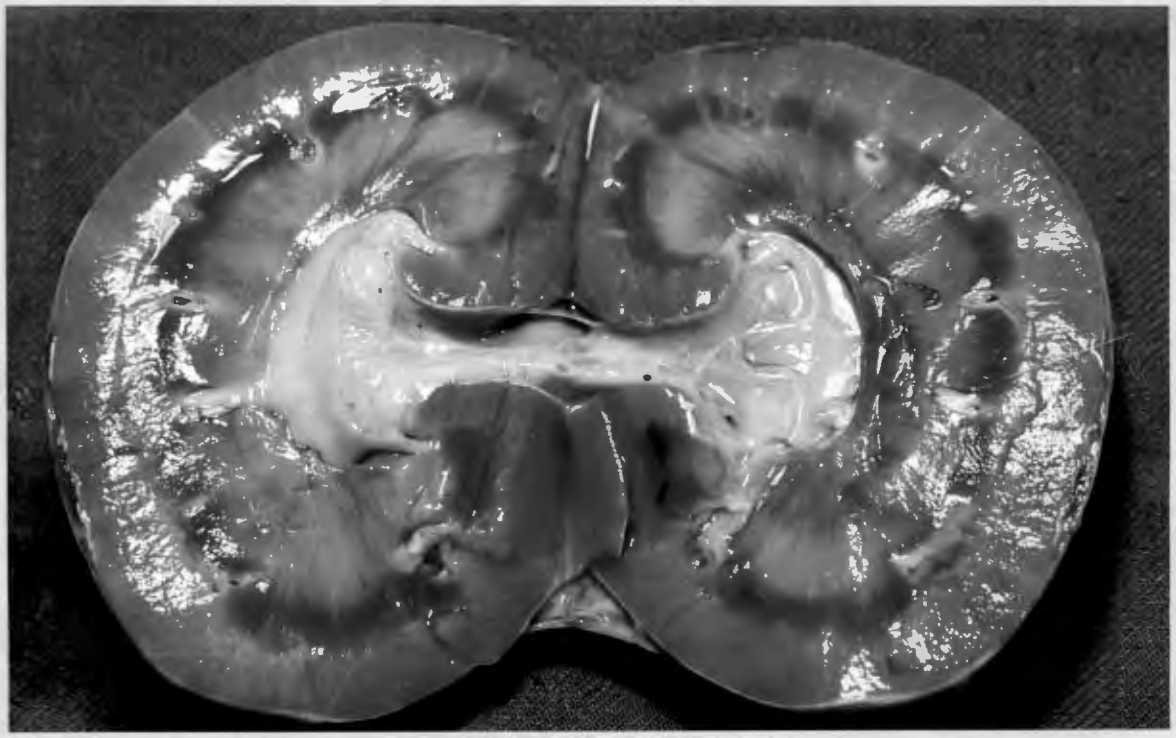


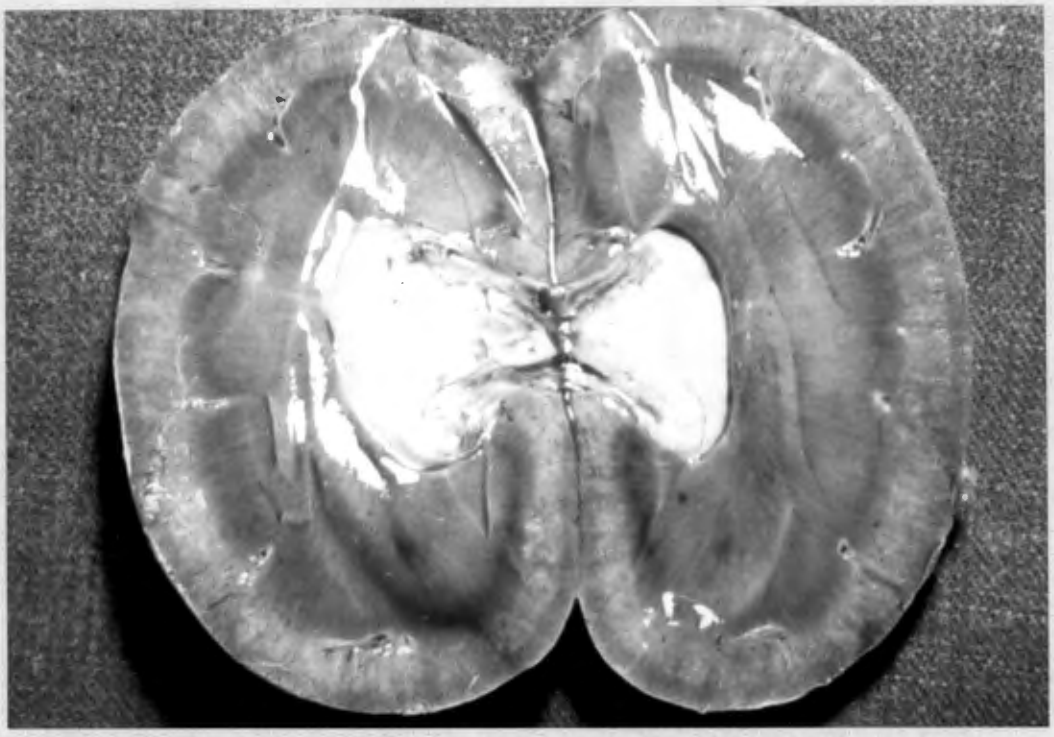
Fig. 34.

Group VI - group I(b): post mortem cut surface appearance of kidney. The dog died 4 days after storage, re-implantation and immediate left nephrectomy. There is evidence of medullary necrosis at the cortico-medullary junction. The cortex is broadened but otherwise appears normal.

Group VI - group II: post mortem cut surface appearance of the stored kidney. The dog died 4 days after re-implantation with immediate left nephrectomy, in renal failure. There is complete necrosis of cortex and medulla.

Fig. 35.

Fig. 36.



