Warm perfusion of ischaemically damaged kidneys: ex-vivo function, viability assessment and preservation efficacy

Matthew Stephen Metcalfe

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From

The University Department of Surgery

Leicester General Hospital

University of Leicester

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ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

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PART 3: RESULTS AND DISCUSSION

This thesis is based on my own independent work, except where acknowledged

M.S. Metcalfe

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Abbreviations

A & E	Accident and Emergency Department
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
AN	Adenine nucleotide
ANOVA	Analysis of variance
AAP	alanine aminopeptidase
AP	alkaline phosphatase
AR	acute rejection
ATN	Acute tubular necrosis
ATP	Adenosine triphosphate
CAPD	Continuous ambulatory peritoneal dialysis
CCU	Coronary care unit
CfHb	cell free haemoglobin
CIT	Cold ischaemic time
Cl^-	chloride ions
CNS	Central nervous system
CO_2	carbon dioxide
CP	cold perfusion
CPP	cryoprecipitated plasma
CS	Cold storage
CT	computerised tomography scan
DGF	Delayed graft function

DNA	de-oxyribonucleic acid
EMS	exsanguinous metabolic support
ESRF	End stage renal failure
F	kidneys not exhibiting PNF
GFR	Glomerular filtration rate
g	gramme(s)
GGTP	γ-glutamyl transpeptidase
(α/π) GST	(alpha / pi) glutathione s-transferase
HBD	heart-beating donor
HD	haemodialysis
Hepsal	0.9% saline with 1unit/ml heparin sodium added
HOC	Hyperosmolar citrate solution
HTK	Histidine tryptophan ketoglutarate solution
HPP	Hypothermic pulsatile perfusion
ICU	Intensive Care Unit
IF	Immediate graft function
ISP	In situ preservation
IRI	Ischaemia-reperfusion injury
IRR	Intrarenal vascular resistance
iv	intra-venous
K^+	Potassium ion(s)
Kg	kilogrammes
KW	Kruskal Wallis test
LDH	Lactate dehydrogenase
LRD	living related donor

Mg^{2+}	magnesium
MHC	major histocompatability complex
MPS	Machine perfusion solution
MRS	magnetic resonance spectroscopy
MW	Mann Whitney U-test
Na^{+}	Sodium ion(s)
NHB(D)	Non-heart-beating (donor)
NO	nitric oxide
O_2	oxygen
^{31}P	phosphorus-31 isotope
PFC	Peflourochemical
PFD	Perflourodecalin
P_i	inorganic phosphate
PME	Phosphorus mono-esther
pmp	per million population
PNF	Primary non-function
POPS	portable organ perfusion system
SGOT	serum glutamic oxaloacetic transaminase
u	units
UW	University of Wisconsin solution
WIT	warm ischaemic time
WP	warm perfusion

Thesis Abstract

Title: Warm perfusion of ischaemically damaged kidneys: ex-vivo function, viability assessment and preservation efficacy

Author: Matthew Metcalfe

Background

The shortage of kidneys for renal transplantation has prompted renewed interest in non-heart-beating donors (NHBD). While this may increase the number of transplants, it also increases the primary non-function (PNF) rate. This is caused by excessive warm ischaemic injury in some NHBD, and has hindered their more widespread use. A reliable pre-transplant test of organ viability, and a preservation method minimising additional ischaemic damage, would allow the PNF rate to be reduced. The aims of this thesis were to explore the potential of warm *ex-vivo* perfusion as a preservation method and a means of diagnosing viability pre-transplantation.

Methods

Warm *ex-vivo* perfusion of ischaemically injured porcine kidneys with an oxygenated emulsion of a perflourochemical in tissue culture fluid was used to measure ex-vivo function and preserve kidneys. A cadaveric model was used to assess the relationship of *ex-vivo* function and warm ischaemic time. An autotransplant model was used to determine the relationship of *ex-vivo* function to post-transplant function, and to compare the efficacy of preservation by warm perfusion with conventional hypothermic techniques of static storage and pulsatile perfusion. Post-transplant outcome measures were survival, renal function and histology.

Results

WIT correlated well with *ex-vivo* function. *Ex-vivo* function correlated with post-transplant function in terms of survival (and therefore the immediate life supporting function of the kidneys), but not to the extent that it could be used to predict viability better than knowing the WIT alone. The efficacy of warm perfusion was indistinguishable from hypothermic static storage. However hypothermic pulsatile perfusion was slightly superior to both other techniques.

Conclusions

Warm perfusion as used in this thesis was broadly equivalent in efficacy to conventional hypothermic organ preservation techniques. Although *ex-vivo* function correlated with post-transplant function, the correlation was not tight enough to support a diagnostic role for *ex-vivo* function in viability determination.

PART ONE

Review of the Literature

Chapter 1

Non-heart-beating donor renal transplantation

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1.1 Introduction

The gold standard in ESRF

Renal transplantation represents the gold standard in the treatment of end-stage renal failure. This is justified by clear advantages of transplantation over dialysis, whether CAPD or HD. For the patient, these advantages are both in terms of survival[1] and quality of life[2-10]. For the health care provider, transplantation is the most cost efficient treatment of ESRF[11], yielding savings within two years of transplant. There is therefore no trade off between the ideal treatment for the patient and the availability of financial resources in the treatment of ESRF. The option of renal transplantation should be offered to any ESRF patient fit enough for the surgery involved and without contraindication to the immunosuppression required.

Organ shortage

However, the use of transplantation in the treatment of renal failure is severely limited by the availability of organs suitable for transplantation. Since the introduction of brain-stem death legislation in 1977 and the acceptance of the concept by the medical profession and the public in Britain[12-15] and around the world[12, 16-21], the majority of organs for transplantation in the UK have come from brain-stem dead HBD on intensive care units. Very often these patients have suffered intra-cranial catastrophe secondary to a spontaneous haemorrhage or to trauma. Largely due to the welcome reduction in deaths from trauma, and the availability of CT scanning early after trauma often preventing potential donors from being ventilated as their prognosis is deemed hopeless, the number of suitable donors from this source has declined year on year over the last decade in the UK[22]. The number of patients on the waiting list for transplantation has continued to grow (figure 1.1) in the UK[23] and abroad[24, 25].

This disparity has driven the exploration of means to bridge the gulf between supply and demand.

Firstly, the rate of potential HBD referral to transplant services may be increased. The rate of conventional cadaveric donation in Spain in 2000 was double that in the UK[23]. There are a variety of means of achieving this. These include education of ICU staff, of the public, the adoption of 'opt out' legislation[26], reimbursement for cost incurred by the donor hospital[22] and a payment per organ system for transplant co-ordinators[27]. These measures must only be used with caution, and particularly with sensitivity to the need to maintain public support for whatever organ donation system is in operation. The option of elective ventilation of potential donors pre-mortem when it is non-therapeutic has been rejected[28].

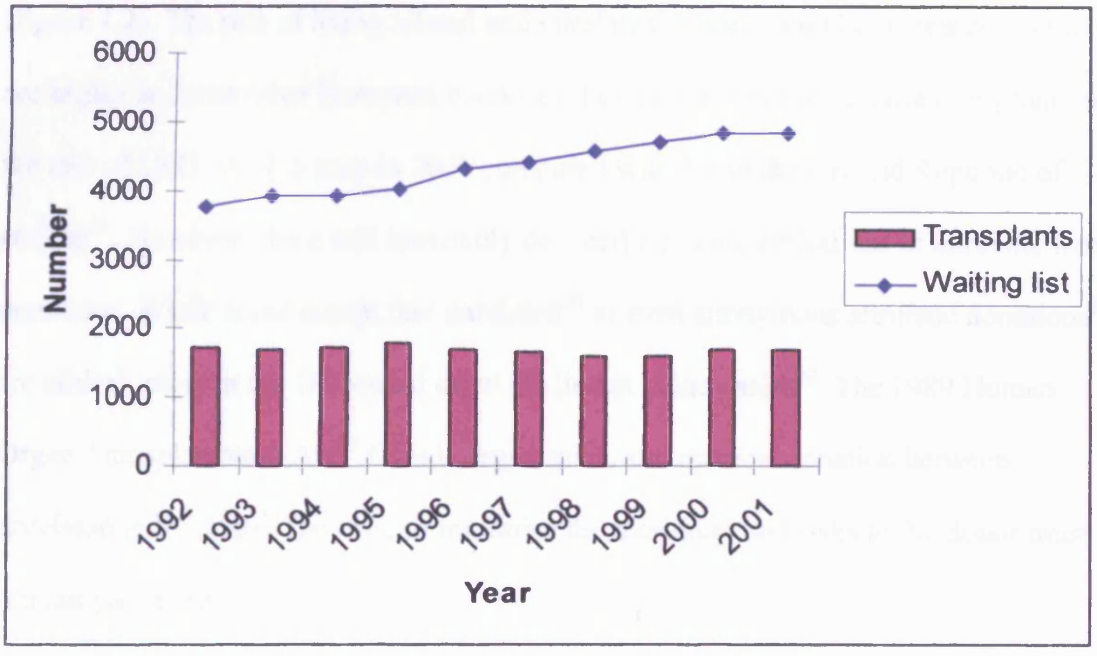


Figure 1.1: The UK transplant figures for the number of kidney transplants performed and dialysis patients on the waiting list between 1992 and 2001 in the UK.

Alternative sources of organs

In addition to these measures to increase the rate of HBD organ transplantation, there are three other potential sources of kidneys for transplantation:

- LRD
- Xenotransplants
- NHBD

LRD, and indeed living unrelated donors, already contribute to renal transplant programmes, with excellent results reported[29]. It is interesting to note that in the UK the number of LD doubled between 1997 and 2000, but that this increased contribution barely maintains the overall transplant rate in the face of falling cadaveric donations[23], (figure 1.2). The rate of living related and unrelated donors could be increased, as rates are higher in some other European countries. For example in the Scandia transplant zone the rate of LRD was 8.5 pmp in 2000 compared with 5.3 in the UK and Republic of Ireland[23]. However, there will inevitably be a ceiling to the ethical use of altruistic live donations. While some accept that unrelated[30] or even anonymous altruistic donations[31] are ethical, most in the UK would draw the line at kidney sales[32]. The 1989 Human Organ Transplantation Act[33] forbids organ sales, and restricts donation between unrelated individuals. The need to minimise the morbidity and risks to the donor must remain paramount[34].

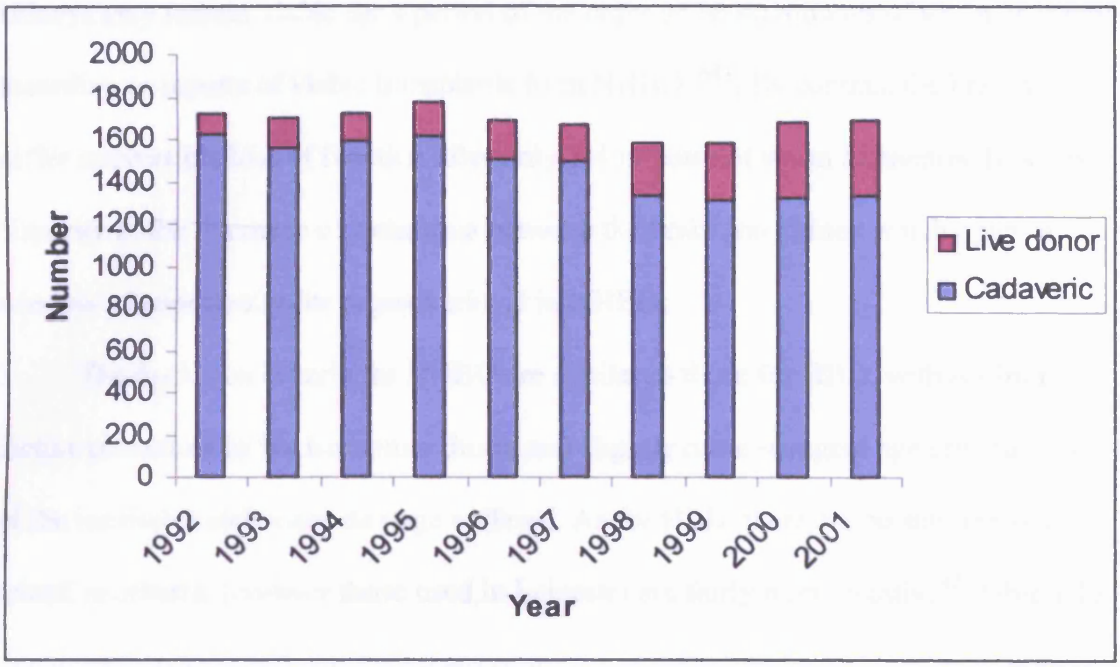


Figure 1.2: The relative contributions of living and cadaveric donors to the total number of transplants performed between 1992 and 2001 in the UK.

Xenotransplantation, whilst offering the tantalising prospect of unlimited organs in the future, is currently bogged down by some major obstacles. These include the cross species discordant immune systems[35-37], and porcine endogenous retroviruses[38].

By contrast, NHBD are a source of organs which are currently available, and under-exploited.

1.2 Definition and Categories of NHBD

Definition

The death of a NHBD is defined by the occurrence of irreversible cardiac arrest. This is in contrast to the HBD, where death is defined on brain-stem criteria, with the heart-beating and the organs perfused with oxygenated blood. After cardiac arrest, the

kidneys may remain viable for a period of the order of 30-40 minutes of warm ischaemia, according to reports of viable transplants from NHBD[39-41]. By contrast the brain will suffer irreversible loss of function after only 3-4 minutes of warm ischaemia. It is this disparity in the tolerance of ischaemia between the brain and kidney which opens a window of opportunity for organ retrieval in NHBD.

The exclusion criteria for NHBD are similar to those for HBD, with additional factors pertaining to the ischaemic times, and slightly more stringent age criteria, in view of the inevitable ischaemic damage suffered. As for HBD, there are no standardised selection criteria, however those used in Leicester are fairly representative[42] (table 1.1).

- **Age < 60 years (cf 70 for HBD)**
- **Warm ischaemic time > 40 minutes**
- **In situ perfusion to retrieval interval > 2 hours**
- **History of renal impairment**
- **Malignancy (except primary CNS tumours)**
- **Systemic sepsis**
- **Complications of diabetes mellitus**
- **Uncontrolled hypertension**

Table 1.1: NHBD exclusion criteria in Leicester

Categories of NHBD

The first international workshop on NHBDs in Maastricht defined categories of donor[43]. *Uncontrolled* NHBD are those in whom cardiac arrest was unexpected. Category I refers to donors who are dead on arrival in hospital. They tend to have a long

and often poorly defined WIT, and are therefore rarely suitable as NHBD. Category II donors arrest unexpectedly in hospital, typically a patient suffering acute myocardial infarction in an A&E department. Their WIT tends to be shorter and better defined then in category I, and therefore make up the bulk of uncontrolled donations.

Controlled NHBD are those in whom cardiac arrest is anticipated or even planned by the withdrawal of life support. Therefore the retrieval team can be prepared in advance and the WIT minimised and accurately documented. Category III donors, typically on an ICU, are those with end stage neurological damage, but who do not meet the criteria for brain-stem death. Category IV refers to brain-stem dead intended HBD, who arrest prior to retrieval. These categories are summarised in table 1.2.

Category	***Control***	***Description***
I	Uncontrolled	Dead on arrival
II	Uncontrolled	Unsuccessful resuscitation
III	Controlled	Awaiting cardiac arrest
IV	Controlled	Brain dead patient undergoing cardiac arrest

Table 1.2: Non-heart-beating donor classification

1.3 Legal and ethical issues relating to NHBD

Naturally many of the legal considerations pertaining to HBD apply also to NHBD. Equally clearly, these considerations vary according to the legal system in force in any country concerned. In some states specific legislation is enacted to cover organ donation, in others general legal principles are applied. Ethical issues are more generic

and 'cross-border', and the international transplant community has been able to establish protocols for NHBD, often ahead of the more cumbersome national legislative processes. Some of the laws pertaining to organ donation in the UK are outdated and generic[44], however they are used to provide legal backing to the principles described below.

The purpose of this section is to outline the broad legal principles which are generally accepted for organ transplantation, and then to use key specific examples of legislation and procedures that highlight issues particularly relevant to NHBD; particularly regarding the determination of death and the initiation of ISP.

General legal principles

The two universal principles are[45]:

(1) The dead donor rule; that a donor must be dead before the retrieval of organs and should not be killed by the act of retrieval itself. Death must be diagnosed by accepted medical standards and determined following legally prescribed procedures.

(2) There must be consent for organ donation, either explicit or presumed (according to local law)

To these universal conditions are often added:

(3) The rights of the next of kin should be respected

(4) Consent of the family may only be sought after the death of the patient.

(5) Conflict of interest between the best care for a patient and their potential as an organ donor should be avoided by having separate medical teams responsible for care of the patient before death and independent confirmation of death prior to the transplant team taking over the management of the donor. These criteria apply in the USA and the UK[46,47].

(6) Invasive organ preservation procedures should only be implemented after death has been determined and consent obtained.