Group VI - group III: post mortem cut surface appearance of kidney removed from a dog which died in renal failure 3 days after re-implantation and immediate left nephrectomy. Note the cortical and medullary necrosis which is present.

DISCUSSION.

D I S C U S S I O N.

It is obvious that any successful method of organ preservation (irrespective of the period of storage involved) must preserve sufficient function for the organ to sustain life alone after storage. In experimental work, where normal kidneys are invariably used, this consideration assumes paramount importance: if parenchymatous damage caused from the storage procedure results in dysfunction incompatible with life, the clinical use of the technique cannot even be considered.

Human cadaver kidney grafts are all subjected to some period of relative ischaemia (sometimes total) and thus to anoxia, both before and after the death of the donor. Further anoxic damage to the organ during storage is clearly proscribed.

In this series of experiments (Group VI) combining organ perfusion, hypothermia and hyperbaric oxygenation, canine kidneys were successfully stored for 24 hours and there was 100% survival after re-implantation with immediate contralateral nephrectomy. Although disordered renal function always followed transplantation, this returned to normal within the following 14 days.

In all cases the stored kidney was returned to its original donor. Thus post-storage function was not complicated by rejection phenomena, and any functional derangement was directly attributable to the storage procedure. Although this is not a true reflection of clinical conditions, for purposes of assaying the relative value of various storage procedures it is certainly the most accurate.

This discussion concerns the factors felt to be of greatest significance in the success of the experiment. Though pathology is described, the animal's survival and the adequate post-storage function of the organ are the main criteria for gauging the success of the technique.

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1. TECHNIQUE.

In discussing the surgical technique, two points bear emphasis. Firstly, the initial nephrectomy must be technically impeccable to ensure minimal parenchymatous trauma to the organ which is to be stored and transplanted. In this way, assessment both during and post-storage will reflect uncompromised renal function, and deviations from normal may be attributed to the ischaemic or anoxic damage caused by storage, without doubt.

The second major point is the length of renal artery which is preserved. The perfusion cannula is tied into the artery during storage, and this portion of the vessel is resected subsequently to permit vascular suture of the normal arterial wall. The greater the length of artery preserved, the easier is initial cannulation and arterial resection post-storage is far safer. Furthermore, the cannula tip must lie proximal to the bifurcation of the renal artery (or only part of the organ will be perfused) which is another reason to preserve a good length of the renal artery.

The technique used for prolonged organ perfusion with hypothermia and hyperbaric oxygenation is simple. The most obvious problem is the incorporation of the pump system into hyperbaric oxygen conditions, with the very real associated dangers of explosion and fire, where ANY type of motor or electricity driven pumping device may not be used. However, using suitable connections and high-pressure tubing, perfusion from outside the oxygen chamber proved convenient and feasible.

Sterilisation of the components used presented no undue difficulties. Detergents were not used for fear of haemolysis.

Specimen collection from the perfusate was facilitated by the 3-way tap in the perfusion circuit; samples for acid-base study and for pCO_2 and pO_2 determination could be withdrawn without risk of air contamination and without decompressing the entire circuit.

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Because of the necessity for decompression in order to collect urine specimens, this was carried out only in representatives in each group and not in every case.

Hyperoxygenation of the perfusate was uniformly effective, irrespective of the perfusate or the flow rate used, although the degree of perfusate oxygenation varied. High perfusate pO_2 levels were required and the hyperoxygenation system proved itself eminently suitable. To ensure an adequate surface for the dissolution of oxygen in the perfusion fluid, a special stand with a corrugated run-off was employed. This stand was also useful in facilitating visualisation of the kidney on decompression. This simple oxygenator system was efficient and effective.

Compressed ivalon was used to cushion the kidney during storage, preventing compression trauma due to contact with the metal stand.

The small recirculating perfusion pump used was ideal in the circumstances of these experiments. Except where whole blood was perfused, the plasma haemoglobin levels hardly rose during storage; this is impressive in view of the very low total volume of perfusate used.

Perfusion flow rate was accurately checked, both at atmospheric pressure and with the whole circuit pressurised to 3 ATA oxygen, at various speeds. No significant deviation from the dial reading could be detected.

The absence of tubing connections within the pump head, coupled with the situation of the reservoir inside the chamber, ensured sterility.

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Rapid compression and decompression of the hyperbaric chamber constituted no danger. In terms of purity, the compression medium was virtually devoid of gases other than oxygen. The hazards of rapid or "emergency" decompression in high pressure medicine stem from the accumulation of nitrogen bubbles in the blood-stream; other gases (particularly oxygen and carbon dioxide) are not responsible for these complications. Thus any possible disadvantage from rapid decompression was insignificant in these experiments.

2. PERFUSATE.

The major difference between the three experimental groups lay in the perfusate. A bloodless perfusate would be ideal for perfusion storage of human cadaver grafts. Homologous blood perfusion, though compatible by transfusion standards, nonetheless carries some risk of immunological and sensitisation problems. However, all the survivors were in the group where 50% haemo-dilution was used. The use of this perfusate was prompted by the success with which a similar dilution was introduced for extracorporeal cardiopulmonary bypass surgery in this centre. U.C.T.

The constitution of the physiological fluid (used in groups I and II) was modified Ringer's lactate solution made up with low molecular weight dextran in saline. The buffering capacity was dependent upon 4% sodium bicarbonate and the electrolyte concentration was standardised with that of the plasma. To prevent vessel spasm, 1% procaine ("Leostesin") was added. Both the blood and the physiological fluid were heparinised to offset thrombosis.

Low molecular weight dextran has been extensively studied. Most of its properties have considerable importance in their potential application to organ storage. No attempt was made to compare other perfusates and no valid conclusions may be drawn or inferred, but low molecular weight dextran combined with blood certainly fulfilled all the requirements of these experiments.

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All three perfusates used were evolved to provide as nearly normal an environment as possible. Nothing need be said concerning whole blood perfusion.