The determination of death

Historically there were no problems regarding the diagnosis of death prior to organ transplantation, the criteria being used being the irreversible cessation of cardiac and respiratory function. These concepts are simple and generally accepted. However, when brain-stem death criteria were first proposed to allow the use of HBD organs for transplantation, these are less easily understood and explained, and hence have been tightly defined in the law pertaining to organ donation to provide the safeguards necessary for ethical practise and the maintenance of public trust and support. However, cardiac criteria have not been clearly defined in law, there previously having been no need for this. This has implications for NHBD, principally in setting a time period for the absence of cardiac and respiratory function which may safely be deemed to be irrevocable.

The University of Pittsburgh protocol[48,49] allows death to be diagnosed after 2 minutes of absence of respiratory and cardiac effort. This abuses the ambiguity in the definition of death on cardiac criteria, particularly stretching the point of irreversibility. This is because of the possibility of cardiac auto-resuscitation after only 2 minutes, and that of persistent neurological function[50]. This casts doubt on the definition of death in legal terms[45]. Also, however cleverly argued in semantic terms by the advocates of the Pittsburgh protocol, there is a serious risk attached to any attempt to bend the dead donor rule, as public opinion is volatile on the issue of organ donation[51, 52]. The Pittsburgh protocol has been infamously dubbed 'an ignoble form of cannabalism'[53]. The first international workshop on NHBD provided the opportunity for the transplant community

to come to a consensus on a variety of issues relevant to NHBD, recognising that 'public education and openness concerning NHBD are mandatory to keep public trust and to prevent backfiring on the HBD programs'. One of their key recommendations was that '....NHBD procedures should only be started 10 minutes after the cessation of cardiac massage and artificial ventilation to "ensure the dead-donor rule"'[54]. Leicester has adopted the 10 minute rule for all NHBD, clearly marking the transition from patient to donor.

In situ preservation

There has been considerable controversy relating to the commencement of *in situ preservation* (ISP) prior to obtaining consent of the next of kin[55]. It is necessary to commence ISP as soon as possible after the elapse of the 10 minutes described above, in order to limit the WIT. However for uncontrolled NHBD the next of kin are often not immediately available, their consent may be unavoidably delayed. Some institutions describe the continued use of artificial circulatory and respiratory support until consent can be obtained[56,57], as under most NHBD protocols ISP procedures require consent[58]. In the UK the situation is ambiguous and left to the discretion of the local coroner. In Newcastle the coroner permits ISP without consent, and the same used to be the case in Leicester until recently following a public consultation process through the local press. Currently consent is required *before* commencement of ISP.

In Spain, presumed consent 'opt-out' legislation allows for ISP without consent, justified by the argument that in the HBD scenario artificial ventilation and drugs are used to support potential donors organs prior to obtaining consent[59].

Perhaps the best clarified position in law occurs in the Netherlands, where since 1998 a clear distinction is made between organ procurement, requiring explicit consent, and ISP which may proceed without it. The advantage of having this policy set out in law

for the transplant team is clear from the discussion above. For the next of kin, ISP may allow time for the relatives to be contacted and their wishes regarding organ donation to be sought. As many relatives find organ donation a source of comfort in their bereavement, ISP may be considered to offer relatives an opportunity, rather than ignoring their wishes.

1.4 NHBD retrieval

The infrastructure

A retrieval programme for uncontrolled NHBD requires many elements to be in place. The local transplant service and the accident and emergency department need to agree referral protocols, procedures and appropriate allocation of resources. A dedicated area for in situ organ preservation in the A & E department is ideal. The transplant team on call need to be based close enough to the A & E department to respond to referral within minutes. The A & E department needs to be in or close to the hospital housing the transplant unit. There is a considerable workload involved in the establishment and maintenance of a NHBD programme[60].

The interval between referral and the arrival of the transplant team

The transplant team are detailed to be available in the A & E department within 15 minutes of a referral being made. After efforts at resuscitation have ceased, and the patient declared dead, then they may be considered as potential NHBD. Once the ten minute rule (see above) has been observed, cardiac and ventilatory support is resumed. In order to release A & E staff from this task a mechanical device may be used such as the

'Thumper'™ (Michigan Instruments, Grand Rapids, USA). This pneumatic device performs external chest compressions and ventilates the patient (figure 1.3).

In-situ organ preservation

Once the transplant team arrive, consent for donation has to be obtained from the next of kin, the coroner and duty pathologist need to give their permission, and a theatre for the retrieval needs to be prepared. Details of the patient history need to be confirmed, to ensure the kidneys will meet the criteria for NHBD, and the family may wish the opportunity to see the donor prior to retrieval. In order to minimise additional ischaemic damage to the kidneys during this time (see also chapter 3.1.1), in situ cooling of the kidneys is commenced immediately.

Intravascular cooling with a DBTL catheter

The groin is prepared and the femoral vessels exposed via a groin incision. The vessels are controlled, and the artery cannulated with a DBTL catheter[61,62] (figure 1.4(a)). The lower balloon is inflated with radio-opaque dye and the catheter withdrawn until it lodges at the aortic bifurcation, which it occludes. The upper balloon is then inflated with dye and the abdominal aorta is thus excluded (figure 1.4(b)). The femoral vein is cannulated to allow venous outflow and then cold kidney preservation solution is run into the abdominal aorta through the third lumen, which has multiple perforations between the two balloons, under a gravitational hydrostatic pressure of one metre. The position of the catheter is confirmed on plain abdominal X-ray (figure 1.4(c)), and if incorrect it may be re-positioned. The perfusate will perfuse all intra-abdominal viscera, including the kidneys, as well as the abdominal wall per the lumbar arteries. Technical problems with catheter placement usually relate to widespread arterial atheromatous

disease, not uncommon, given that many uncontrolled NHBD die of myocardial infarction.

Perfusates used for in situ cooling in this manner include HTK[62,63] in Newcastle and Maastricht, and HOC in Leicester[64]. Up to 15 litres of preservation solution may be used during this stage of retrieval, and therefore cost is an important determinant in the choice of solution, in addition to efficacy[62]. The ISP may run for up to 2 hours (figure 1.4(d)). This is the ISP technique used in Leicester.

Intraperitoneal organ cooling

In this approach, a chest drain and a foley catheter are introduced through a supra-umbilical incision[65], and the defect closed by inflation and withdrawal of the catheter balloon. Cold Ringer's lactate is infused continuously through the chest drain and drained by the catheter. This approach has been modified by Light et al, by incorporating a cooling coil immersed in an ice-alcohol mix and a coolant pump to create a closed intraperitoneal circulation. This increases the rate of cooling and is reported to reduce rates of DGF[66-68]. Intraperitoneal cooling has also been used in conjunction with intravascular cooling, and this is also reported to produce more rapid organ cooling[69-71].

Total body cooling

Femoro-femoral cardio-pulmonary bypass circuits have been used to achieve rapid total body cooling in the same manner in which this is achieved for aortic arch surgery[56, 57]. Perfusates used for this include saline-gelatine hydrolisate[57] and Ringers lactate-colloid combination with mannitol, heparin and bicarbonate[56,72].

By running water through the heat-exchange unit of the circuit at 4^0C, core temperatures of 15^0C are obtained rapidly and may be maintained for many hours if

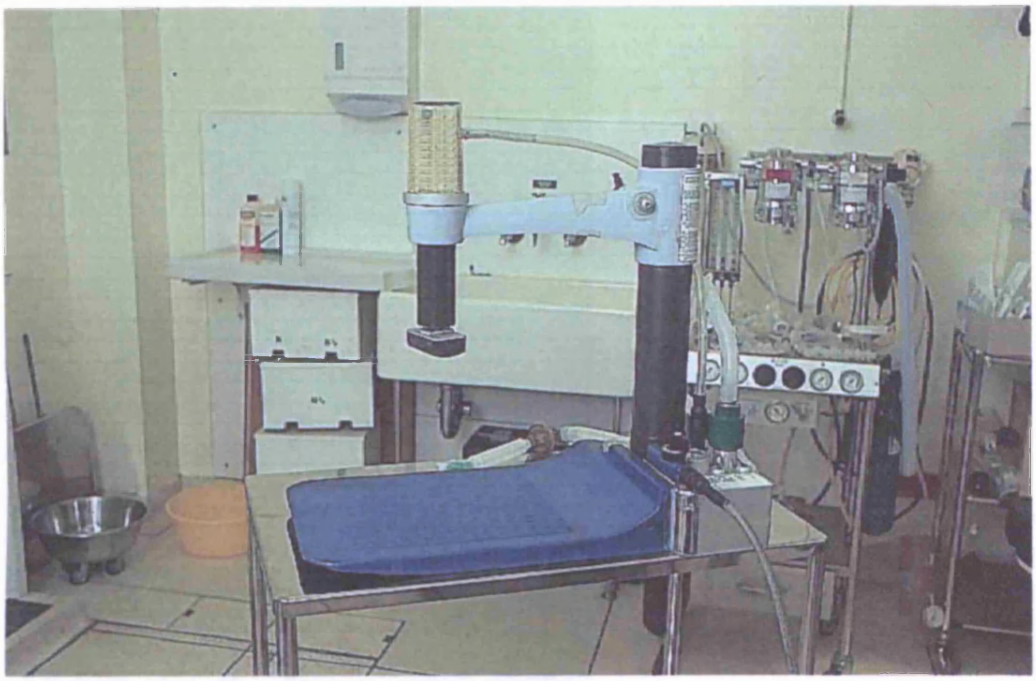


Figure 1.3: "Thumper" used to perform external cardiac massage and mechanical ventilation prior to ISP.

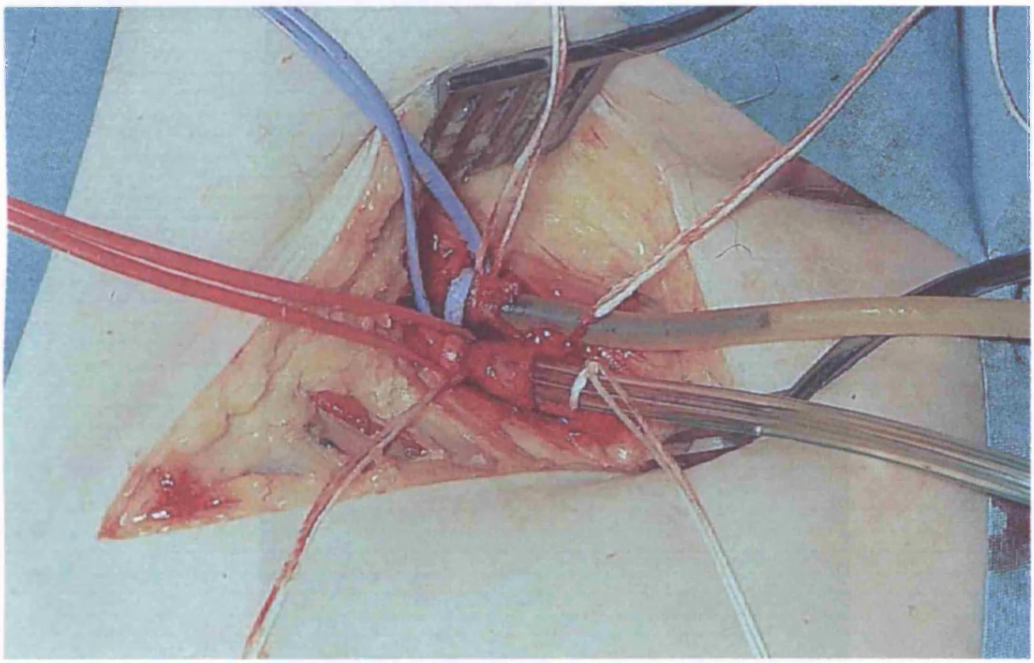
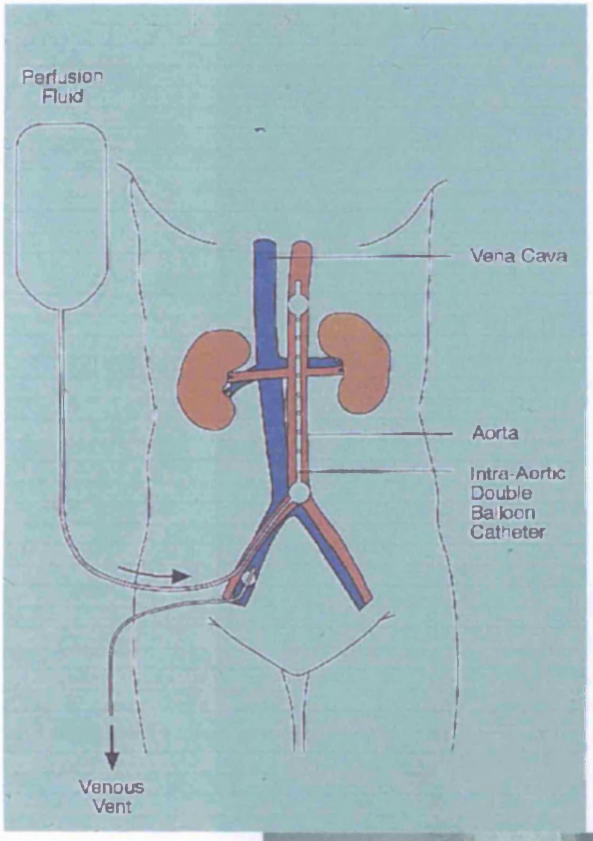


Figure 1.4 (a): The femoral vessels are dissected and controlled via a groin incision and the artery is cannulated with a DBTL catheter (the Vein with a Foley catheter).



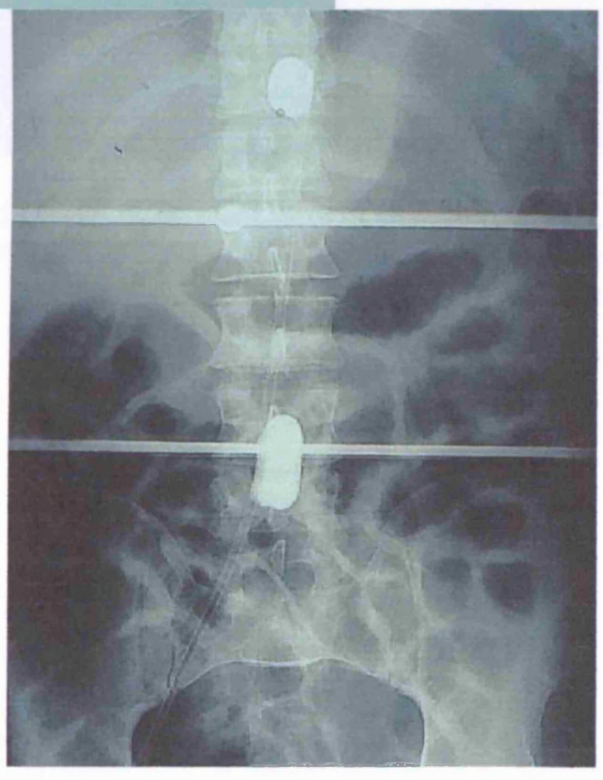


Figure 1.4 (b) and (c): A diagrammatic representation of the correct lie of the DBTL catheter in the abdominal aorta, with balloons inflated to exclude the abdominal aorta, including the renal arteries (b), and an X-ray confirming the correct position of a catheter during in situ perfusion.

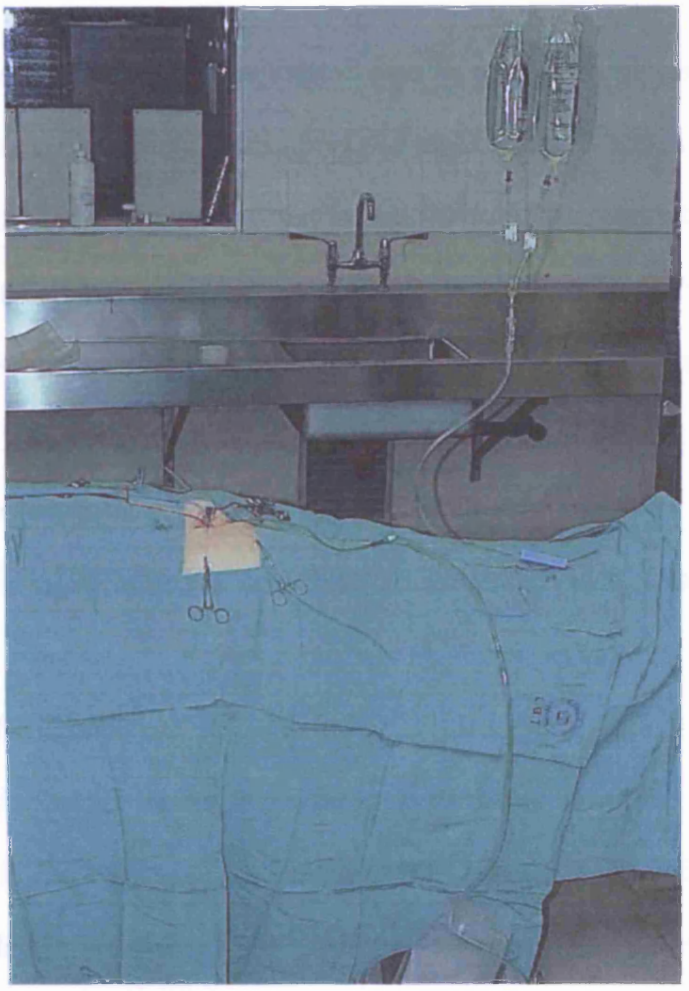


Figure 1.4(d): ISP in progress, up to 20 litres of cold preservation fluid may be used whilst awaiting consent for retrieval.

necessary[72]. In addition to the problems caused by atheromatous vessels seen with DBTL balloon placement, which may also affect this technique, disruption of large blood vessels secondary to major trauma may prevent adequate venous return required for the circuit[56]. The technique is complex and expensive compared with the other *in situ* cooling techniques describe and requires trained perfusionist personnel.