Both with 50% haemodilution and, to a greater extent, using a bloodless perfusate the pCO_2 levels and the base buffering ability of the perfusate were abnormally low. This produced a noticeable base deficit but the pH was maintained within the narrow limits of normal. From the results achieved, the idea is provoked that one of the most important factors of successful organ perfusion storage is that the cells be bathed in and nourished by a fluid of normal pH.

The diluted perfusate and the blood-free fluid were deficient both in calories and in essential proteins. However, over a short period such as 24 hours and at such profound levels of hypothermia, it was considered improbable that the metabolism would require more.

No antibiotics were ever necessary during storage as the sterility of the entire circuit was beyond doubt.

3. ACID-BASE BALANCE.

The acid-base balance and electrolyte studies during storage proved to be most interesting. As expected, the more dilute the perfusion fluid, the higher the pO_2 levels and the more rapid was hyperoxygenation within the chamber. However, it was surprising to note a significant further rise after some hours of exposure to high pressure oxygen, the increase continuing until the end of the storage period.

None of the kidneys perfused without blood survived. This raises the question of the ability of the tissues to utilise dissolved oxygen directly. At very high (but sub-lethal) pO_2 levels, renal ischaemic and anoxic changes were more marked in bloodless perfusate organs than in those perfused with 50% haemodilution.

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Vasoconstriction cannot be excluded as a cause for this, but there is the possibility that tissue cells rely on haemoglobinated oxygen only for survival. This hypothesis does not disallow any benefit from oxygen under pressure. De-oxygenated haemoglobin can be re-oxygenated directly from dissolved oxygen in the serum without gaseous exchange across the pulmonary alveolar membrane. In this way the tissues can be supplied with far more oxygen than in normal conditions.

Purely mechanical factors also may be implicated, considering the lack of survivors in group II (using blood-free perfusate). Although the perfusate is isomotic with blood, it does not contain cell bulk in the form of red and white blood corpuscles, and these spherical bodies may constitute an essential for the maintenance of small vessel patency. The histological findings in these kidneys, indistinguishable from anoxic or ischaemic tubular degeneration, support this theory. Correction may well be achieved using faster perfusion flow rates, producing a sufficiently greater perfusion pressure to ensure medullary supply.

The medullary necrosis similar to that observed in the blood-free perfusate group is easily explicable in group III, where whole blood was used for perfusion. Very high plasma haemoglobin levels were recorded in this group, indicating gross red cell destruction with resultant mechanical blockage of the distal and therefore of the medullary small vessels. The inter-relationship of perfusion pressure with both rate of flow and with differences in perfusate constitution are more fully discussed later.

In all the Group VI experiments the perfusate temperature was between 15°C and 20°C after 30 minutes of storage. However, it must be remembered that immediate post-nephrectomy hypothermic perfusion (at 5°C) had been performed, and the renal core temperature was very probably significantly lower than the reservoir temperature. Levels of 5°-10°C were easily maintained with a refrigerator.

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In view of the low pCO_2 and standard bicarbonate levels (with a pronounced base deficit), it was surprising that the hydrogen ion concentration was maintained within so narrow and normal a range. This largely is explicable by the balancing effect of low pCO_2 and bicarbonate.

In the successful group, three points were noted during storage which did not appear in the other groups:-

1. The perfusate pCO_2 showed a slight but uniform significant rise,
2. The perfusate potassium level remained constant, and
3. The urea concentration of the perfusate increased slightly, but significantly.

In contrast, the unsuccessfully stored kidneys showed quite different findings. No increase was noted in the pCO_2 or the urea and, by the end of the storage period, the potassium levels grossly exceeded normal and had risen to higher levels than in the early stages of storage.

The importance of these observations lies in the possibility that they may express normal but metabolically depressed cellular function on the one hand, and cell death on the other.

pCO_2 and urea formation not only reflect the general state of metabolism but, in particular, renal tubular function. Metabolic processes cease with cell dysfunction and, when actual destruction occurs, the predominant intracellular ion K^+ is released. In the unsuccessfully stored kidneys there was not only suppression of metabolism but also liberation of intracellular ions from dead cells, which probably caused the very high potassium levels at the end of the storage period.

Base deficit was never more pronounced at the end of storage in the successful experiments. In all the other groups, however, base deficit increased and was most marked during the last 12 hours of storage.

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4. URINARY OUTPUT.

Urinary electrolyte determinations during storage confirmed the already widely recognised depression of tubular function which is produced by hypothermia.

Sodium re-absorption and potassium excretion were reduced (if not absent) and, electrolytically, the 'urine' was in fact unaltered glomerular filtrate. As the volume output was not recorded, the actual glomerular filtration rate could not be deduced, but in the conditions of the experiments it was clear that glomerular filtration was grossly diminished, though not abolished. Two explanations come readily to mind: the relative inhibition of glomerular filtration by hypothermia, and the quantitative diminution of filtrate caused by low perfusion flow and pressure.

The incidence of proteinuria during storage is more difficult to explain. There are three possibilities: (1) by depressing glomerular function, hypothermia may produce a simple protein escape into the filtrate; (2) parenchymatous renal damage from the nephrectomy may also be implicated (a routine immediate post-transplantation finding in other transplantation studies done here without storage); (3) cellular destruction resulting from the damage produced by the storage technique per se.

The third possibility was conclusively demonstrated in the unsuccessful experiments where there was far more protein in the urine than in the successful group. With haemodilution or whole blood perfusates, glomerular filtration could be responsible, although gross proteinuria was present only after some hours of storage. However, there was a quantitative similarity in the organs perfused without blood and the perfusate contained no protein; the urinary protein thus must have originated within the kidney itself, from cellular breakdown.

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Catalase is a reducing enzyme in rich supply in the renal tissues. It is postulated that catalase estimation (qualitatively or preferably quantitatively) would be useful in the diagnosis of threatened renal homograft rejection at an early stage. It was hoped that the appearance of catalase would indicate cellular damage in these experiments. However, though catalase was never found in the urine excreted by the successfully stored kidneys, likewise it was never isolated in any of the unsuccessful groups either, except in the kidneys perfused with whole blood (where the urine was heavily blood-stained); as red blood corpuscles have a high catalase content, no significant conclusions may be deduced.