The retrieval operation

Retrieval is performed at laparotomy in exactly the same was as for HBD.

Controlled NHBD retrieval

Category IV donors are treated in the same way as categories I and II, as they also require rapid in situ cooling of the kidneys whilst preparing for retrieval. However, as category III donors are planned, everything is in place for the retrieval prior to withdrawing life support. As the donor is already in theatre, it is possible to proceed directly to in situ cooling at laparotomy. The remainder of the retrieval proceeds as for a HBD.

1.5 Theoretical potential and practical limitations of NHBD

Theoretical calculations

Numerous studies reviewing mortality statistics have shown for HBD that the theoretical numbers of donors available are far in excess of donation rates achieved[24,73-79]. The more optimistic forecasts project over 100 HBD per million population per year. There are considerable variations in the estimated potential, due to the inclusion and

exclusion criteria used for HBD and the methods used to extrapolate analyses to larger populations. The proportion of hospital in-patient deaths that meet brain-stem death criteria whilst supported on an ICU is approximately 6%[77,80]. Therefore the potential pool of NHBD may be much larger. Nathan ran records of hospital in-patient deaths through an algorithm on computer to determine the proportion that would meet selection criteria for NHBD. He estimated from this that in the USA 123 NHBD per million population were theoretically available[81]. Terasaki made similar calculations, concluding that NHBD could eliminate the waiting list for renal transplantation, and the need for LRD[82]. However a more detailed appraisal of the situation is of necessity more conservative.

Practical limitations

Daemen et al[83] conducted an elegant study into the potential supply of NHBD kidneys in Maastricht. In this a retrospective review of deceased patient's records was undertaken. In addition to assessing whether or not a patient met the inclusion/exclusion criteria for NHBD, their medical and logistical suitability was assessed and given scores. The medical score was based upon aspects of the donor's medical condition likely to impact on transplant outcome, the logistical score on the Maastricht category (see below) and the hospital unit the patient died in.

Cardio-respiratory support and in situ preservation are easier and more effective on ICUs and A & E departments than on general wards. They extrapolated their findings to the death rates in the other Dutch University hospitals only, recognising that NHBD transplants will need to be concentrated in specialist centres. Attrition due to known rates of refusal to consent by next of kin, and technical difficulties in establishing in situ preservation was built into the results. Taking only the highest scoring potential NHBDs,

they calculated there was potential for NHBD to provide an extra 9.4 kidneys per million of the population in 1994. In that year in Holland there were 26.4 kidneys transplanted per million population, therefore even this guarded and conservative estimate of the potential of NHBDs to expand the donor pool by 36%.

A further relevant finding of the study is that 70% of the most medically suitable donors were located in the most favourable environments (A & E, ICU, CCU). This suggests that concentrating efforts aimed at NHBD retrieval on such units may be the most appropriate use of resources.

Indeed, resources represent a further consideration in the use of NHBD. Maastricht has reported up to 40% of their renal transplantation programmed supplied by NHBD[84]. However, this does not necessarily result in a 40% rise in the rate of transplantation. The resources of the transplant unit in terms of finances and personnel need to be adequate to run a NHBD programme without detracting from the rate of HBD and LRD transplantation.

1.6 NHBD renal transplant results

Patient and graft survival

One year patient survival rates are reported to be between 75-100% and graft survival between 40-100%[60, 64, 65, 72, 84-109]. Increasingly 1 year graft survival rates of greater than 80% are being reported[86, 87, 89, 91, 94, 101, 103, 110-112], and although the better rates have tended to be amongst controlled NHBD transplants, uncontrolled NHBD can achieve similar results[86, 103, 110]. Some carefully matched studies have been performed to compare the results of NHBD with HBD[42, 85]. 57 kidney recipients from the Maastricht NHBD programme were compared with 114 HBD recipients, matched for a variety of

potentially confounding variables known to impact upon renal allograft survival. No differences were found in the 1 or 5 year graft survival rates in this study between the cohorts. The Leicester study used similar matching criteria, and compared 72 NHBD with 105 HBD, finding no differences between the Kaplan-Meier curves for graft survival on log rank analysis. A large study of 229 NHBD and 8,718 HBD renal transplants found 1 year graft survival rates of 83% and 86% respectively. The results of NHBD, HBD and LRD transplants in Leicester have also been compared[41], and the one and five year graft survival rates are statistically indistinguishable from one another, and exceed the standards set in the British Transplantation Society[113] and Morris report guidelines[114]. Series reporting poorer results for NHBD than for HBD may reflect the early part of a learning curve in new techniques, such as the selection of NHBD kidneys suitable for transplantation. For example, Newcastle report three distinct phases in their NHBD programme[63], the latter 2 phases using uncontrolled NHBD. The results their initial use of organs from uncontrolled NHBD were poor, with only 45% of patients achieving dialysis independence. However after the introduction of viability testing they subsequently improved their success rate to 92%.

Rates of PNF

The incidence of PNF amongst NHBD transplants is higher than for HBD, and over 5% in an overwhelming majority of series reported[42, 56, 64, 84-86, 90-98, 110]. Some series report a zero PNF rate for NHBD[100, 111, 115, 116], and surprisingly 2 of these include kidneys from uncontrolled NHBD[111, 116]. The aetiology of PNF varies according to donor type, with ischaemic cortical necrosis responsible for the majority in NHBD, and primary allograft thrombosis in HBD and LRD. Clearly the raised incidence of PNF is a serious

deterrent to the wider use of NHBD kidneys, and is the main drive behind efforts to develop a pre-operative viability test.

Rates of DGF

Whilst severe ischaemic injury will result in cortical necrosis and therefore PNF, relatively mild injury will cause ATN, the clinical correlate in the transplant recipient being DGF. For this reason rates of DGF are much higher in NHBD than in HBD, reported at between 50-100%[42, 60, 64, 85, 86, 89, 90, 92, 98, 99, 102, 103, 110, 117] compared with 20-60%[42, 85, 92, 110, 118, 119] respectively. The DGF rate for controlled NHBD has been reported to be similar to that in HBD series[65] presumably reflecting the shorter WIT. Whilst the deleterious effects of PNF are obvious, it is worth considering the implications of DGF in more detail, particularly as they apply to NHBD. If a patient is prepared in advance for the possibility of a delay between surgery and dialysis independence then it is unlikely to cause any undue psychological trauma when it occurs. There is considerable controversy as to whether DGF is a poor prognostic sign for the long-term graft function in HBD recipients, with evidence to support[120, 121] and refute[122, 123] the supposition. It is certainly accepted that the occurrence of acute rejection is detrimental to long-term function[123, 124], and acute rejection is one cause of DGF[124]. One study finds that both acute rejection and DGF predict poor graft survival, and that they are particularly detrimental in combination[125]. However for NHBD, one study finds no difference in graft survival between kidneys exhibiting DGF and those with immediate function[126]. One possible interpretation of why DGF may be of differing significance for HBD and NHBD relates to the differing aetiologies, with ischaemic ATN responsible for DGF in NHBD, with drug toxicity and acute rejection responsible more commonly in HBD. An alternative explanation is suggested by animal models, in which brain death induces, through a

cytokine cascade, peripheral organ dysfunction[127, 128]. The clinical correlate of this may adversely affect transplant outcome from brainstem dead HBD, but not NHBD, thus counterbalancing the damage done by warm ischaemia. Although the explanation is not proven, there is certainly indirect evidence to support the notion that DGF does not imply poor long-term function for NHBD, as the long-term results of NHBD are as good as for HBD[42, 85].

Rates of acute rejection

In all but one NHBD series that have compared results with HBD, no differences have been found in rates of acute rejection[42, 85, 90, 93, 94, 116], despite the theoretical expectation that rates may be higher, ie that MHC class II expression is increased by ischaemia[129]. Cho et al used[130] the UNOS database to compare large numbers of HBD and NHBD renal allograft recipients, and found a significantly higher rejection rate in the NHBD (19% versus 14%), however there were many potentially confounding variables in this study. For example, as NHBD kidneys are often used locally within the retrieval centre to reduce additional ischaemic injury from a prolonged cold time, they are therefore often not as well matched as those those transported in organ sharing schemes according to tissue type. In a case control analysis[42] the HLA-DR mismatch was greater in the NHBD group than the HBD controls, but the acute rejection rates were lower in NHBD recipients. However neither difference was significant.

Rates of chronic allograft nephropathy

The level of tissue inhibitor of metalloproteinase 1, which is pro-fibrotic, has been found to be elevated in routine biopsies of renal cortex taken one week after renal transplantation in NHBD compared to HBD[131]. This raises the possibility that warm

ischaemia induces pro-fibrotic genes, which may in turn increase allograft fibrosis and therefore increase rates of chronic allograft nephropathy. However the same study found no differences in the levels of other pro-fibrotic gene expression, and the significance of the finding is unclear in the context of reported rates of chronic allograft nephropathy of 8-25%[59, 89, 93, 111, 130], which do not exceed those reported for HBD. The study by Schlumpf et al[89] is perhaps most instructive as recipients were matched for donor age and sex and time since transplantation.

Renal function

Some series report that normal serum creatinine values are attained post-transplantation in patients receiving NHBD kidneys[87, 99, 102, 112, 115], whereas others demonstrate higher mean serum creatinines than would be expected for HBD at a variety of different time points post-transplant[62, 63]. In the paired study comparing the results of HBD with NHBD in Leicester the mean serum creatinines over the first five years post transplant were marginally higher in NHBD than for HBD[42]. Uncontrolled NHBD tend to have poorer post-transplant renal function as estimated by serum creatinine compared with controlled donors[132].

Quality of Life

The quality of life of renal transplant recipients is proven in a multitude of studies to be superior to that on dialysis[2-10]. However since the renal function is marginally poorer for NHBD than HBD recipients, it was necessary to examine whether quality of life was also improved by a NHBD transplant. The KDQOL-SF questionnaire was used to compare quality of life of NHBD, HBD and LRD with haemodialysis patients on the transplant waiting list as controls[133]. This demonstrated that NHBD recipients

experienced a quality of life both better than the waiting list controls, and no worse than for the other organ sources.

Health care economics

NHBD transplantation incurs some costs that are unique, such as the equipment and consumables needed for ISP[61], and also some which are more prevalent with NHBD than other sources, such as dialysis post-transplant. However other costs of HBD and LRD are not incurred, including the cost of ITU support of donors in the former, and the operative costs and after care for the latter. It has been established that HBD[11] and NHBD[134] transplants are cheaper than dialysis. A direct comparison between the cost of NHBD and other donor types has not been performed. It is a moot point whether this should be measured against HBD cadaveric transplants or the cost of dialysis, as NHBD kidneys are performed in addition to, rather than as replacements for, HBD organs.

1.7 Challenges for NHBD transplantation

Viability assessment

A major hurdle to the broader acceptance of the use of NHBD organs for transplantation is the concern that the warm ischaemic injury may have been severe enough to cause cortical necrosis, resulting in primary non-function. This is born out by the higher rates of PNF amongst NHBD recipients than for HBD (above), despite the otherwise excellent results of NHBD transplants, comparable with their HBD counterparts. In the case of uncontrolled donors, it is particularly difficult to be sure as to the precise duration of WIT, and the effectiveness of cardiac and ventilatory support. It is

also necessary to consider the 'pre-mortem' state of the donor kidneys, including donor age, hypertension and diabetes in determining the suitability of organs for transplantation, as these influence transplant outcome[135]. Therefore it is difficult to predict from (estimated) warm ischaemic time alone whether a NHBD kidney may recover function following a warm ischaemic insult. A reliable viability test would help overcome widespread resistance to the use of NHBD organs[136]. The development and current state of renal viability assessment are described in detail in chapter 3.

Preservation

One of the problems with organ sharing schemes is that they tend to increase the cold ischaemic time, which is an adverse factor to transplant outcome, offsetting the benefits afforded by improved matching. There is indirect evidence from the machine perfusion of porcine kidneys that the effects of warm and cold ischaemia are additive[137]. Kidneys subjected to significant WIT tolerate prolonged CIT poorly[138, 139]. Therefore the broader use of NHBD is limited by the requirement to minimise CIT and so the total ischaemic damage. For this reason NHBD kidneys tend to be used locally. If preservation techniques were evolved to reduce the detrimental effects of CIT, or even to reverse some of the warm ischaemic injury then it would be possible for NHBD to be used in wider geographical organ sharing schemes, thus improving the immunological matching to recipients. The evidence suggesting that hypothermic machine perfusion preservation reduces the impact of ischaemic damage for NHBD, and that normothermic perfusion preservation may reverse it is considered in chapter 2.

1.8 Summary

In transplant centres who have established NHBD renal transplant programmes, excellent results can be achieved, often equivalent to those achieved by HBD and LRD. This holds true when the results are examined from a number of perspectives, including patient and allograft survival, recipient quality of life, and economic savings. Also in these centres NHBD make up a significant contribution to increasing the donor pool, in the face of declining availability of HBD organs. If NHBD programmes were adopted by the broader transplant community, then a real impact could be made on the waiting list for renal transplantation.

There is a considerable workload involved in establishing a NHBD programme and inevitably a learning curve associated with learning the techniques specific to reducing WIT in NHBD kidneys.

Concerns over the viability of NHBD organs are a major bar to general acceptance of NHBD transplants. This may be addressed by the development of a reliable pre-transplant viability test.

Chapter 2

Renal Preservation

Contents **Page number**

"If one could substitute for the heart a kind of injection...of arterial blood, either natural or artificially made, ...one would succeed easily in maintaining alive indefinitely any part of the body whatsoever."

Le Gallois, 1812[140].

2.1 Introduction

In order to consider the requirements for organ preservation, and thence the strategies by which they may be best met, it is helpful first to review cellular metabolism and the regulation of cellular homeostasis, and how these are disrupted by ischaemia. The subsequent additional injury of reperfusion is also described, along with the amelioration of these effects by hypothermia.

From the basis, the principles of organ preservation are explained, along with their limitations and drawbacks. Then issues of preservation that relate to NHBD are considered specifically.

Finally the potential for future strategies to improve organ preservation are discussed, striving for the 'holy grail' described by Gallois.

2.2 Pathophysiology of ischaemia, hypothermia and reperfusion

Cellular homeostasis during normal metabolism

There is a tendency for sodium ions to enter a cell down the concentration gradient, taking chloride ions with them and also water into the cell. Water also enters cells passively by the Donnan effect. These effects would tend to cause cellular swelling.

Potassium and magnesium ions leave down concentration gradients. Calcium ions diffuse into cells, also down a concentration gradient.

These effects are countered by active, ATP-dependent processes. The main energy consuming process in a non-contractile cell is the membrane NA^+/K^+/ATPase, which pumps 3 Na^+ ions out of a cell in exchange for 2 K^+ ions in the other direction, for each ATP consumed. This not only helps to maintain intracellular ionic concentration homeostasis, but also maintains the negative membrane potential, which resists the influx of chloride ions. An Na^+/Ca^{++}/ATPase exchanges sodium and calcium ions across the cell membrane against their concentration gradients, helping to maintain homeostasis of these ions.

These energy dependent processes convert ATP into ADP and P_i, which are recycled to ATP, largely in mitochondria and by oxidative phosphorylation[141].

The effects of ischaemia

Ischaemia is defined as the *'inadequate flow of blood to a part of the body, caused by constriction or blockage of the blood vessels supplying it'*. The initial effects of ischaemia are due principally to the lack of oxygen, as most cells have some cytoplasmic energy reserves. Ischaemic effects are therefore first manifest as a result of the interruption of oxidative phosphorylation and consequent ATP depletion[142] (figure 2.1(a)). This has two direct effects, firstly a reduction in the rate of ATP dependent processes, and secondly, the generation of a small amount of ATP by anaerobic metabolism causes intracellular acidosis. The acidosis acts to further reduce the activity of the enzymes responsible for cellular homeostasis.

The direct effect of the inhibition of these enzymes can be deduced from the above. The inactivation of the Na^+/Ca^{++}/ATPase causes loss of the cellular membrane potential, and therefore allows greater influx of Cl^- ions, and the influx of Na^+ ions is not countered. K^+ and Mg^+ leak out of the cell. Water enters the cells as described above, causing intracellular swelling and tissue oedema. Calcium diffuses into the cytoplasm from the extracellular fluid unchecked by the Na^+/Ca^{++}/ATPase and from intracellular stores[142-146]. This is exacerbated by the opening of voltage gated calcium channels caused by membrane depolarisation.

The failure to synthesise ATP results in the accumulation of ADP. 2 ADP are converted to 1 ATP and 1 AMP, the ATP is then available for energy consumption. The AMP is converted by xanthine dehydrogenase to adenosine which diffuses out of the cell. Adenosine is there further broken down through inosine to hypoxanthine[142, 143, 147]. Hypoxanthine is normally converted to xanthine and thence uric acid. This causes a depletion of the substrates necessary for the generation of ATP, even if adequate perfusion was restored. This depletion has been linked to the irreversibility of ischaemic damage, and has been the focus of viability assessment research (see chapter 3).

The deleterious effects of these changes are numerous. The influx of water causes cellular swelling which in the kidney causes renal tubular obstruction and further impairs renal perfusion by extrinsic compression of the vasculature[148]. The increase in cytoplasmic calcium concentration has the following results[149]:

1) activation of phospholipases which damage the cell membrane, further increasing it's permeability to water and ions.

2) activation of lysosomal proteases[145].

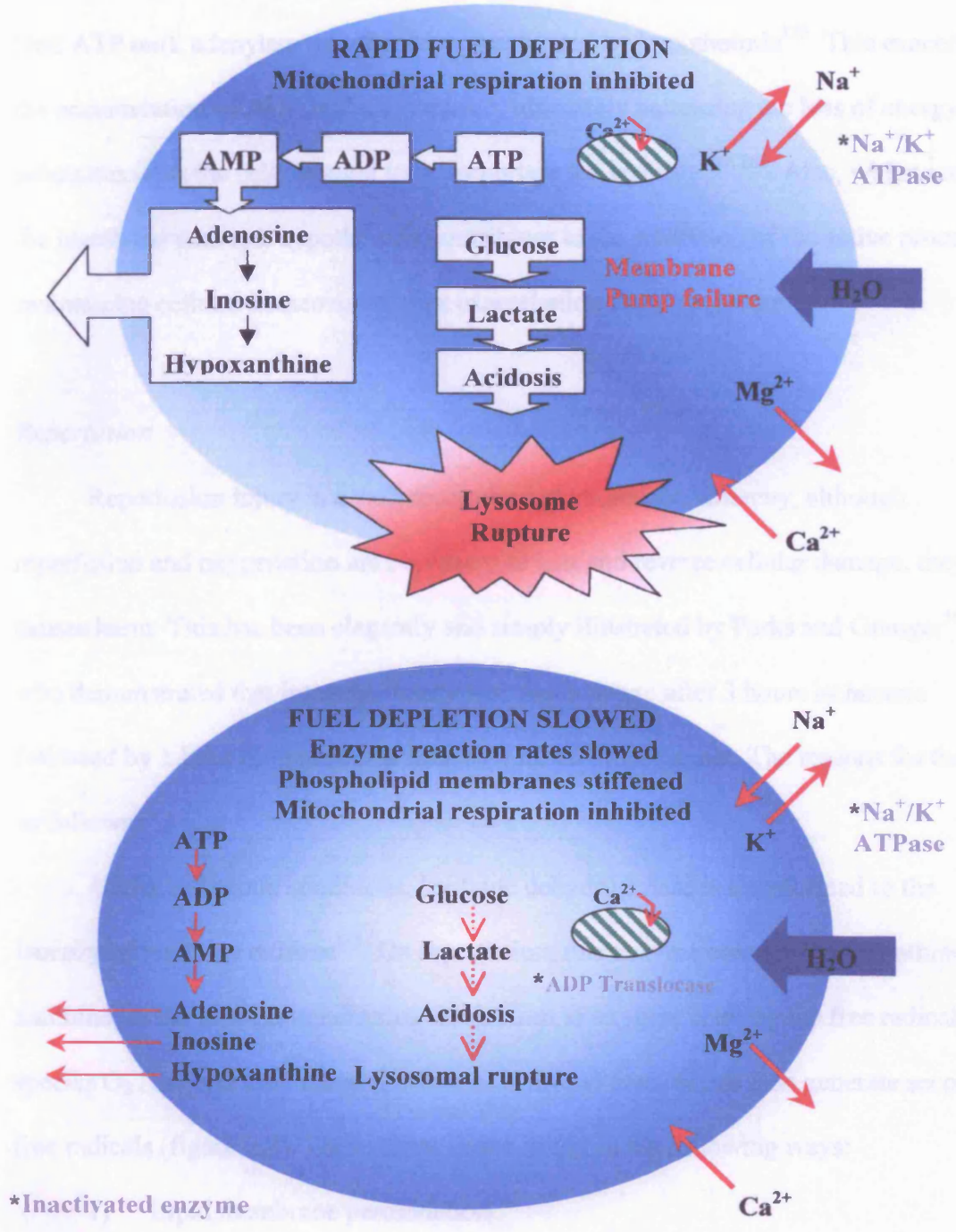
3) inflexibility and paralysis of the cytoskeleton.

4) cell membrane rigidity[150].

The further membrane permeability allows more water into the cell, which together with the intracellular activation of proteases may result in cytolysis. The rigidity of cells secondary to the cytoplasmic and membrane effects is of particular relevance to erythrocytes in the microvasculature of an ischaemic organ, as they need to deform to pass through capillaries, and so they get trapped when rigid, contributing to red cell sludging seen in ischaemia[145], further inhibiting perfusion, or reducing re-perfusion.

The effects of hypothermia

The principal effect of hypothermia in an ischaemic organ is to reduce the metabolic rate. The principal difference between warm and cold ischaemia being the rates at which cell injury occur (figure 2.1 (b)). This principle is employed in clinical spheres outside transplantation, including aortic arch surgery where profound hypothermia allows circulatory standstill with no cerebral perfusion for 30 minutes, with subsequent recovery of function[151]. Porcine experiments demonstrate that the limit for recovery of function after warm ischaemia in kidneys is around 120 minutes[152, 153], compared with up to 111 hours of CIT[154]. Cellular oxygen requirements fall exponentially with decreasing temperature[155]. This is due to the induction of conformational changes in enzymes altering their catalytic activity. Although this is different for each enzyme, as a rough rule of thumb, enzyme activity decreases 2-3 fold for every 10^0C below normothermic conditions[156,157]. Below 15^0C the Na^+/K^{++}/ATPase, responsible for 85% of renal tubular cell metabolism, ceases to function[146], and the metabolic demand is reduced by 90%[156]. There are also disadvantageous effects of hypothermia however. The cell membrane is less fluid, and more permeable to water[158], thus exacerbating the problem of cellular swelling. Also the enzyme responsible for transferring ADP into mitochondria



Figures 2.1 (a) and (b): The changes occurring in cellular metabolism with ischaemia (a), and how these are attenuated by hypothermia (b). *(After Marshall[142])* This pair of figures illustrates that the main protective effect of hypothermia is achieved by slowing enzyme dependent processes. Therefore the depletion of energy substrates and the activation of lysosomes are inhibited or slowed preventing early cytolysis.

(and ATP out), adenylate transferase, is inactivated by hypothermia[155]. This exacerbates the accumulation of ADP in the cytoplasm, ultimately increasing the loss of energy substrates from the cell, thought to be important for viability[159, 160]. Also, whilst reducing the metabolic demand, hypothermia contributes to the inhibition of the active processes maintaining cellular homeostasis, thus exacerbating those problems.

Reperfusion

Reperfusion injury is a well recognised phenomenon whereby, although reperfusion and oxygenation are necessary to halt and reverse cellular damage, they also causes harm. This has been elegantly and simply illustrated by Parks and Granger[161], who demonstrated that intestinal injury was more severe after 3 hours ischaemia followed by 1 hour of reperfusion than by 4 hours of ischaemia. The reasons for this are as follows.

Under ischaemic conditions, xanthine dehydrogenase is transformed to the isoenzyme xanthine oxidase[145]. On reperfusion, this enzyme converts hypoxanthine to xanthine, in the process transferring an electron to oxygen, creating the free radical species O_2^-, and via the Fenton reaction to hydroxyl ions, which then generate secondary free radicals (figure 2.2). These cause tissue injury in the following ways:

1) Lipid membrane peroxidation
2) Protein denaturation
3) DNA cross linkage and scission[143]

Reperfusion injury is greatly exacerbated by neutrophils[143] in the reperfusing blood, which are attracted to ischaemic tissue by released chemotactic agents, including leucotriene B4, oxygen free radicals and platelet activating factor[143], and generate free radicals as described above through the actions of xanthine oxidase[143].

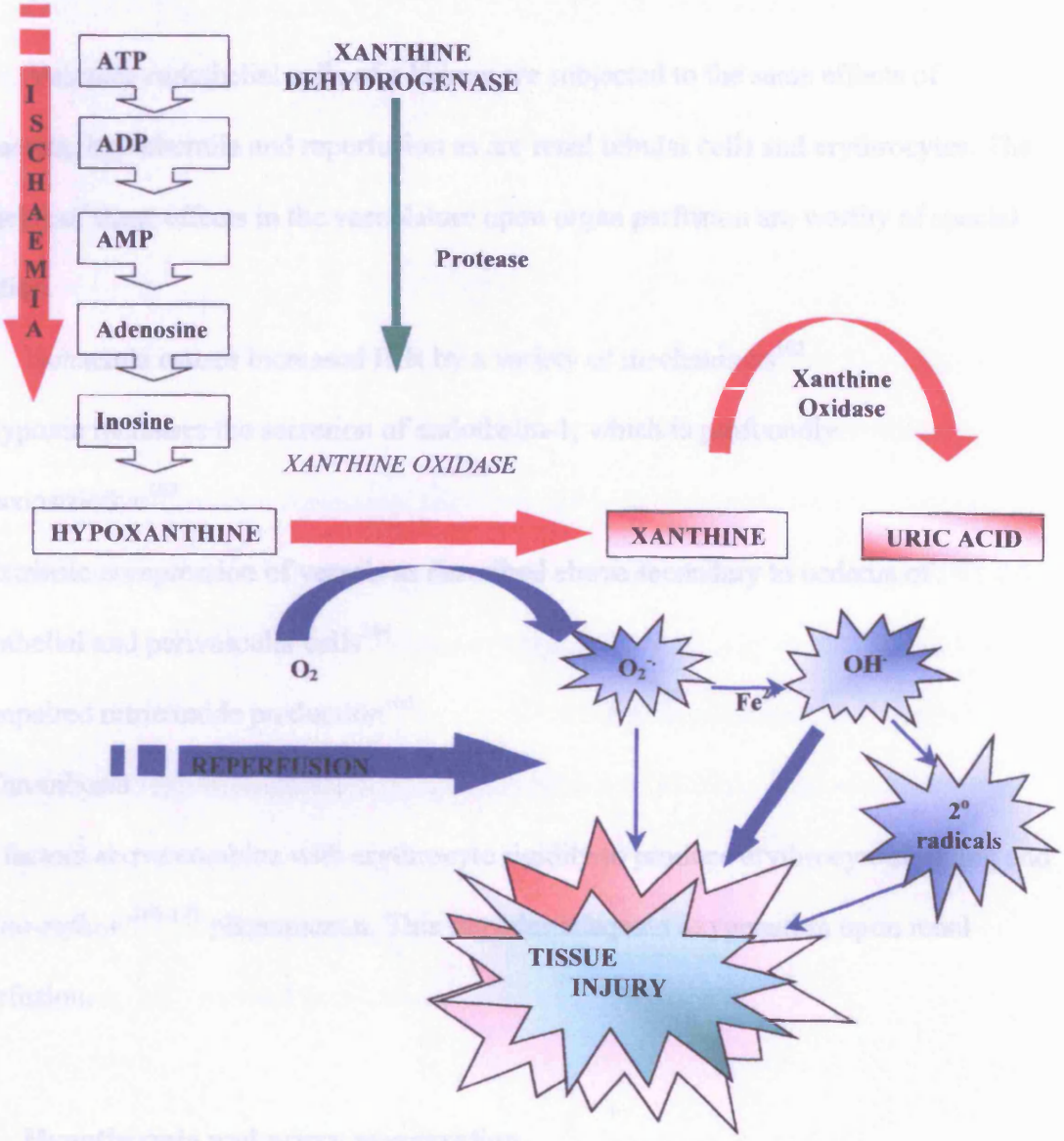


Figure 2.2: Schematic representation of the principal mechanisms of reperfusion injury. *(Grace [145])*

Renal vasculature under ischaemic conditions

Vascular endothelial cells of a kidney are subjected to the same effects of ischaemia, hypothermia and reperfusion as are renal tubular cells and erythrocytes. The sequelae of these effects in the vasculature upon organ perfusion are worthy of special mention.

Ischaemia causes increased IRR by a variety of mechanisms[162].

- Hypoxia increases the secretion of endothelin-1, which is profoundly vasoconstrictive[163]
- Extrinsic compression of vessels as described above secondary to oedema of endothelial and perivascular cells[164]
- Impaired nitric oxide production[165]
- Thrombosis

The factors above combine with erythrocyte rigidity to produce erythrocyte sludging and the *'no-reflow'*[166-171] phenomenon. This impedes adequate oxygenation upon renal reperfusion.

2.3 Hypothermia and organ preservation

As described above, hypothermia dramatically reduces the metabolic demand of an ischaemic organ, and historically hypothermia has been the basis of organ preservation. In 1956 it was found that cooling to $<26^0$C markedly reduced the damage done to canine kidneys by 2 hours cross clamping of the renal pedicle[172]. Calne found in 1963, again with canine kidneys, that simple immersion into ice-cold water preserved

recoverable function for up to 12 hours[173]. One consequence of cooling and reducing the metabolic activity of organs is that they inevitably cease to function under such conditions. This was first noted in 1937, during experiments reducing the temperature of kidneys during *ex vivo* perfusion with blood[174].

2.4 Hypothermic static storage

The problem of diffusion of ions down their concentrations gradient unopposed by active homeostasis mechanisms was first addressed in 1966, when a preservation solution high in potassium and magnesium and low in sodium and calcium was used. This reduced the fluxes of ions across the cellular membrane[175], compared with kidneys preserved with isotonic saline, essentially using a solution with intracellular rather than extracellular ionic composition[176]. Osmotic agents were also used to counter cellular oedema. This is the basis of solutions used for static hypothermic storage. Collins successfully transplanted canine kidneys subjected to 30 hours of hypothermic static storage using an intracellular type preservation solution, after an initial flush of the organs to clear the vasculature of blood[177].

2.5 Hypothermic pulsatile perfusion

An alternative approach to obviating the need to support even the reduced metabolic demands of hypothermic tissue, as above, is to provide for them. Belzer quickly followed up early work on hypothermic perfusion preservation, successfully transplanting canine kidneys after 72 hours of CIT[178], and then using the same techniques in clinical practice[179]. Perfusion of hypothermic organs has the potential advantages of

washing away toxic metabolic waste products, and chemotactic agents that may recruit neutrophils to worsen subsequent reperfusion injury. It may also wash out erythrocytes sludged in the microvasculature, and reverse the intense vasospasm of ischaemia[162]. It may also deliver oxygen and nutrients to provide for the reduced metabolic demands of hypothermic organs[180, 181], and improve the integrity of the microcirculation compared with cold storage[182]. As the metabolic demands are provided for, the cell can undertake some homeostatic activity, and so an extracellular type of fluid can be used as a perfusate.

A theoretical disadvantage of pulsatile perfusion is that the perfusate may wash away the adenosine and other energy substrates that diffuse out of ischaemic cells, further depleting these and therefore the capacity for ATP regeneration post-reperfusion (see chapter 3).

Damage to the vascular endothelium depends on the perfusion pressure, and careful studies have determined that in hypothermic conditions physiological perfusion pressures are injurious. A maximum systolic perfusion pressure of 60mmHg seems to be compatible with minimising endothelial damage, whilst most adequately perfusing an organ[183-187].

Pulsatile perfusion also allows measurement of the intra-renal vascular resistance, which varies with ischaemic insult, and therefore has been proposed as a predictor of organ viability in the NHBD setting (see chapter 3).

2.6 Preservation solutions – hypothermic static storage

The eponymous Collins solution was low in sodium and high in potassium to reduce the ion fluxes associated with ischaemia. In order to prevent mass movement of

water, glucose was added at a concentration of 140mmol/l, making the solution isosmotic with intracellular fluid[177]. The original solution was high in Magnesium, also to reduce ion flux, but as the solution is phosphate buffered, and the concentrations of magnesium and phosphate in the solution exceeded their solubility product, resulting in crystal deposition in the renal parenchyma. Although never proven to be harmful, a modification to the solution was made, removing the magnesium. The modified solution was known as EuroCollins[188].