The absence of catalase in the unsuccessful groups is not felt to reflect the presence of entirely undamaged cells. It is more likely yet another expression of the effects of hypothermia on cellular metabolism, and on enzyme activity and release.

Examination of the urinary sediment smears provided corroboration of tubular dysfunction, various casts and cellular debris having been observed. These abnormalities were marked in the unsuccessfully stored kidneys, as was to be expected.

5. PERFUSION PRESSURE.

The relationship of flow rate to perfusion pressure is clearly shown in these studies. Doubtless other factors also exert an effect on the perfusion pressure at a standard flow rate. In fact, it is the problem of perfusion pressure which has made isolated organ perfusion (especially at profoundly hypothermic levels) such a complicated and frequently unsuccessful storage technique.

A great deal has been written about the optimal perfusion pressure at various temperatures for kidney storage; a review of the salient points was presented earlier in this thesis. It bears repetition that there is no uniformity as regards either observation or conjecture of the correct pressure at temperatures between 5°C and 10°C (the operative level in this study).

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In group I, using haemodilution, the correlation between flow rate and pressure suggested a linear relationship, which was felt to be significant. Only at very low perfusion flow rates (and therefore low pressures) was there success. The pathological findings support the necessity for low flow/low pressure perfusion at the levels of hypothermia used. Oedema was more marked at faster flow rates and, at 1,000 ml./hr. flow there was actually rupture of the stored organ; there was also evidence of medullary necrosis at these higher flow rates.

The importance of renal oedema in the pathogenesis of "acute tubular necrosis" - especially of the anoxic or ischaemic variety - has only relatively recently been recognised. The intra-renal vasculature is such that the medulla is the most peripheral, or distal, area of supply. Interstitial oedema, whatever the cause, produces obstruction by compression of the tenuous peripheral arterioles and renders the tubules ischaemic, and so anoxic.

This explains the appearance of florid signs of anoxia in the tubules, occurring more markedly at high perfusion flow rates and pressures and always associated with severe interstitial oedema. The degree to which the interstitial tissues were water-logged was directly proportional to perfusion flow and pressure during storage.

Hypothermia must provide at least part of the answer for the outpouring of fluid at low perfusion rates, compared with normal renal flow rates in the intact, normothermic animal. The reduction of cellular metabolism and activity by hypothermia applies in particular to the highly specialised renal tubular epithelium. Although the cell is not lost as an integral, viable entity, many of its functional characteristics are markedly (or totally) depressed, e.g. the conservation ofsodium and the excretion of potassium. Hypothermic depression of function in vascular endothelial cells may well allow a massive fluid loss to the tissues above a certain critical pressure, and this pressure is probably very low.

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Another aspect of organ perfusion storage which has stimulated considerable interest is the increase in renal resistance which occurs in time, reflected in the perfusion pressure. Whatever the temperature, this has been observed in all cases at stabilised rates of flow. The term "arterial bed block" has been coined to describe this phenomenon and is most appropriate.

This phenomenon occurred throughout the experiments in this study. The progressive pressure increase was evident earlier in storage, as well as being more pronounced, when faster flow rates were used.

The most likely cause is vasoconstriction. There are two reasons. Firstly, microscopy failed to show any organic lesion within the tubular lumina. Secondly, the pressure rise is reversible to some extent, and is partly avoidable. Microscopic examination localises the site to the small vessels which supply the tubules, by the effects of vessel spasm, distal ischaemia and anoxia. The glomeruli are unaffected. It may be concluded that this phenomenon occurs at efferent arteriolar levels.

Many factors may be implicated in vasoconstriction. In a few cases the stage at which some of these factors operate has been noted, the factor identified, and its effects specifically prevented or reversed. These factors are discussed below.

(1) In few (if any) storage experiments performed elsewhere has perfusion been incorporated with hyperbaric oxygenation of the perfusate. Investigation of the perfusate acid-base balance routinely showed very low pCO_2 levels associated with very high pO_2 values. It is well recognised physiologically that, in both the brain and the skin, carbon dioxide is a powerful vasodilator and is indispensable for adequate tissue perfusion. Although other tissues are less sensitive perhaps, this effect still exists.

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Carbon dioxide is not added to the compressed oxygen in this study. The kidneys survived with oxygen alone in the successful group, where the initial pressures produced by perfusion were low and the rise during storage did not produce macroscopic oedema or microscopic necrosis.

Hyperoxygenation itself might produce small vessel spasm. Local tissue factors affect the calibre of feeding vessels, perhaps on the basis of supply and demand. Direct monitoring of arterial flow to the brain in particular confirms this, as a marked reduction in cerebral blood flow occurs at raised arterial pO_2 levels. As no other cause can be found, vasoconstriction is credited. It is easy to appreciate that a similar reduction of oxygen (and thus of blood) is necessary in kidney storage once the tissues are super-saturated with oxygen. There is no disadvantage provided the spasm is relieved as soon as the tissue requirements increase again.

It has been suggested in the field of hyperbaric medicine that the convulsions and other neurological manifestations which are labelled 'oxygen toxicity' may actually be caused by cellular anoxia due to intense vessel spasm and ischaemia. This acute tissue ischaemia and anoxia fail to produce any improvement in blood supply, although initial diminution could have been caused by vasoconstriction (mediated by local factors) the tissues being in no need of oxygen at that stage. In the intact animal the addition of carbon dioxide to the hyperbaric atmosphere delays these cerebral signs.

In this study, hypothermia may protect the cells from anoxia by slowing metabolic processes and oxygen utilisation in the presence of the poor tissue perfusion caused by severe and persistent arterial spasm. This possibility gains weight with the recollection that these cells initially are super-saturated with oxygen.