Hydroxytryptophan ketoglutarate (HTK) solution, originally developed for cardioplegic preservation by Bretschneider is also effective for renal CS preservation[189]. This is the IPS preservation solution of choice for NHBD kidneys in Maastricht[190] and HTK has the theoretical advantage of reducing tissue acidification during ischaemia, due to the buffering capacity of histidine hydrochloride[191].

Marshall devised another cold storage solution in which the oncotic pressure to reduce cellular oedema was provided by a hyper-osmolar solution of the impermeant citrate ion[192]. This has the theoretical advantage of improving pH homeostasis[193]. Hyper-osmolar citrate solution is used for ISP and CS preservation in the Leicester NHBD programme[42].

Ironically the current 'gold standard' in cold storage solutions was developed by the most pre-eminent advocate of perfusion preservation of kidneys. Belzer designed University of Wisconsin solution, with the recipe designed specifically to support various cellular requirements[141]. High concentrations of adenosine and phosphate reduce leaching of energy substrates during preservation[194,195]. The impermeant anion gluconate was used initially, the high molecular weight compared with chloride reducing translocation into the cell when the membrane potential is lost. Subsequently, this beneficial effect was increased by using lactobionate rather than gluconate, as this has an

even higher molecular weight, and a study in liver preservation suggested that the impermeability of the anion is the most important protective factor in UW solution[196]. Glutathione is also added, as a reducing agent of proven benefit in renal[197] and hepatic[198] preservation. Other constituents of UW are of unproven benefit in preservation, but theoretically advantageous, for example the addition of steroids as a membrane stabiliser[199-201]. Experimental data from a rodent model of renal preservation suggests that the hydroxyethyl starch, insulin and dexamethasone in UW are unlikely to be important, whilst confirming the beneficial effects of anti-oxidants glutathione and allopurinol and energy substrate, adenosine[202, 203].

UW seems to allow longer preservation of kidneys and other organs, with lesser degrees of post-transplant renal dysfunction than Euro-Collins[204, 205], HTK[204], or Marshall's solution[206]. UW has also been shown to be superior to Euro-Collins for NHBD kidneys[207]. UW is considerably more expensive than the other solutions. However as the results are reported to be better, particularly in reducing delayed graft function and therefore post-operative dialysis, the overall costs of using UW in renal transplantation have been calculated to be cheaper than Euro-Collins[208].

2.7 Preservation solutions – hypothermic perfusion

In addition to the extracellular ionic requirement for perfusion preservation solutions, a perfusate needs oncotic pressure to counter the hydrostatic force of perfusion, which unopposed would increase tissue oedema, especially as ischaemic endothelium is 'leaky'. The oncotic agent used by Belzer and colleagues in early experimental models was plasma-derived protein. Although successful 72 hour preservation was achieved, aggregates of lipoproteins formed during perfusion, and

eventually obstructed the microvasculature[209]. The lipoprotein content of plasma can be removed by ultrafiltration after cryoprecipitation, and cryoprecipitated plasma CPP became the standard perfusion preservation solution, with excellent results in dogs initially[178], and subsequently in clinical transplantation[179]. However the use of human plasma as a perfusate entailed the problems of batch to batch variability, limited shelf life, complex and expensive preparation and the risk of disease transmission[210].

A variety of different solutions have been tried to reduce these problems and improve upon the efficacy of preservation compared with CPP. These have included lipoprotein removal by silica gel extraction of a plasma based solution[211], Ringer's solution with human albumin as an oncotic agent[212], and the use of plasma protein fraction[210]. However no improvement upon CPP based perfusate occurred[213] until the development of a perfusate with a synthetic oncotic component. Belzer's machine perfusion solution (MPS) has an ionic composition similar to extracellular fluid and uses hydroxyethyl starch (HES) to provide oncotic pressure[214]. A carefully controlled animal study was used to approach optimising some of the other constituents of MPS[215]. This showed that contrary to the situation in CS, Na^+ a better cation to use than K^+, and that Gluconate outperforms lactobionate.

MPS has advantages in longer shelf life and no risk of infection. Also MPS permitted longer preservation of kidneys, for up to 7 days , and was associated clinically with lower rates of DGF than CPP[216-219] and albumin-gluconate based solution[220]. One possible explanation for superiority of HES over CPP and albumin based solutions is that albumin is denatured during prolonged perfusion preservation, and is then toxic to renal tubules[221]. Albumin is the major protein component of CPP, and all other plasma derived perfusates. Whatever the reason MPS remains the gold standard hypothermic preservation solution for renal transplantation.

2.8 Comparison of the Results of Static and Pulsatile Hypothermic Perfusion

The most important outcome measures of renal transplantation are patient and graft survival, and rates of PNF. These are compared between CS and CP below. DGF and AR may affect graft survival, and so the influence of preservation method on the rates of these complications of transplantation is also considered. Finally the levels of renal function achieved after transplantation are compared. Specific attention is drawn to studies pertaining to NHBD renal transplantation.

Patient and graft survival

There are reassuringly no differences in recipient survival between CS and CP[222, 223]. Several studies have demonstrated superior graft survival for organs preserved by CP compared with CS[224-226]. A paired study of 54 kidneys with one of each pair preserved by each method found that when DGF occurred post-transplantation, it was of graver prognostic significance for allograft survival in recipients of CS than CP preserved kidneys, with one year graft survival rates of 74% and 89% respectively[224].

In contrast, some large multi-centre studies, although not controlled, found one year graft survival to be worse in CP preserved kidneys than CS[227-229]. The majority of studies however have shown no difference between CS and CP in graft survival[222, 223, 230-235]. Whilst some of these studies date from early days of renal transplantation, others are more recent. The latter encompass the modern era of preservation solutions, and other advances that effect graft survival, such as the introduction of cyclosporin to immuno-

suppression regimens. They are therefore more reliably relevant to kidney transplantation today. Although most of these studies have been retrospective examination of large databases, a small prospective study in 1994 or 18 pairs of kidneys was unable to distinguish between CS and CP in terms of one year graft survival[233]. The only study of patient and graft survival specifically relating to NHBD is a small (n=11 in each arm) paired study of controlled NHBD, where patient survival was 100% in each arm[236]. The graft survival was not significantly different between groups, and although there was a tendency towards better survival in the CP group, the numbers are too small to draw reliable conclusions.

Rates of PNF

There is very little in the literature comparing PNF rates between CP and CS perfused kidneys and the two recent reports in the literature are only small studies[231, 237]. The more recent of these in 1991 reports rates of 14% and 9% respectively[231]. Whilst appearing to favour CP in this regard, the results are not significant as there were only 11 kidneys in the CP group. Other concerns in interpreting this study are that the MP perfusate and parameters are not defined, and two different CS solutions are used. Finally, the rates of PNF are higher than those generally reported or accepted[114]. Although smaller, the study reported by Merion et al in 1990 is a tighter, better designed[237]. For 18 pairs of kidneys, the rate of PNF was 2% for those preserved by CP and 6% for CS. This difference is not significant, and the rates are consistent with the majority of the literature on PNF rates (see chapter 1). In short, it would be fair to conclude that no difference has been demonstrated in rates of PNF between CP and CS, however, the possibility of type II error remains due to the small numbers studied. Once

again, for NHBD there is very little evidence on the influence of preservation method upon the rate of PNF. Just as for patient and graft survival a solitary small paired study of CS versus CP (n=13 in each arm) for controlled NHBD finds no difference in the rate of PNF[236]. Once again the possibility of type II error is difficult to discount.

Rates of DGF

Canine renal autotransplant models with contralateral nephrectomy have been used to compare early post-transplant function after CP and CS. After 24 hours preservation, 7/7 MP and 9/9 CS preserved kidneys were adequate to provide immediate life-supporting function[238]. Although the renal function was superior in the CP group during the first 2 post-operative weeks, there was no difference after one month. A similar study evaluating prolonged preservation found that after 96 hours preservation all 7 dogs with CS preserved kidneys died of renal failure, whereas 6 of 8 in the CP group survived, and were therefore immediately dialysis independent[216]. These studies suggest that, particularly for prolonged ischaemia times, CS results in higher rates of DGF than CP. The use of the auto-transplant model and young animals eliminates or controls for other factors implicated in the aetiology of DGF.

There are several well-constructed, clinical prospective paired studies comparing CP and CS. The majority of these demonstrate a tendency towards higher rates of DGF in CS compared with CP preservation, without achieving significance[224, 233, 237], and two studies in which this tendency reaches significance[222, 239]. The majority of larger database analyses also find MP to reduce rates of DGF compared with CS[231, 240-242], although a minority find no difference[234, 243]. Some studies are large enough to look at

the interaction between the preservation method and other DGF risk factors. For example, donor age over 55 and CS in combination are predictive of DGF[244].

The overwhelming weight of the available evidence is that CP does protect against the occurrence of DGF. As discussed in chapter 1, the prognostic significance of DGF for allograft survival is disputed.

The evidence is even stronger in studies specifically of NHBD kidneys, with one animal model[162] and all clinical reports[236, 245-249] finding unambiguously that DGF was more common in CS preserved kidneys compared to CP. By contrast, the significance of DGF is even more controversial[126].

Acute rejection

If pulsatile perfusion caused endothelial damage, then it may also predispose to acute rejection in perfusion preserved kidneys[250]. Theoretically, this may be compounded in NHBD, as expression of class II major histocompatability complex antigens is increased by ischaemia[129].

The only study to compare rejection rates between CP and CS preserved HBD kidneys, although small with only 38 kidneys in each arm, found no difference in the rates of acute rejection overall, or of steroid resistant or vascular rejection[251].

The only study comparing NHBD kidneys preserved by CP and by CS found no significant differences in AR rates, although the study was too small to draw firm conclusions[236].

Renal function

This has not been analysed for the very early post-transplant period, as this would add nothing to the evidence provided by the DGF rate comparison, reflecting the same phenomenon, the presence of acute tubular necrosis. Rather, the function of 'established' renal transplants is considered, reflecting any irreversible damage suffered during preservation. The only study to compare established renal function between preservation modalities[251] found at 1 and 2 years post-transplantation mean serum creatinine levels were higher in the CS arm than in the CP kidneys.

For NHBD one study found that the best serum creatinine achieved by CP and CS preserved kidneys was indistinguishable[236]. There was no mention of time post-transplantation in this study. There are no other reports detailing renal function according to preservation method.

A caveat to the comparisons made between CP and CS

The main problem associated with interpreting the studies above is that there are no standardised or generally accepted protocols for either CS or CP[246], and this is particularly important for the latter. There is clearly a need for large-scale multi-centre trials of CS versus CP, using protocols and solutions that are considered optimal on the basis of animal experiments and the clinical studies carried out so far. Until the data from such trials becomes available, the comparisons between preservation modality must be made with caution. Daemen et al[245] compared CS with UW and CP with Belzer's MPS, and this probably represents optimisation of both methods. Similarly Gage et al[242],

Johnson et al[243], Kosieradzi et al[251] and Kwiatkowski et al[252], all compared at least some groups using CS with UW and CP with MPS.

2.9 Normothermic preservation

Normothermic or warm perfusion (WP) preservation is the antithesis of all that has been discussed above. The central tenet of hypothermic preservation techniques is reducing metabolic demand to delay and minimise ischaemic damage. WP relies by contrast in supplying enough oxygen and nutrients to meet tissue demand, and so theoretically prevent ischaemic damage, or even reverse that which may occurred prior to commencing WP. In addition, normothermia permits restoration of organ function *ex-vivo*, and therefore offers a potential method of viability assessment. This is reviewed extensively in chapter 3. This section is limited to consideration of the requisites for a WP solution, the different options available to meet them, and the results of recent WP experiments in terms of efficacy of preservation.

The requisites for warm perfusion

A WP solution needs to carry oxygen and nutrients, an oncotic pressure agent to counter the hydrostatic pressure of perfusion, just as in CP, and antibiotics, for such a nutritious solution under normothermic conditions makes for a perfect culture medium.

The solubility of oxygen in aqueous solution is very low, quite inadequate to support renal metabolism even at 25^0C[156], so causing damage during perfusion with an

acellular perfusate[253]. Therefore in order to support metabolism the perfusate needs a specific oxygen-carrying agent. There are two main classes of oxygen carrier available: haemoglobins and perfluorochemicals.

Haemoglobin based warm perfusates: autologous blood

The most obvious route for the delivery of haemoglobin containing perfusate to an organ is to use autologous blood, and this is usually readily available from a NHBD. Historically however, experiments using of autologous blood as a perfusate has been found to be deleterious to an organ[254-263]. The main reasons for this are that the pumps used for perfusion pressure generation are traumatic to erythrocytes, and microemboli from damaged cells clog the renal microvasculature and impair perfusion[260-263]. Even perfusion of cell free plasma degrades lipoproteins, causing their aggregation and obstruction of the vascular bed, just as for CP[209].

There are some recent encouraging reports of autologous blood based perfusate being used successfully for prolonged WP with good *ex vivo* function for kidneys and hearts[264, 265], with successful auto-transplantation of kidneys subsequently *(unpublished work)*. The major advance made in these experiments is the use of a left-ventricular assist device to drive perfusion, with a dramatic reduction in haemolysis in the *ex vivo* circuit as a result. Hassanein et al also dilute blood to a haematocrit of about 30% with a crystalloid solution based on Ringer's with mannitol added to reduce oedema, and a continuous infusion of nutrients, vitamins and minerals. The blood is filtered to remove leukocytes prior to organ perfusion in order to reduce the severity of reperfusion injury. This approach is similar to that reported by Telander in 1964, with a rare early report of successful blood based organ preservation of up to 7 hours with subsequent successful

transplantation in large animal models[266]. Imber et al also report encouraging findings using blood based perfusate for porcine liver preservation[267, 268], although they have yet to report successful transplantation back into pigs. This work on perfusing livers with blood has been taken a step further by Schon et al who transplanted ischaemically-injured livers back into pigs, having been preserved either by CS with UW solution, or by WP with a blood based perfusate[269]. All 6 of the WP animals survived, all the CS animals died.

Haemoglobin based warm perfusates: cell free haemoglobin

Cell free haemoglobin (CfHb) offers an alternative to blood based perfusates, and the micro-embolic problems associated with them. However in solution the $\alpha_2\beta_2$ tetramer dissociates to $\alpha\beta$ dimers. These are nephrotoxic[270, 271], even when purified[272-279], poisoning tubular cells after being filtered by the glomerulus[280]. This problem has been countered by the polymerisation or cross-linking of CfHb to prevent glomerular filtration[281-286], but even these preparations have been found to be slightly nephrotoxic, although one study found improved renal function in rat kidneys when perfused with polymerised haemoglobin compared with HES solution alone[287].

However CfHb behaves differently to Hb contained in erythrocytes in a number of other important respects. The 2,3 diphosphoglyceride in erythrocytes plays a role in reducing Hb O_2 affinity in the tissues to facilitate oxygen release. This effect is absent with CfHb preparations, so impairing oxygen delivery to the tissues[288]. Many CfHb preparations cause vasoconstriction[289], with two possible mechanisms having been proposed for this. Firstly, CfHb acts as a scavenger for NO, as even the macromolecular forms are able to pass through endothelial cell junctions and enter the interstitial space[290].

This would reduce the relaxing effect of NO on vascular tone, causing spasm. In addition, the presence of very high oxygen tensions in the afferent arterioles may cause constriction by autoregulatory mechanisms[288, 291].

Finally, and of particular importance when considering the need in organ preservation to minimise reperfusion injury, the absence of erythrocyte regulatory enzymes permits the accumulation of methaemoglobin, which in addition to losing O_2 carrying capacity, increases the production of free radicals[288].

Perfluorochemcial based warm perfusates

Perfluorochemicals (PFC) are organic compounds analogous to hydrocarbons, with fluorine substituting for hydrogen[292]. The high energy of the C-F bond renders PFCs chemically inert to an extraordinary degree[292, 293], and so they do not react chemically with other elements in a perfusate. PFCs are immiscible in aqueous solution, and require emulsification with a surfactant. PFCs have a high solubility for O_2 and CO_2, which occupy spaces between PFC molecules in emulsion droplets[293, 294]. Unlike Barcroft's sigmoid dissociation curve of haemoglobin, oxygen association with PFCs approximate to Henry's law, with a linear relationship between O_2 saturation and concentration[294]. O_2 delivery to the tissues is facilitated by the lack of chemical bonding to the PFC and the large surface area of the emulsion droplets, the diameter of which is approximately 0.1μm (cf 8 μm for erythrocytes). An additional advantage of the small droplet size is that they may pass into vessels which for reasons of constriction or sludging may be impassable for erthrocytes[294]. Therefore the emulsion may oxygenate tissues that would remain ischaemic if reperfused by blood. PFCs used in blood substitute research are cleared by leucocyte phagocytosis and ultimately exhaled[295]. The phagocytosis of

perfluorochemicals by neutrophils tend to reduce their activity, as they become distended and foamy, and this may be of benefit in reducing the reperfusion injury[296]. The use of PFCs has even been reported to improve the results of xenotransplantation, presumably by the same mechanism[297].