(2) Mechanical blockage of small vessels would hinder tissue perfusion and increase the pressure required for adequate intra-renal circulation. Such vascular aggregation and thrombosis was observed repeatedly and was particularly noticeable in group III where whole blood perfusion was used.

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Experiment has shown that perfusion with low molecular weight dextran significantly improves small vessel circulation, by preventing red cell adherence and thrombosis. The onset of pressure rise has been restricted, where this is the cause, by the use of low molecular weight dextran by others.

With whole blood perfusion storage, large scale small vessel thrombosis was a prominent histological feature in the organ post-storage. In the kidneys perfused with the physiological solution only (group II) or with haemodilution (group I) this feature was absent.

The use of heparin in the perfusate is a limited solution to this problem.

(3) It has been shown that vasoconstrictors are released merely by perfusing blood through glass or tubing. However, this factor cannot be excluded in perfusion storage unless a blood-free perfusate is used. In the Group II experiments the initial perfusion pressure was certainly lower than in the other groups, and the subsequent rise was less pronounced.

(4) The hypothesis has been propounded by one group of workers (Kenyon (1965)) that serotonin is liberated during isolated organ perfusion, and is at least partly responsible for vasoconstriction. Adding an anti-serotonin agent to the perfusate, some improvement in "arterial bed block" was obtained.

Other tissue vasoconstrictors are probably similarly involved but have not yet been identified.

On purely empirical grounds, following the practice of others, known vasodilator agents were used. However, no effects were directly attributable to their action and the feeling is that they are of limited benefit only.

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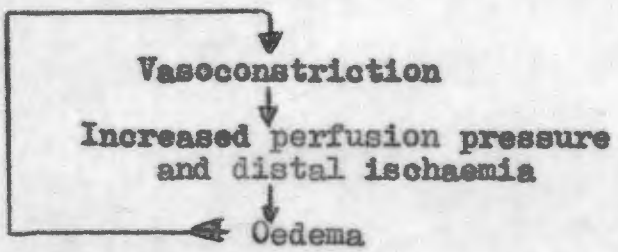
Much of the preceding discussion, regarding perfusion pressure, is conjectural. However, there is no doubt that a rise in perfusion pressure occurs during prolonged isolated organ perfusion. This is currently best explained on the basis of small vessel constriction although other factors are undoubtedly also involved. It is unlikely that any single factor is responsible.

Some possible reasons for the rise in perfusion pressure have been postulated but the vital significance of this factor in successful organ storage must be clarified. Small vessel occlusion (functional or organic) causes distal ischaemia. At low temperature and high oxygen tension the damage may not be extensive enough to cause cell death, especially where vessel obstruction is only partial. The effect of pressure on interstitial fluid accumulation has been discussed. Above a critical pressure fluid escapes from the vessels and the resultant oedema aggravates vessel spasm (or occlusion) by mechanical pressure from without. A vicious cycle results: vasoconstriction leads to increase in perfusion pressure, which causes oedema, and this aggravates the already impaired blood supply; a further increase in perfusion pressure ensues, with an even greater out-pouring of fluid.



This discussion highlights some of the causes of failure in the unsuccessful experiments in Group VI: technique, perfusates, acid-base balance observations, urinary output as evidence of renal function, and the importance of perfusion pressure during storage.

The effects of perfusion pressure in the three groups of Group VI are briefly summarised below.

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Group I - Haemodilution perfusion

(a) High flow rates/pressures:-

Haemodilution was sufficient to prevent intravascular aggregation and thrombosis. Initial pressures were understandably on the high side and the subsequent rise was relatively greater at faster flow rates. Similarly, oedema was more severe at faster flow rates and higher pressures. In other respects, the findings were similar to those in the successfully concluded experiments.

(b) Low flow rates/pressures (group I (d)):-

This was the only group in which the kidneys were successfully stored, without exception. The success achieved appears to be closely related to the extremely low perfusion flow rate (150 ml./hr) and pressure, using haemodilution. The perfusate contained 50% blood and was electrolytically and metabolically similar to normal homeostatic environment.

In the histology of these organs, no attention was paid to dessication of the kidney. Using perfusion without immersion of the organ during storage, there was no evidence of any drying-out.

Group II - Physiological solution perfusate.

No blood was used for perfusion, which was carried out at low flow rate/pressure. The following points are significant:-

(1) Unrecordable partial pressure of carbon dioxide in the perfusate; any vasodilator effect of carbon dioxide thus did not operate;
(2) The pO_2 values were extremely high (+2,500 mm.Hg): oxygen toxicity is not thought to apply but there was persistent vasoconstriction secondary to an initial diminished demand;
(3) There was the possibility that dissolved oxygen could not be directly utilised by the tissues;

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(4) The absence of cells in the perfusate: cell bulk may be essential to maintain the patency of small vessels (if true, the deficit may be compensated for by increasing the flow rate, thereby increasing pressure and preventing vascular collapse).

There was no success in this group.

Group III - Whole Blood Perfusion at low flow/pressure:

There was no success in this group either. Any benefit derived from the higher pCO_2 and slightly lower pO_2 levels was offset by the high initial perfusion pressure which occurred, with a considerable increase subsequently.

Histologically, small vessel thrombosis was an invariable finding and was clearly responsible for the pressure rise during storage, and its attendant changes. Markedly raised plasma haemoglobin levels during storage were indicative of the extent of red cell destruction.

6. POST-STORAGE FUNCTION.

After perfusion storage and subsequent autotransplantation of the kidney, investigations were performed to assay renal function and to exclude graft rejection. No criteria of threatened graft rejection were ever detected and this indicates that the storage technique used does not complicate the diagnosis of rejection.

Renal function post-storage was invariably disordered. In the successfully stored organs this was never very severe and function returned to normal within 14 days of re-implantation and contralateral nephrectomy. The last parameter to return to normal was creatinine clearance, usually some days after stabilisation of the BUN.

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It is not claimed that, using this storage technique, post-storage renal function was immediately normal (or even near normal) in the successful group of experiments. There was evidence of parenchymatous organic destruction on histology 14 days after re-implantation or even longer, in these organs, although not in every case. However, in these and in the histologically normal specimens in the successfully stored group of kidneys, renal function was normal at the time of sacrifice.

Not one kidney in the successful group was incapable of life-sustaining function, unassisted, after storage and transplantation. In every case in group I (d) the animal was dependent on the stored organ from the time of re-implantation with simultaneous contralateral nephrectomy.

7. PATHOLOGY.

The pathological changes which occurred and were evident immediately after storage showed oedema. The degree of interstitial oedema and capsular dilatation was a reliable index to subsequent function of the stored organ. The least evidence of oedema was observed in the successfully stored kidneys, where haemodilution perfusion at very low flow rate/perfusion pressure had been used.

The only other pathological finding was the presence of small vessel thrombosis, prevalent in the kidneys perfused with whole blood and in those perfused at high flow rates. Oedema was gross in these cases.

Vacuolation was the only suggestion of actual tubular cell destruction, seen mainly in the proximal tubular epithelium. This was never found in the successful group. If it is fair to conclude that these pathological changes result from ischaemia or anoxia, then the presence of blood in the perfusate is essential for the prevention of vacuolation, since this finding was most common in the group perfused without blood (group II).

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In the successful group, microscopy was performed only after the 14 day survival period, by which time renal function was invariably within normal limits. In most cases the picture was indistinguishable from normal. In a few there were isolated areas of tubular regeneration, which is felt to indicate reversible ischaemic damage inflicted during storage.

The greater the flow rate, the more severe was damage to renal parenchyma, the ultimate result being complete necrosis.

It is clear that the perfusion flow rate/pressure are the most important variables in the storage procedure used. Their inter-relationship is close but, more important, their relationship to the success or failure of perfusion storage is striking. The association is found in the pathological picture as well as in the functional capabilities of the organ during and after storage.

The pathological features most evident in the unsuccessful groups are those of necrosis, involving both cortex and medulla, or only the latter. It is interesting that necrosis was most extreme at the cortico-medullary junction.

In whole blood perfusion experiments the perfusion pressures were exceptionally high. The association between high pressure and rising pressure, and inadequate tissue perfusion, is proven by the histology. In all these organs there was necrosis of both cortex and medulla.

A similar picture was found in the kidneys where a blood-free perfusate was used. The probable causes have been discussed already.

8. COUNTER-BALANCE.

Renal counter-balance (first observed by Minnar) is a phenomenon of considerable fascination and has stimulated a good deal of speculation, controversy and discussion.

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The suggestion is that a traumatised kidney in the presence of its normally functioning fellow undergoes a progressive diminution in function, ultimately reaching disuse atrophy.

Although no satisfactory explanation exists, there is presumably some mechanism which wrests function from the damaged organ (either by super-function on the part of the normal kidney, or by active prevention of function in the damaged one). It might be argued that the cessation of function results from the trauma, but it can be proven that this is not the case. By removing the normal kidney after varying intervals, the damaged kidney can sustain life adequately up to a certain period - a remarkable and almost instantaneous increase in urine flow from the damaged kidney occurs and functional capability is clearly present. In those experiments, renal function was adequate after storage and re-implantation provided contralateral nephrectomy was performed without delay. However, if the opposite kidney was allowed to remain in situ for 24, 48 or 72 hours after re-implantation, almost no function could be obtained in the stored organ until contralateral nephrectomy was performed. Immediately thereafter there was adequate function in the re-implanted kidney.

The importance of this observation in human nephrology is obscure. The experimental demonstration of renal counter-balance is invariably performed in dogs. In marked contrast - with careful differential appraisal of function after unilateral renal disease, trauma or surgery - confirmatory evidence in man has been limited, if indeed present at all. It would seem that the canine kidney is hypersensitive to the condition of its fellow in some way. The nature of this relationship is unknown and further study is strongly indicated, mainly to facilitate its recognition and exclusion in human renal disease.

In 1960, Faulkner et al undertook to evaluate the possibility of kidney preservation with anastomosis to the iliac vessels. They also wished to determine the effect exercised by the opposite normal kidney on an autogenous graft in these circumstances.

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The concept of renal counter-balance suggests that a damaged kidney cannot compete functionally with its healthy mate (Addis, 1954). This suggestion frequently dictates the advisability of nephrectomy rather than a restorative procedure when the surgical problem involves a severely damaged kidney. Due to its functional shortcomings, the damaged kidney may not be salvaged surgically if the healthy organ progressively assumes the entire excretory load.

The importance of this concept in renal transplantation is obvious. If the trauma incidental to grafting renders the transplant functionally inadequate, disuse atrophy will occur.

Evidence indicates that, the greater the amount of nitrogenous wastes presented to a kidney (or the greater the parenchymatous blood flow) the greater will be the tendency to renal hypertrophy. This is consistent with the enlargement of the remaining kidney after nephrectomy.

With the fairly extensive vascular and cellular trauma inflicted in grafting a kidney, the opposite organ might assume more and more excretory function - leading to loss of the graft. From Faulkner's study (1960) this phenomenon appears to merit some consideration. In 8 of his animals intravenous pyelography showed good function of the unoperated kidneys and poor or no function of the grafts. Upon removal of the unoperated kidney, however, graft function improved markedly, evidenced by radiology and clearance studies.

These observations make the functional survival of kidney autografts in cases of massive ureteral loss doubtful, particularly when the opposite kidney functions normally. Early removal of both host kidneys in transplants may be of benefit, to allow the donor kidney to reach the hypertrophy which occurs in response to its assumption of the entire excretory load.

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9. HYPOTHERMIA.

The benefits derived from the use of hypothermia in prolonged organ preservation are easily understood. In this entire series of experiments (Groups I - VI) profound hypothermia was singly the most important factor in maintaining isolated organ viability.