Oncotic pressure

The advantages of HES over albumin for the provision of oncotic pressure in CP are described above. However in WP a protein oncotic agent, or more specifically albumin is necessary not only as an oncotic agent, but also as a carrier with binding sites for nutrients and drugs[298-300]. Studies involving CP and WP have used HES in the MP perfusate, and albumin for WP[301]. Albumin has been used as an oncotic agent by Brasile et al in their work on the WP of kidneys[153, 302-309].

Aqueous solution

The aqueous phase of the WP perfusate needs to contain the nutrients and any other factors necessary for organ support in solution. As mentioned above, Hassanein et al used a relatively simple crystalloid solution and infused nutrients and other factors as required[264]. Others have suggested using a tissue culture like fluid, on the basis that a fluid designed to support cell culture is a good starting point for whole organ support[310]. This ideas has been taken up recently by Brasile et al, who have used a variety of different oxygen carriers for WP of kidneys, emulsified with a tissue culture like medium containing nutrients, vitamins, energy substrates, antioxidants and growth factors amongst other constituents[153, 307].

2.12 Results of normothermic perfusion

Initial experiments with WP of kidneys used blood or blood based perfusates, as blood was the only available oxygen carrier. In 1903 a perfusion system was devised oxygenating blood with air, however poor renal blood flows were obtained, presumably due to high renal vascular resistance[254]. In 1914 an *ex vivo* circuit for renal perfusion was devised using a lung to oxygenate the blood, and to remove presumed vasoconstrictive materials. This resulted in normal renal blood flows, but the kidney deteriorated over a few hours of perfusion[255]. Subsequent studies persisting with 'pump-lung-kidney' or 'pump-oxygenator-kidney' models over subsequent decades similarly resulted in a gradual decline of organ condition, demonstrated by gross appearance, increasing resistance to flow and declining *ex vivo* function[256-263]. Subsequent attempts at transplantation of kidneys preserved in these experiments were unsuccessful[311, 312], until the work of Telander as described above, with his careful preparation of a blood-based perfusate. These kidneys were allowed three weeks to recover from presumed acute tubular necrosis, then their functional capacity was assessed by delayed contra-lateral nephrectomy.

Kootstra et al contrived an alternative to the problem of blood perfusion preservation, using short bursts of warm perfusion with the circulation of an anaesthetised dog intermittently throughout a prolonged period of renal preservation by cold storage[313], prior to transplantation. In these experiments, viability could be considerably prolonged when compared with cold storage alone. Although not a practical solution amenable to clinical application in transplantation, these experiments elegantly

demonstrated the principle that warm perfusion may resuscitate organs *ex-vivo*, provided the correct environment can be created.

As described above, recognition of the role of cellular and lipoprotein degradation during perfusion played in renal deterioration *ex vivo* led to the use of blood free perfusates. Although it proved possible to prevent the rise in perfusion pressure and tissue oedema, organ condition deteriorated throughout perfusion on functional and histological assessment and when transplanted were found to be non-viable. This is unsurprising in the absence of oxygen carriers in the solution.

Stubenitski et al have successfully used 3 hour WP with a polymerised CfHb dissolved in a tissue-culture like medium to improve post-transplant function of kidneys subjected to a combination of warm and cold ischaemic insult in a canine model[307]. The post-transplant renal function was better than controls transplanted with the same combination of WIT and CIT, but without WP. The long-term effect on renal function is not presented, but this work supports the earlier findings by Kootstra that WP may resuscitate ischaemically-damaged kidneys.

Early work using the alternative class of oxygen carriers, PFC, supported the theory that oxygen delivery and renal metabolism may be better supported by the use of PFC emulsions than by solutions without oxygen carriers[314]. Histological evidence of deteriorating function was evident however on perfusion for more than a few hours[315].

Renal viability did not appear to be improved by the addition of PFC in an experiment perfusing kidneys hypothermically at 10^0C, in fact, the PFC free perfusates (plasma only) proved better at restoring adenine nucleotide content to kidneys in one study[316].

Although it proved possible to stabilise perfusion parameters for longer periods of perfusion using PFC containing perfusates (12 hours)[317], these experiments were not backed up by subsequent renal transplantation as a test of viability.

Perfusion with PFC containing perfusates has been extended to 18 hours, but the organs were not viable after this process, with significant weight gain indicating oedema[318].

Early support for the notion that effective normothermic perfusion with PFC may resuscitate ischaemically damaged tissues came from a rabbit isolated kidney perfusion model, in which the ATP levels in the tissue were depleted by 2 minutes WIT, but largely restored by 2 hours of PFC perfusion[319].

The specific PFC used to augment a perfusate may also be important. In a canine autotransplant model comparing perfusion preservation with no PFC, and with 2 different PFC emulsions in plasma based perfusate, one PFC, FC-43 (47) improved outcome, with 5 of 5 animals surviving compared with only 3 of 11 surviving using FX-80, which was worse than the control group, in which 5 of 8 survived[320].

Brasile et al[153, 302-309] have used PFC supplemented solutions for WP, and found that they permit restoration of oxidative metabolism and renal function *ex-vivo*. Subsequent transplantation back into animals not only confirms viability, but suggests that WP resuscitated organs that have been subjected to warm ischaemia. Similar findings have been reported for warm *ex vivo* perfusion systems used to preserve extrarenal organs with substantial WIT[264, 267-269, 321].

2.13 Summary

Hypothermia reduces the rate at which ischaemic injury occurs, and has been the cornerstone of established organ preservation technologies. The two different approaches to hypothermic preservation, by static storage or by pulsatile perfusion have been explained and reviewed, and compared with regard to their efficacy for organ preservation. On balance CP would appear to offer slightly superior preservation and this would seem more clear-cut for NHBD than for HBD. This is consistent with the observation that kidneys subjected to WI injury are particularly poorly tolerant to CI[137-139]. The additional potential for viability assessment during CP is reviewed in chapter 3. WP preservation presents an as yet experimental alternative to hypothermic preservation, and although more complex than hypothermic techniques, some very encouraging reports suggest that appropriate WP may actually be able to partially reverse ischaemic damage, so called 'ex-vivo resuscitation'[153, 321].

Chapter 3

Viability Assessment

Contents **Page number**

3.1 Introduction

The primary purpose of renal viability assessment prior to transplantation is to reduce the incidence of primary non-function. Historically the interest in viability assessment of kidneys as reflected in the literature has been biphasic. Early in the era of renal transplantation there was much interest in the subject, due to two main factors. Firstly, all donors were non-heart-beating, and therefore all kidneys retrieved suffered significant periods of warm ischaemia. Secondly, perfusates for organ preservation were relatively primitive and consequently viability was further compromised during prolonged preservation. The introduction of brain-stem death legislation effected the virtual elimination of a warm ischaemic insult prior to retrieval from heart-beating cadaveric donors. Thus kidney viability could be reliably anticipated on the basis of donor history and investigations. Further damage was much reduced during the interval between retrieval and transplantation by improvements in preservation technology.

However in the drive to bridge the gap between supply and demand in renal transplantation, the option of harvesting kidneys from non-heart-beating donors (NHBD) has been revisited. Most, although not all, series of NHBD have reported higher incidences of primary non-function than HBD transplants. This is presumably secondary to severe irreversible ischaemia-reperfusion injury, since acute rejection and vascular complications are not reported to have higher incidences in NHBD transplants than their HBD counterparts. The pressing need to expand the donor pool without increasing PNF rates has stimulated a renewed interest in viability assessment. These areas have been covered in detail in chapters 1 and 2.

The principal areas of research into potential pre-transplantation viability assessment have been as follows:

- Depletion of energy substrates
- Magnetic resonance spectroscopy
- Tubular enzyme release
- Changes in extracellular ion concentrations and metabolites
- Perfusion characteristics
- Renal function

This chapter represents the current state of knowledge of renal viability assessment in each of these areas. Consideration is given particularly to the limitations of current techniques, and future avenues down which research may be channelled in order to develop clinically applicable, objective and reliable tests of renal viability.

3.2 Depletion of energy substrates

Adenine nucleotides (AN) constitute the universal energy substrates in cellular metabolism, and become depleted during ischaemia (see figure 2.1). Were there to be a critical level of AN below which the restoration of metabolism was impossible, and cell death inevitable, then this level may serve as a test of viability for ischaemic organs. Levels of different AN species can be assayed directly in biopsies taken from a kidney, although the tissue sample needs to be snap frozen immediately to arrest AN metabolism.

A canine model of renal warm ischaemia has been used to demonstrate rapid reduction in the total levels of adenine nucleotides from the onset of warm ischaemia[322]. Further analysis of the different AN species demonstrates an early rise in ADP levels mirrored by a fall in ATP levels over the first 15 minutes of warm ischaemia, with

subsequent decline of both species. From the outset, total AN (ATP, ADP and AMP) levels fall, with a corresponding rise in the oxypurines, hypoxanthine and xanthine. This study did not then go on to transplant kidneys back into dogs, and so did not establish any link between total AN and renal viability.

Also employing a canine model, Garvin et al[323] demonstrated not only that renal cortical levels of ATP were depleted by both warm and cold ischaemia, but also that ATP levels could be increased by an intra-aortic infusion of ATP-$MgCl_2$ at the time of renal harvesting. However in this study only ATP levels were measured, and not total AN concentrations. The authors merely speculate that "any manipulation that restores cortical ATP should be beneficial". This seems reasonable conjecture since canine kidneys subjected to critical warm ischaemia (35 minutes) then perfused for 24 hours prior to auto-transplantation function better when ATP-$MgCl_2$ is added to the perfusate[324]. Renal function was defined three days post-transplantation on the basis of radio-isotope GFR measurements.

While investigating the mechanism of action of the beneficial effect of retrograde oxygen persufflation during renal preservation by cold storage, Pegg et al found that tissue ATP levels were reduced to approximately 25% of normal in rabbit kidneys subjected to 60 minutes warm ischaemia. With the use of persufflation this value could be improved to 42%[325]. This was also correlated with reduced tissue oedema and cell swelling, and the conformation of mitochondria (which reflects the energy status of the cell). The improvements in ATP levels were abolished by addition of respiratory chain toxins such as cyanide, in which marked cellular swelling was seen. Conversely ATP was increased by the addition of ouabain, an inhibitor of the membrane Na^+/K^+/ATPase pump. Also noted with ouabain was an increase in cellular swelling. This beautifully constructed series of experiments suggests that tissue levels of ATP are depleted by

ischaemia, and confirm that the lack of tissue oxygenation is responsible for this. They also demonstrate that hypothermically stored kidneys consume ATP, and that this use of ATP is at least in part to drive the membrane Na^+/K^+/ATPase pump. This is suggested to be important for the regulation of cell volume, and this suggestion is in turn corroborated by the histological findings.

This work, although not directly pertaining to viability assessment was motivated by the results of Fischer[326], Rolles[327] and Ross and Escott[328] who found that retrograde oxygen persufflation of cold stored canine kidneys could render viable organs subjected to 30 minutes warm ischaemia, and reduce the depletion of ATP.

Bore et al[329] and Tatsukawa et al[330] demonstrated that the viability of a rat kidney could be predicted by the ability of the organ to regenerate ATP. Calman[159, 160] found a stronger correlation between renal viability and total AN using the rat clamped kidney model than between viability and ATP specifically. These findings are consistent. ATP levels fall quickly during a warm ischaemic injury, but as initially ADP levels rise, the total AN levels fall more slowly[322]. Total AN represents ATP and the substrates required for regeneration of ATP. Once total AN is depleted by the xanthine oxidase system, the ability to regenerate total AN is lost, and with it, tissue viability.

Recently, Coremans et al[331] have pointed out that the ability to regenerate ATP is largely dependent upon the respiratory chain in mitochondria. The first step in the respiratory chain involves the oxidation of NADH to NAD+. The ratio of NADH to NAD+ can be measured by UV fluorimetry. In a rat model the authors compared the NADH/NAD+ ratio in kidneys subjected to either no or 60 minutes warm ischaemia. The groups had significantly different ratios, and indeed the groups did not overlap. Two out of six animals in the warm ischaemia group died, compared with none in the control

group. The average serum creatinine values in the surviving rats were higher in the ischaemia group compared with the controls.

Further indirect evidence for the relationship between total AN and renal viability can be drawn from the protective effect the xanthine oxidase inhibitor allopurinol has been reported to have upon ischaemically damaged kidneys in some studies[332-335], although this is disputed by others[336]. Similar protection can be afforded by other free radical scavengers, such as superoxide dismutase. Distilling the anti-oxidant effect of allopurinol from the total AN preserving effect is difficult, as both result from the same biochemical pathway[337].

Against these findings are those of Pegg et al[316] using a canine model, in which 60 minutes of warm ischaemia produced expected falls in ATP and total AN levels, largely reversed by 48 hours perfusion with oxygenated colloid perfusates. However this restoration of total AN and regeneration of ATP did not confer viability on the organs when auto-transplanted with simultaneous contralateral nephrectomy. A potential criticism of this paper, as observed by the authors themselves is that the model used was a severe test of organ viability. 30 minutes WIT has previously been described as a critical model for canine kidneys with immediate life-sustaining function possible after a maximum of only 24 hours subsequent cold perfusion[338], and a delayed contralateral nephrectomy would have been a better test of ultimate viability.

Maessen et al[313] suggest that although adenine nucleotide levels correlate with viability in dog kidneys, viability is lost with increasing ischaemia in a substantial number of organs before severe depletion of total AN occurs. However they also find that the increase in viability in an auto-transplant model brought about by the technique of intermittent autologuous normothermic perfusion correlates with a recovery of total AN levels.

The problem with total AN and ATP levels as indicators of renal viability is that although there is undoubtedly a relationship between the two, the nature of the relationship is such that it is impossible to set a 'cut-off' value for either that accurately delineates between viable and non-viable organs. It may be possible to set a range of values that constitute 'dubious' viability, and by discarding these most non-viable organs will be eliminated. No such range has been defined for renal preservation.

This contrasts with the situation in pancreas preservation where in a canine model a tissue concentration of 6μmol/g ATP has been used as a 'cut-off' value to discern organ viability. This produced a sensitivity of 100%, a specificity of 84.6%, a positive predicitive value of 91.7% and a negative predictive value of 94.3%[339]. However, even a specificity of 84.6% for viability compares unfavourably with the primary non-function rate of 7% reported in a clinical NHBD renal transplant programme[42].

3.3 Magnetic resonance spectroscopy

More recent interest in measurements of adenine nucleotide status of ischaemic tissues has predominately been directed at Magnetic Resonance Spectroscopy (MRS). ^{31}P-MRS emission spectra enable discrimination to be made between relative concentrations of ^{31}P containing intracellular metabolites, including ATP, ADP, phosphomonoesters (PME) and inorganic phosphate (Pi). This has the advantages of being non-invasive, and a relatively rapid procedure, that could conceivably be performed within the time required to complete tissue typing, cross-match and preparation of patient and kidney for transplant.

Radda and Sehr et al[340-342] pioneered work in this field, employing a rabbit isolated kidney model. With this they demonstrated both that the ATP peak seen on spectroscopy

virtually disappears after 8 minutes of warm ischaemia, and then subsequently almost completely recovers within a further 4 minutes of reperfusion with autologous oxygenated blood. The changes in ATP seen on MRS were noted to correlate well with the restoration of renal function, as determined by urine production, GFR, sodium and water re-absorption. They also report that the intracellular pH (detectable by shifts in the absorption spectra) reaches a minimum after approximately 50 minutes warm ischaemia. This appears to be the outer limit consistent with renal viability in the rabbit.

Bretan et al pursued this line of research using a variety of animal models and clinical studies subjecting kidneys to a variety of warm and cold ischaemic insults, and correlating the MRS findings with subsequent renal function.

They performed two parallel experiments using a rat model, with kidneys from the first group of animals being subjected to warm ischaemic times between 0 and 120 minutes, and assessed by MRS and electron microscopy during cold storage over the following 72 hours. In the parallel group, left kidneys were subjected to the same durations of warm ischaemia as above, at the end of which the right kidney was removed. Renal function was then assessed by serum creatinine values. Both PME/Pi and NAD/Pi ratios fell with increasing WIT and cold storage times. These falling ratios correlated with worsening features of ischaemic damage seen on electron microscopy. All animals from the second group with WIT of 0 or 20 minutes survived to 4 weeks, and had normal serum creatinines 72 hours post-operatively. Animals with 60 minutes WIT also all survived for the duration of follow-up, but had significantly raised serum creatinines after 72 hours. None of the rats with 120 minutes renal warm ischaemic time survived 48 hours post-operatively[343].