Direct observations of the metabolic effects of low temperature are sparse but all indicate that, predominantly, the function of the tubular epithelial cells is affected. Our results confirm this. Tubular function is so depressed that the glomerular filtrate is virtually unaltered.

The greater the reduction in metabolic requirements, the less is anoxic damage and the longer the period for which an isolated organ may be stored. Theoretically it should be possible to hibernate an isolated organ indefinitely, but so low a temperature would be needed that the organ would be destroyed by injury from freezing and thawing.

At the hypothermic level used in these experiments (5°-10°C) metabolic requirements were reduced but not to the extent of producing a state of suspended animation. Over the 24 hour storage period, consequently, gross anoxic parenchymatous damage resulted.

In the hope that there would be adequate diffusion of oxygen to maintain cellular viability, the whole immersion system of cooling was subjected to 3 ATA pure oxygen (Group V). This in fact did occur: cell destruction was not so severe or widespread and it was possible for regeneration to take place. After two or three weeks these kidneys were capable of life-sustaining function. However, none retained sufficient function to allow immediate contralateral nephrectomy.

10. PERFUSION.

When the Group V experiments had been concluded, it seemed logical to incorporate a further method of preserving kidney viability during storage. In Group VI organ perfusion was combined

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with hypothermia and hyperbaric oxygenation as a method of organ storage for 24 hours. Successful storage, with immediate function of the kidneys, was achieved using 50% haemodilution with a low perfusion flow rate and perfusion pressure together with hypothermia and high pressure oxygen.

As already mentioned, perfusion pressure is the all important variable using this storage technique. At suitable levels of profound hypothermia and hyperoxygenation the flow can be reduced sufficiently to ensure a low enough pressure to avoid damaging the organ being stored.

In general, perfusion of the organ contributed the following benefits to the successful outcome of the experiments:

(1) Immediate post-nephrectomy perfusion cooling, which has already been discussed, is themost efficient method for rapid and uniform reduction of renal core temperature, and is important for depressing cellular metabolic rates. With prolonged perfusion cooling the core temperature was maintained at this low level and the entire kidney was kept cool - not only the surface.

(2) The individual renal cells were maintained in a normal, homeostatic environment by means of perfusion.

(3) Although renal cellular metabolism was markedly depressed it still takes place in the temperature range of 5°-10°C, and oxygen requirements must be met. Perfusion of the organ during storage was the most efficient method of supplying oxygen to the cells ofthe kidney.

(4) Cellular metabolism continuing during storage, the break-down products had to be removed by a satisfactory disposal system. Perfusion was best suited to this purpose.

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11. HYPERBARIC OXYGEN.

The role which is played by hyperbaric oxygenation of the organ during in vitro storage is a controversial one. As yet little or no investigations have been performed in this aspect of organ storage.

From published reports it appears that perfusate oxygenation at normal pressure provides inferior results in organ storage as compared with oxygenation at increased ambient oxygen pressures.

It has been suggested that the oxygen requirements of the tissues are actually raised when hypothermia is used during storage. No such conclusion may be drawn from the results of our studies. It seems more probable that certain enzyme systems are inhibited at increased partial pressures of oxygen, this rather in the same way that hypothermia depresses but does not obliterate cell metabolism. Such effects have been demonstrated but their application in the circumstances of this study is unproven.

Whatever additional benefit is derived from the use of hyperbaric oxygenation in this storage technique, there is no doubt that the diffusion oxygenation of the perfusate was simple, efficient and highly successful.

The use of high pressure oxygen was prompted initially by its reported efficacy in preventing thermal injury. It is unlikely that the low pressures used in this study would exert any such effect, however, and it is appreciated that damage from cooling and thawing is improbable at the hypothermic levels used in Group VI.

The possibility of high perfusate pO_2 causing intense and persistent vasospasm has been mentioned. Although this is likely, when other possible causes are considered in the circumstances of these experiments, vasoconstriction was not expected to preclude

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success and did not in fact do so.

It is possible that vasospasm could be eliminated by the addition of carbon dioxide to the oxygen which is supplied under high pressure during storage.

12. ASSESSMENT OF RENAL FUNCTION DURING STORAGE.

Using the combination of perfusion with hypothermia and hyperbaric oxygenation during storage of the kidney (Group VI), it was possible to assess renal function during the storage period. The significance of metabolic discrepancies observed between the successful and unsuccessful experiments in Group VI has been discussed already.

The variations which were noted probably reflect cell death and loss of function, resulting from inadequate oxygenation during storage, in the unsuccessfully stored kidneys. In retrospect, the accurate and continuous monitoring of the parameters studied would facilitate the diagnosis of anoxic destruction during storage. The extent of renal damage would be appreciated before re-implantation and the unnecessary transplantation of the already destroyed, afunctional kidney would be obviated.

The clinical application of function assessment during storage is significant. Cadaver grafts are unavoidably subjected to variable periods of relative ischaemia and anoxia. If this causes irreversible impairment of renal function, transplantation of the organ into the patient is a dangerous procedure performed without benefit to the prospective recipient.

If it were possible to store the cadaver graft without the risk of further anoxic damage, at the same time assessing its functional capacity, the patient would be guaranteed a graft providing immediate post-transplantation benefit. The damaged kidneys would be discarded without material loss.

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Many clinical cadaver transplants of kidneys have failed not because of rejection but as a result of anoxic damage. Using the storage technique as described, these failures could be avoided.

At the risk of repetition, the important point is not that satisfactory organ viability be maintained for 24 hours but that the stored kidney can be exhaustively studied during this period. Though the attempt to gauge function during storage was incidental to the experiments performed in this project, mainly depending on negative metabolic studies, the active assessment of function by established procedures may well be feasible.

Other exciting possibilities with satisfactory kidney storage techniques also spring to mind. By the nature of a cadaver transplant programme, donor and recipient are unrelated. Currently the time factor is all important and - with the exception of standard blood compatibility tests - no other course is possible. Given more time, histocompatibility might be more exactly investigated and the least dissimilar candidate selected from a panel of prospective recipients.