The authors concluded from these results that PME/Pi and NAD/Pi ratios reflect organ viability, and set values for both to distinguish between organs that will function

perfectly immediately, those that will function adequately but with raised creatinine, and those that will be too damaged to function. However there are a number of assumptions made in extrapolating between parallel groups, which may not be valid. The authors have recognised the combined influence of warm and cold ischaemic times on organ viability, and constructed nomograms in the form of graphs of MRS ratios against cold storage time, with lines drawn to denote boundaries of perfect function, impaired function and non-function. Even on the nomogram presented by the authors, 3 of 5 kidneys subjected to 120 minutes of warm ischaemia had PME/Pi ratios above the cut-off value set for organs able to sustain life-supporting function. In the parallel group of 9 animals with kidneys subjected to 120 minutes warm ischaemia then contralateral nephrectomy, none survived. On this basis, there is a 60% chance that such a kidney would appear viable on MRS criteria, when the real probability of viability is 0%. Also the model used does not completely reliably distinguish between viability and non-viability. In the clinical situation NHBD kidneys usually undergo a period of acute tubular necrosis resulting in delayed graft function (and therefore dialysis dependence) that can last up to two months. DGF does not have the adverse effects on long-term graft survival in NHBD transplants with which it is associated in HBD transplants[126]. Models of renal viability in which the contralateral nephrectomy is delayed and therefore allow resolution of ATN may be fairer tests of viability.

Bretan and colleagues also used a canine model to examine changes in the PME/Pi ratio seen with ischaemia and reperfusion of kidneys[344]. Dog renal pedicles were cross-clamped for 45 minutes, during which time the ATP levels fell to approximately half of their starting values. On reperfusion the ratio was restored to normal values within 2 hours of perfusion. Another group of animals underwent left nephrectomy followed by immediate cold flush and storage for 24 hours. They were auto-transplanted at the end of

this period with a simultaneous right nephrectomy. MRS was performed on these kidneys at the end of the cold storage period and then at three hours post-reperfusion. Similar falls and rises in PME/Pi were noted with ischaemia and reperfusion respectively as with the ATP levels in the warm ischaemia group. The exception to this being when there were technical failures in the transplant operation. In these circumstances, PME/Pi ratio continued to fall to "non-viable levels". The authors inferred from these results that PME/Pi ratio was a "strong renal viability parameter". However there was no viability endpoint reported for these experiments, other than the PME/Pi ratio. The correlation of this ratio with viability was presumed from the rat models described above and therefore subject to the same caveats in addition to that of the caution required to interpret results across species. The continuing fall of PME/Pi on technically inadequate auto-transplants seems an axiom of re-warming without reperfusion.

Bretan et al also reported the clinical use of MRS to evaluate renal viability in 16 cold stored cadaveric human kidneys[345]. 13 of the 16 kidneys had high PME/Pi ratios and 3 had low ratios. Those with high ratios had good urine output and falling serum creatinine on day 3 post transplant. Those with low ratios were oliguric post-transplant and had raised serum creatinines post-operatively. However, the outcome beyond 3 days post-transplant is not specified, and therefore PME/Pi may reflect the risk of delayed graft function rather than viability. In this paper the authors also refer to using PME/Pi determined on MRS in addition to anatomical information gleaned from MRI to determine the viability of kidneys they suspect of being damaged by donor disease (recurrent urinary tract infections and megaureters). This illustrates the clinical problem of the multitude of factors which need to be considered in determining renal viability outside tightly controlled animal models, and therefore the difficulty in deriving a viability test based on just one factor.

A subsequent larger series of 40 cadaveric renal transplants[346] was also analysed by MRS and PME/Pi was found again to predict the need for post-operative dialysis with a sensitivity of 75% and specificity of 87%, when a 'cut-off' value of 0.5 was used. Once again, no reference was made to primary non-function. Also there was little information given on other parameters known to affect the outcome of renal transplantation between the kidneys requiring dialysis and those with immediate function. For example, it was not stated whether there was any differences between the groups in the donor or recipient ages, sexes, degree of HLA mismatch, warm or cold ischaemic times or re-vascularisation times. Interestingly, in the discussion of their results, the authors suggest that the primary role of MRS spectroscopy in renal transplantation may be as a tool in research into improving renal preservation. They also note that if ATN is predicted by MRS, immunosuppressive therapy may be tailored accordingly to provide a lesser additional nephrotoxic insult. These are sensible suggestions, and the latter is consistent with the practice in Leicester where all non-heart-beating donor kidney transplants are treated with low doses of calcineurin inhibitors compared with HBD counterparts for precisely this reason.

Pomer and colleagues[347] pertinently identify that in using MRS to evaluate renal 'viability' the 'important question is if it can be used for prediction of post-transplant functional recovery'. However they do not go on to answer that question. Using a rat model they subjected kidneys to periods of warm ischaemia much shorter than that thought likely to be critical to viability[348], assessed them by ^{31}P MRS, then isografted them into bilaterally nephrectomised animals. They demonstrated a good correlation between the PME/Pi ratio and the peak serum creatinine concentration seen on day 2-3 post transplantation. All the kidneys were viable.

Pomer went on to examine ten human cadaver kidneys by MRS prior to transplantation[349]. All had detectable ATP, which Bretan had found only in kidneys that had high PME/Pi ratios and good post-transplant function. Pomer also found good post-transplant function as assessed by isotope renography and serum creatinine, and also a correlation between these outcome measures and PME/Pi ratios.

Kunikata et al also report that ATP becomes undetectable by MRS after only just over two hours cold storage in a rabbit model, and thus decline in ATP is too sensitive for viability assessment[350]. They note that PME/Pi ratio declines more slowly over time, as do MRS derived calculations of intracellular pH. They suggest that these may therefore be more useful as determinants of renal viability. Although they do not demonstrate this in a transplant model, they use the ability to restore MP/Pi ratio on perfusion with a fluorocarbon emulsion as a surrogate marker of viability. In this way they observer that MP/Pi ratio can be fully restored after 24 hours cold storage at 4^0C but only partially after 48 hours.

The suggestion by Kunikata that intracellular pH may be useful to determine viability is controversial in the literature. Radda et al[340] noted, again in rabbits that intracellular pH declined, until bottoming out after 50 minutes warm ischaemia. They felt that this time represented a 'cut-off' at the limit of renal viability in their model.

However, Bretan and Somer found no correlation between intracellular pH and post-transplant renal function, in rat or canine models or in human cadaver kidneys[343, 344, 347-349].

MRS may also be used to examine urine produced by a kidney for the presence of Trimethylamine-N-oxide (TMAO), which is released by the renal medulla in response to ischaemic injury. Urine and plasma levels of TMAO have been used to compare the efficiency of different preservative solutions, and it has been correlated with post-

transplant GFR[351]. However, in order for this to be a useful technique in the assessment of renal viability prior to transplantation, the kidney must produce urine, and therefore needs to be perfused. In an *ex vivo* perfusion model (see later) the level of TMAO in the urine has been associated with increasing cold ischaemic times, although the relevance of this to ultimate viability is not proven, since in this model, there was no transplant performed following perfusion[352, 353].

In summary, whether measured biochemically in tissue biopsies, or by MRS it would appear that ATP itself disappears far more rapidly than renal viability. The ratio of PME to Pi (MRS) or tissue total AN levels (biochemical evaluation) decline more slowly and certainly appear to correlate with the degree of ischaemic insult suffered by a kidney. Total AN includes the substrates necessary to generate ATP on the restoration of perfusion. Therefore depletion of total AN by xanthine oxidase during ischaemia beyond a critical level may render a cell incapable of regenerating ATP, and therefore surviving. The difficulties in using total AN or PME/Pi for the assessment of renal viability are twofold. Firstly although a correlation exists between these and ischaemic insult, the variability of this relationship is too wide to be able to set a level of either that is sensitive or specific enough to be useful clinically. Secondly, different cell types within the kidney differ in their susceptibility to ischaemic damage, with proximal tubular cells being most sensitive. Acute tubular necrosis would result in delayed graft function, and many of the transplant models above use this as an outcome measure. However, it is the occurrence of cortical necrosis that results from more severe ischaemia, and would result in primary non-function if transplanted, that needs to be predicted in order to determine viability.

3.4 Tubular Enzyme Release

The previous section demonstrates how the assessment of renal viability has been tackled directly by quantifying cellular energy state and capacity. Pettersson et al took a sideways look at cellular energy state, by looking at the activity of the membrane bound Na^+/K^+/ATPase[354]. Since this is responsible for up to 90% of energy consumption in a resting cell, the function of it may reasonably be expected to reflect the energy state of a tissue. Also the enzyme is essential for maintaining transmembrane sodium and potassium gradients and preventing cell swelling. A canine autotransplantation model was employed in which a left nephrectomy was performed and the kidney subjected to different cold preservation conditions and durations. It was subsequently autotransplanted at the time of contralateral nephrectomy. At the end of the storage period a wedge biopsy was taken, from which slices were prepared for estimation of Na^+/K^+/ATPase activity. Of 32 dogs transplanted, 19 of the kidneys functioned post-transplant and 13 did not. Of the 19 that functioned, 18 had detectable enzyme activity in the biopsy slices, and 1 had none. Of the 13 that did not function 7 had no detectable enzyme activity. Of the 6 with detectable activity but non-functioning transplants, two had renal vascular thrombosis and must be considered technical failures and three had continuous urine production, but nevertheless were not immediately able to provide life supporting function. Therefore the presence of enzyme activity has a sensitivity of 95% and a specificity of 63%. Clinically, although this would be very useful in preventing the discard of viable kidneys, transplanting 37% of non-viable kidneys would be unacceptable. However, if the three kidneys which made urine continuously but did not clear the plasma creatinine were suffering delayed graft function rather than primary

non-function, then the specificity would rise to 91%, which would certainly be of an order to be clinically useful. In order to demonstrate this, however, the model would have to have been modified to incorporate a delayed contralateral nephrectomy, perhaps for three weeks. Three weeks is suggested, because in the surviving animals there was a good correlation between GFR as measured by insulin clearance at this time post-transplantation, and enzyme activity. Unfortunately this promising work does not appear to have been carried further forward.

With the solitary exception of the example given above, the approach of investigators to enzyme analysis as indices of renal viability has been as markers of damage when enzymes are released, rather than direct markers of cellular metabolism and function. This approach as the theoretical disadvantage that measuring damage *per se* does not necessarily permit an assessment of the ability to recover from damage. Nevertheless, gauging damage by measuring enzyme release has been extensively studied, with the stated aim of determining renal viability.

Starling et al[355] found that when canine kidneys were preserved by cold pulsatile perfusion under differing conditions, the rise in perfusate levels of betaglucuronidase and cathespin D correlated with the increase in vascular resistance seen during perfusion. Betaglucuronidase and cathespin D are lysosomal enzymes, the release of which may be triggered by ischaemia, and which increase vascular smooth muscle tone[209]. These findings by no means prove that the release of these enzymes results from damage, let alone suggest a level of betaglucuronidase or cathespin D that may predict viability. However the correlation with vascular resistance is interesting, as vascular resistance is inversely correlated with post-transplant performance (see later). This at least suggests that these lysosomal enzymes merit further research, for their potential diagnostic value.

The membrane bound tubular enzymes alkaline phosphatase (AP), alanine aminopeptidase (AAP) and γ-glutamyltranspeptidase (GGTP) have been analysed by Heinert et al[356]. They used computer-assisted histomorphometry to quantify staining of these enzymes by histological techniques dependent upon enzyme activity in sections of normal and hypothermically stored kidneys. In rats, they found significantly less AP activity in 24 hour cold stored kidneys than in fresh kidneys, but no difference in GGTP activity. Similarly in human kidneys there was no difference in GGTP or AAP activity between fresh and cold stored specimens. The only exception to this was in a perfused human kidney, accidentally warmed to over 15^0C, in which a dramatic fall in AAP activity was found. There is no comparison drawn between these results and transplant function, so the use of these enzymes in viability assessment is unclear.

Liebau et al examined the levels of the tubular enzymes lactate dehydrogenase (LDH) and serum glutamic oxaclacetic transaminase (SGOT) levels in the perfusate of canine kidneys preserved by hypothermic pulsatile perfusion[357]. The endpoints to their experiments were renal function immediately on autotransplantation, or 'significant signs of deterioration during perfusion' as determined by weight gain or increasing vascular resistance. The authors noted that there was higher enzyme release in the 8 kidneys that did not function or were not transplanted than in the eight that did function immediately on autotransplant. On the basis of this they set limits to the amount of LDH and SGOT that could be released into the perfusate during preservation of kidneys, within which 'the viability of the preserved kidney can be assured'. However although the values given are consistent with the results presented, no statistical calculations pertaining to the confidence or significance of the values were presented and therefore it does not appear that this conclusion was validated.

Indeed, Newmann and Shenton found LDH in perfusate samples to be of no use in predicting renal viability. In a clinical series of 23 transplants, 9 patients had immediate graft function, 7 had delayed graft function and 7 had primary non-function[358]. Although all the immediate function kidneys had LDH levels of less than 400 units/litre, 5 kidneys with delayed graft function would not have been transplanted using this cut off and 3 of the 7 primary non-function kidneys would have been transplanted. For this reason LDH would appear not to be specific enough a test of renal viability for use clinically.

Those authors who argue otherwise who do not provide firm evidence to back up their position. Danielwicz et al measured LDH during hypothermic pulsatile perfusion of 86 human cadaver kidneys[247]. The recipients were divided into an IF group (n=54) and a DGF group (n=26). The remainder either had PNF (n=2) or died in the early post-transplant period (n=4) and were excluded from analysis of results. An LDH value for each kidney during perfusion was calculated and was significantly higher in the DGF group than the IF group. The authors report that a detailed analysis of their results reveals levels of LDH that predict 'with high probability' that a kidney will exhibit IF or DGF if transplanted. Unfortunately they do not present the derivation of these values. The limit of LDH for IF kidneys given is >10.5 and for DGF is <5. They do not comment on the function of kidneys with an LDH level of between 5 and 10 during perfusion. They also set ranges for other perfusion characteristics, including vascular resistance and perfusate lactate concentration. Using all three reference ranges, they state that they can predict DGF or IF for a kidney in 60% of cases. Not only does this seem rather low to be of use clinically, but also they do not attempt to predict PNF. They report themselves that there is no difference in serum creatinine levels between the IF and DGF recipients at one year post-transplant.

Kohn and Ross describe a canine model in which ten animals had both renal pedicles clamped for the same length of time (0 to 120 minutes), and then had a unilateral nephrectomy followed by releasing the clamp on the other pedicle[359]. The removed kidney was flushed and the venous effluent collected for LDH estimation. They found a strong correlation between warm ischaemic time and maximum post-operative blood urea (correlation coefficient 0.67, $P<0.05$), and an even stronger correlation between LDH and peak post-operative urea value (correlation coefficient 0.88, $P<0.001$). They point out that all three of the animals that died within the first month after surgery had LDH values above a certain level, and all seven surviving were below this value. No degree of confidence in this level is calculated and the numbers are very small. Also, of the three deaths, one demonstrates a late rise in creatinine, over a week post-operatively, and this raises the suspicion that the animal may have succumbed to an insult other than or in addition to the warm ischaemia. Finally, again, this model is able to differentiate between IF and DGF, but not identify PNF.

The most extensively investigated enzyme in renal viability assessment is glutathione-s-transferase (GST). Originally identified as an intracellular protein confined to renal proximal tubular cells, and name ligandin[360], GST had been shown to be released into the urine in a rat model of acute tubular necrosis[361]. It seemed also that ligandin might be a very sensitive marker of renal tubular injury, as it was detected in the urine of patients given contrast reagents for renal arteriography who had no clinical or routine biochemical evidence of renal insult[362]. GST has also been found to be released into the urine of transplant kidneys during acute rejection and cyclosporin toxicity[363]. These observations lead Feinfeld et al to hypothesise that ligandin might be released into the perfusate of ischaemically damaged kidneys preserved by machine perfusion[364]. They assayed the presence of ligandin by Ouchterlony immunoprecipitation and the GST

activity quantified spectrophotometrically by the conjugation of glutathione with 1-chlor-2, 4-dinitorbenzene. They correlated the presence and activity of ligandin to the post-transplant function of 13 human cadaver kidney transplants. Post transplant renal impairment was defined for the purposes of their study as a combination of the following three conditions: oliguria (urine output less than 200ml/24 hours), no evidence of hyperacute rejection and a renal scintiscan demonstrating good renal perfusion but poor clearance of isotope. According to these variables and outcome measures, 8 kidneys had delayed graft function requiring at least three post-operative dialyses and 5 had immediate graft function requiring no dialysis after transplantation. All eight of the DGF kidneys had ligandin detected by Ouchterlony, and GST activity detected. None of the 5 IF kidneys had ligandin detectable, and only one had GST activity, barely detectable at the limit of sensitivity of the spectrophotometric method. Fisher's exact test demonstrated that ligandin presence or absence discriminated between IF and DGF with a P value less than 0.001. The authors reported that the warm and cold ischaemic times are known for only 3 and 7 of the retrieved kidneys respectively, and that they are unable to demonstrate any correlation between ischaemic times and ligandin presence or activity. However, from the perspective of viability assessment, this is irrelevant. The presence of ligandin, or GST in the perfusate of ischaemically damaged kidneys would appear to predict accurately the occurrence of delayed graft function. The authors were unable to correlate the degree of impairment of graft function with the level of GST activity detected because the kidneys had been preserved for different lengths of time, with different volumes of perfusate, and different weights of kidney. No mention is made of ultimate graft viability.