Perhaps the most fascinating possibility is that, during the storage period, treatment to attenuate the antigenicity of the donor kidney could be instituted in the isolated organ before transplantation. If successful, this could lead to the partial (or complete) prevention of rejection.

SUMMARY AND CONCLUSIONS.

SUMMARY AND CONCLUSIONS.

Combining profound hypothermia, hyperbaric oxygenation and low flow/low perfusion pressure continuous organ perfusion, kidneys were stored for 24 hours with 100 per cent success. These kidneys were all capable, immediately following storage, of sufficient function to maintain life unaided.

The use of profound hypothermia for organ preservation is well recognised, owing to its ability to lower markedly the metabolic processes and oxygen requirements of the tissues. The exact benefit derived from high pressure oxygenation in these experiments is difficult to define. Various theories are presented, perhaps the best substantiated being that tissue oxygen demands at very low temperatures are comparatively high.

Perfusion is the most logical method of organ preservation. However, when attempted, the technical problems were usually insurmountable. In this study these hazards were overcome. At low temperature and with hyperoxygenation of the perfusate, low perfusion flow rates and low perfusion pressure were found to be essential for successful organ storage. Furthermore, although blood proved to be a necessary constituent of the perfusate, haemodilution was equally essential for post-storage survival and function.

The success of this storage technique depended on the solution of the problems posed by perfusate, rate of flow and perfusion pressure. These three factors are closely inter-related and are equally important. Only when correctly combined was successful storage achieved.

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To obtain low perfusion pressure, it was necessary to use extremely low flow rates and haemodilution.

The possibility that hyperoxygenation is essential to meet tissue requirements at the low flow rates used cannot be overlooked, and may explain the apparent benefit derived from high pressure oxygenation.

Two comparative studies warrant special mention:-

(1) The comparison of all the experiments performed in Groups I - VI (Table V), demonstrating that only in Group VI was it possible to achieve success after 24 hours organ storage and autotransplantation with simultaneous contralateral nephrectomy;

(2) The comparison of the results achieved in these experiments with those obtained by others in similar studies (Table VI).

TABLE V.

24 HOUR IN VITRO KIDNEY STORAGE – ALL AUTOTRANSPLANTS. SURVIVAL TIME: 14 DAYS POST-CONTRALATERAL NEPHRECTOMY.

GROUP.	NUMBER OF DOGS	STORAGE TEMPERATURE	OXYGEN AVAILABILITY	STAGING OF CONTRALATERAL NEPHRECTOMY.	SURVIVORS
1	9	5°C. TO 10°C	—	4 IMMEDIATELY AFTER RE-IMPLANTATION 5 AT 20-28 DAYS	— 1
2	4	-5°C. TO -10°C.	—	THESE KIDNEYS WERE FUNCTION-LESS AND LEFT NEPHRECTOMY WAS NEVER POSSIBLE.	—
3	4	-5°C. TO -10°C.	3 ATA.	AS IN GROUP 2.	—
4	5	±37°C	3 ATA	1 AT 26 DAYS POST-REIMPLANTATION	—
5	10	5°C. TO 10°C.	3 ATA.	4 IMMEDIATELY 6 AT 20-28 DAYS.	— 4
6	12	5°C. TO 10°C.	3 ATA.	IMMEDIATELY.	12

GROUP 6 — WITH PERFUSION.

An overall picture of the results of in vitro kidney storage for 24 hours (Groups I - VI). Only by using organ perfusion with hypothermia and hyperbaric oxygenation was 100% survival possible after re-implantation of the stored kidney with immediate contralateral nephrectomy.

TABLE VI.

NAME AND YEAR	HYPOTHERMIA (°C)	PERFUSION	OXYGEN	STORAGE TIME (Hrs)	OPPOSITE NEPHRECTOMY (Wks.)	SURVIVORS
TELANDER, (1962).	37	YES	YES	7 24	1 2-3	7 OF 7 0 OF 2
HUMPHRIES ET AL., (1962)	4	YES	YES	24	3	2 OF 41
MATLOFF & GOWEN, (1962)	4	YES	NO	3	IN VIVO	2 OF 2
CALNE ET AL., (1963)	4	NO	NO	12 17 24	2-3 2-3 2-3	1 OF 2 0 OF 4 0 OF 3
HUMPHRIES ET AL., (1963)	4	NO PLASMA SERUM BLOOD	NO YES YES YES	24 24 24 24	NEVER 3 3 3	0 OF 6 2 OF 9 2 OF 11 0 OF 3
HOFFMAN ET AL.,	4	NO	NO	12 24	2-4 2-4	8 OF 14 2 OF 16
TELANDER, (1964)	NO	YES	YES	7 24	2-3 2-3	8 OF 11 0 OF 1
MANAX ET AL., (1964)	4 37 4	NO NO NO	NO 3 A.T.A. 3 A.T.A.	24 24 24	NEVER NEVER 2-4	0 OF 10 0 OF 10 6 OF 30
HITCHCOCK ET AL., (1964)	4 4 37	NO NO YES	NO NO YES	8 20 5	1-3 1-3 1	4 OF 6 2 OF 6 7 OF 7
HUMPHPIES ET AL., (1964)	4-10	YES	YES	24	3	5 OF 7
ACKERMANN ET AL., (1965)	-10 -10 25 5-10 5-10 5-10	NO NO NO NO NO YES	NO 3 A.T.A. 3 A.T.A. NO 3 A.T.A. 3 A.T.A	24 24 24 24 24 24	NEVER NEVER NEVER 3 3 IMMEDIATE	0 OF 4 0 OF 4 0 OF 5 1 OF 9 4 OF 10 12 OF 12

This is a comparative table depicting the published results of experimental in vitro kidney storage, and the results obtained in the experiments described here. From this it will be seen that the only success previously obtained with 24 hour storage was dependent on delayed contralateral nephrectomy.

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