Unfortunately no such clear-cut results are reported by Cho et al, who also evaluated human cadaver kidneys preserved by hypothermic machine perfusion[365].

Perfusate was collected from 12 machine perfusions of pairs of kidneys. Ligandin was assayed by GST activity as described above[364]. The eight pairs of kidneys that exhibited immediate function had very significantly lower levels of ligandin than the four that exhibited DGF (p=0.0002). However activity was detected in every perfusate sample, and no level was suggested as a cut-off to delineate between transplant outcomes. The 'urine' or ultrafiltrate produced by a further 12 kidneys during cold perfusion preservation was analysed for levels of GST activity which was found to be higher in kidneys that subsequently exhibited DGF compared to IF, although this was not significant. Once again, a familiar picture is emerging; a parameter that can be measured pre-transplant correlates with the early post-transplant performance of a kidney. However the variability of this is too great to be of adequate prognostic accuracy for clinical use. Again also, ATN is predicted (by release of a tubular enzyme), with consequent DGF, but the ultimate potential of the kidney to render a recipient free of dialysis is not evaluated.

Daemen et al[366] assayed LDH and α-GST (ligandin or basic GST, the isoform of GST present in proximal tubular cells, which are the most susceptible to ischaemia) in 67 hypothermically perfused human cadaver kidneys from NHBD. They transplanted 46 of these, rejecting the others on the basis of donor history, macroscopic appearance, perfusion characteristics or histological findings. They separated those that were transplanted into functioning (n=37) (either immediately or delayed) (F) and never-functioning (n=9) (PNF). They found that LDH was not able to differentiate between these groups, and nor did it correlate with recorded warm ischaemic times. α-GST correlated with WIT after 4 and 8 hours of perfusion (p=0.009 and 0.011 respectively). GST levels were significantly lower in the F group than in the PNF group (p=0.004 after 8 hours perfusion). Interestingly, they found that LDH may differentiate between IF and

DGF in the F group (p=0.0003) which in these experiments α-GST failed to achieve. This is consistent with the experiences of Kohn et al as described above[359]. The authors correctly identify the distillation of PNF from F as the most important outcome measure of renal transplantation, rather than IF from DGF, and therefore that α-GST may be of use for prognosis, whereas LDH is unlikely to help. They suggest an upper limit for perfusate α-GST which in their series would have resulted in 37 kidneys being transplanted, 4 of which would never have functioned. The specificity of α-GST for viability assessment at this cut off is 89%, however the sensitivity may be as low as 56%. They also suggest that 3 of the 4 kidneys that did not function despite levels of α-GST below the cut-off may have failed for other reasons to the warm ischaemic injury. The evidence cited for this is that the other kidney from the same donor in each of these three cases went on to function. However, in the experience of the author, the in situ preservation of kidneys from NHBD with a DBTL catheter often results in disparate quality of perfusion between the two kidneys. Therefore the warm ischaemic insult may have been more effectively arrested in one kidney than the other.

The same group (Kootstra, Maastricht) have broadened this work pertaining to α-GST in both NHBD (n=91) and 'marginal' (high donor creatinine, advanced donor age) HBD (n=16) human cadaver kidneys[367]. The level of α-GST in HBD, functioning and non-functioning NHBD were significantly different in the perfusates after 8 hours of perfusion. Only 59 of the 91 NHBD kidneys were transplanted, the remainder being discarded on the basis of donor history, macroscopic appearances at retrieval, unacceptable intrarenal resistance on perfusion, or unacceptable LDH levels in the perfusate. All the HBD kidneys functioned, compared to 49 (83%) of the NHBD, with 10 never functioning. 8 hour α-GST levels were available for 45 of the functioning NHBD transplants, and 9 of the non-functioning grafts. All of the HBD had relatively low levels

of α-GST. The NHBD kidneys had a much wider distribution of α-GST which overall was significantly higher in the non-functioning recipients that the functioning group. However the distribution in both these latter groups was very wide and even by selecting an optimum cut-off value of α-GST the authors would have been unable to prevent 3 of 45 functioning transplants being discarded (7%), or 6 of 9 of the non-functioning transplants being transplanted (67%). No HBD transplants would have been excluded on the chosen cut-off. If the NHBD transplant decision had been made on α-GST levels, after exclusions had been made on the other criteria listed above, then 50 (89%) of the 56 transplants would have been performed, with 43 (86%) of them functioning. Therefore for an increase in the rate of transplant function from 83% to 86%, a price is paid in a reduction in NHBD transplant rate of 11%. The authors attempt to break down the non-functioning NHBD transplants according to the cause of the failure. It is reasonable to suggest that non-function secondary to renal vascular thrombosis should be excluded from subsequent analysis. However to exclude a non-functioning graft because of acute rejection is of dubious validity, as the non-function may arise as a result of the warm ischaemia, rejection or a combination of both. Were these to be excluded from the analysis, so too should be those functioning grafts that suffered acute rejection episodes. Certainly the combination of acute rejection and delayed graft function is particularly deleterious to renal allograft function. Also it is difficult to fully evaluate the worth of a viability test when it is used on kidneys that have already been pre-selected by other criteria, some of which are inherently subjective. However this presents an extremely difficult ethical problem, as the pre-selection influence can only be abolished by eliminating pre-selection. This would result in the transplantation of kidneys thought likely to be non-viable. Until a high degree of confidence can be placed in a new

viability criteria (in this case α-GST) this is unacceptable. The results above do not instil quite such confidence.

α-GST, as the isoenzyme found exclusively in the proximal tubules has been shown to correlate with warm ischaemic insult as detailed above. The other isoform found in renal tubules, π-GST is distributed in the distal convoluted tubules and the collecting ducts. This has been demonstrated not to correlate with WIT[368]. These findings are consistent with the observation that the proximal tubule is more susceptible to ischaemia than the distal tubule and beyond[369]. The authors also find in the same study that π-GST does not predict transplant function, whereas α-GST does. The evidence given in support of this claim is that α-GST is significantly higher in the perfusates of transplants that go on to function than in those that do not, whereas there is no difference in π-GST levels. The study numbers however are very small, with 19 functioning grafts, and only 3 non-functioning. In fact the mean level of π-GST was 42% higher in the non-functioning group than in the functioning group, and it is easy to imagine that such a disparity would quickly achieve significance if maintained with larger numbers. However Polak et al[370] were unable to differentiate between kidneys with ATN (n=15) and those with immediate function (n=40) using π-GST, whereas there were significant differences between these groups in their α-GST levels. This study was not able to suggest cut-off levels for renal viability for either enzyme.

To summarise the evidence for the use of enzymes in predicting organ viability, much as with the research into metabolite substrate levels, several enzyme levels or activities in tissue, perfusate or urine have been correlated with viability. Some authors have even gone so far as to suggest cut off levels to differentiate between viability and non-viability, or between immediately functioning and delayed functioning grafts. However, none would appear to be sensitive and specific enough to be used alone.

Candidate enzyme levels are found to be elevated in non-transplanted organs, however that these organs are not transplanted suggests that their non-viability is obvious without recourse to these enzymes. Recent studies into organ viability assessment by enzyme analysis have concentrated heavily on GST and LDH, and it would seem likely that the release of tubular enzymes will more directly predict the occurrence of DGF secondary to ATN than PNF secondary to cortical necrosis.

3.5 Extracellular Ions

The concentration of various ions may change during ischaemic damage secondary to direct release from injured cells, or impaired trans-membrane concentration gradient homeostasis.

Ogden et al used a canine autotransplant model to assess whether the rate of release of various metal cations into perfusate during 24 hours of cold machine perfusion preservation correlated with post-transplant fucntion[371]. He divided the animals into 2 groups according to whether they had good or poor post transplant function. He found that zinc concentrations in the perfusate rose significantly faster in the poor group than in the group that functioned well, and also correlated very closely with release of LDH into the perfusate (r=0.91). By contrast they found no significant changes in concentrations of magnesium and calcium during perfusion or between groups.

In contrast, more recently in the clinical situation Polyak et al have found that perfusate concentrations of calcium are significantly higher in kidneys that function immediately (n=102) than in those that do not (n=48), but found no differences between the groups in perfusate concentrations of potassium, sodium or chloride[372]. This study was on expanded donor criteria kidneys, defined as those that the retrieving surgeon

decided to biopsy. Why these authors should report such differing findings for perfusate calcium is not clear. Ogden perfused for 24 hours with cryoprecipitated plasma, compared to 12 hours with Belzer's II solution for Polyak.

Ion selective electrodes placed directly on the kidney can be used to monitor ion concentrations in the vicinity of the cell membrane, and may therefore provide information regarding cellular capacity to maintain transmembrane concentration gradients, rather than merely reflect gross leakage of ions from disrupted cells. Also, such a technique may be used in cold stored kidneys. Abendroth et al assessed 87 cadaveric transplants with a multi-channel sensor, measuring potassium, sodium, calcium and hydrogen ion concentrations at the organ surface. Potassium levels were significantly elevated in PNF and DGF kidneys compared with IF kidneys, whereas the other ions did not distinguish between the groups[373].

3.6 Metabolites

The accumulation of products of anaerobic metabolism may reasonably be expected to reflect the duration of ischaemia and particularly of warm ischaemia, and therefore to relate to organ viability.

Pursuing this line of reason, Baxby et al measured lactate levels and pH in 28 cadaveric kidneys and divided the recipients into those with IF (n=12) and DGF (n=14). They found that both parameters were significantly different between the groups. The distribution of pH was so wide that there was large overlap between the groups and thus was of no use in predicting transplant outcome[374]. However, lactate levels were so different that the authors were able to set a lactate level which would include only one 'false negative' result, excluding an IF kidney from transplantation, and only false positive, passing a DGF kidney as fit for transplantation. It is noteworthy that these

kidneys had already been pre-screened according to their flow characteristics during machine perfusion, with 5 kidneys excluded from the study due to poor flows. Also, the authors contended that the transplantation of DGF kidneys could be excluded if a larger number of cadaver kidneys became available, because of their experience of the relatively poor outcome of transplants that exhibit DGF[375]. This association of DGF with poor outcome is disputed (see chapter 1). Furthermore, the trend in organ availability has been in the opposite direction to that which they anticipated. The aim in the current climate must be to exclude PNF organs from transplantation, rather than DGF.

Johnson et al assayed the lactate levels of 69 perfused kidneys[376]. They found significant differences in these between IF and DGF kidneys, and the levels were even higher in the PNF group, however he was unable to determine the significance of the latter due to a small group size (only three kidneys had PNF).

3.7 Vascular Resistance

Ischaemia causes renal vasoconstriction as a result of several mechanisms[162]. Endothelin secretion is increased[163]. There is decrease in the production of NO[165]. Thus increasing warm ischaemic times have been associated with increasing renal vasospasm[377]. Insult to the vascular endothelium also causes an increase in permeability, resulting in the extravasation of any perfusate into the interstitial space, reflected in weight gain during perfusion[357]. The oedema also increases interstitial hydrostatic pressure, compressing vessels and thereby further increasing measured intra-renal vascular resistance. Increasing IRR reduces blood or perfusate flow to the tissues, thus exacerbating ischaemic damage, further increasing vasospasm.

Vasospasm has therefore been purported to reflect WIT and thus viability and can be assessed visually, by renal scintigraphy, or mechanically, by calculating the resistance to flow during organ perfusion.

Scintigraphy

In a canine auto-transplant model Anaise et al. found that the length of cold ischaemic time correlated with the cortical distribution of ^{99}Tc labelled albumin microspheres, which in turn correlated with post-transplant function, as measured by the proportion of animals surviving[378]. No cut off values were set for viability, however the assessment of cortical perfusion was semi-quantitative, expressed as a ratio of cortical activity to total renal activity. This quantitation would permit a cut-off value to be sought in further experiments.

In collaboration with the same group, also using a canine auto-transplant model, the efficacy of different preservation solutions, pharmacological interventions and durations of cold ischaemia were compared by Sato et al[379]. ^{99}Tc renal cortical scintigraphy was also correlated with animal survival. However the cortical perfusion was only described qualitatively, and therefore subjectively, offering no advantage over other subjective assessment of organ viability.

In his work on MRS using a canine model, Bretan perfused cold stored kidneys with ^{99}Tc labelled albumin, and also analysed this semi-quantitatively in the manner described above[345]. He concluded that there was a good correlation between cold ischaemic time and PME/Pi but none between either of these and scintigraphy. However, on the basis of only two kidneys this cannot be a valid conclusion.

Bretan et al subsequently reported, again in a canine auto-transplant model, that ^{99}Tc DTPA scanning revealed a correlation with PME/Pi ratio on MRS and renal

perfusion, although, as the model did not include a contralateral nephrectomy, it was not possible to draw any conclusions regarding renal viability[380]. Also, the groups compared in this study had right nephrectomies followed by cold flush then immediate transplant. One group had no renal vascular obstruction, another had apparently intentional renal pedicle obstruction, designed to cause renal ischaemia and thrombosis. It seems hardly surprising that this latter group should have demonstrably poorly perfused kidneys.

Chin et al[381] have used magnetic resonance imaging to assess renal perfusion by flushing in and then washing out the paramagnetic agent gadolinium DTPA. The failure to wash out the agent results from loss of vascular endothelial integrity, and therefore extravasation of the agent into the interstitium, this in turn is detected on MRI. These measurements correlated with the duration of cold ischaemia, however, there was not a transplant end-point to assess the relationship of MRI/gadolinium DTPA to viability.

These indirect radiological assessments of the state of the vasculature have the advantage that they are possible on cold stored kidneys. However in kidneys preserved by machine perfusion direct, immediate and dynamic data on the state of the vasculature can be obtained.

Machine perfusion

MP permits direct measurement of IRR, by recording the mean pressured used during perfusion and dividing it by the renal flow. According to Poisseulle's law, the resistance is proportional to the length of a vessel and the viscosity of the fluid and inversely proportional to the fourth power of the diameter. In the perfusion of any one kidney, the viscosity and length of renal vessels perfused is constant and the diameter of vessels will alter with the vascular tone. Therefore changes in vascular tone will be sensitively reflected by changes in resistance. The resistance is dependent on the basis of

the total cross-sectional area of vessels within the kidney and there are many small vessels within the kidney in parallel. Therefore the resistance will vary with the size of kidney, tending to decrease with increasing organ size. In order to compare IRR between kidneys and correctly infer information about vascular tone, it is necessary to calculate resistance per given weight of kidney. This was recognised by Kootstra's group in Maastricht[366, 382], who present results of IRR in mmHg/ml/min/100g of kidney. Many papers in the literature cite values for resistance (or flow or pressure, as determinants of resistance) and ascribe to them significance, such as thresholds for viability[135, 247, 249, 366, 383-385]. These however cannot be translated between different perfusion systems, unless the same perfusate is being used and therefore having the same viscosity. This echoes the need for standardised protocols for renal perfusion to be established in order that values may be compared between centres and the results of one usefully interpreted by another. In chapter two this was argued for the purpose of determining optimum preservation efficacy. Here standardisation would allow the resistance of large numbers of kidneys to be compared and the casual factors and consequences better elucidated.

Despite this there is evidence supporting the notion that IRR increases with increasing WIT[377, 386] and that higher IRR values are associated with increasing probability of DGF and PNF[135, 247, 249, 338, 357, 366, 383—385, 387]. There are very few dissenting voices with only one study reporting no correlation between IRR and WIT[388] and others reporting a decreased correlation between IRR and post-transplant function in kidneys that have suffered WIT[388] . Knight et al have used a porcine model of warm ischaemia to investigate the effect of WIT on IRR[377]. This demonstrated that IRR at the beginning of CP increased with WIT, but that during perfusion, the IRR tended to decrease, and irrespective of WIT, IRR tended towards similar values by 6 hours, by which time the IRR had stabilised. The reduction in IRR may be due to the wash out of

sludged erythrocytes, or vasoconstrictive substances, which would be consistent with the improved early post-transplant graft function compared with CS. On the basis of this it is suggested that the IRR at the beginning of perfusion may reflect viability more accurately than at the end. This was not tested by transplantation of the kidneys at the end of perfusion. The finding that with ischaemically damaged kidneys the resistance at the beginning of perfusion differentiates best the degree of ischaemic damage is consistent with the earlier observations of Johsnon et al[390].

In clinical practice, Henry[210], Danielewicz et al[247] and Polyak et al[249] all report a correlation between increasing IRR and probability of DGF. Tesi et al[135], Matsuno et al[384], Kozaki et al[385], Daemen et al[366] and Balupuri et al[383] associate high IRR with increased probability of PNF. However they do not provide data on the positive and negative predictive values of their scoring systems and this is probably a result of the variability of IRR with viability, despite the significance of the correlation, the same problem that dogs all the other candidates of viability discussed so far. Also, the clinical practice of discarding kidneys with high IRR because they are suspected to be non-viable is an ethical necessity, but hinders attempts define cut off values for transplantation. Animal studies are required to address this flaw. However, animals used are likely to be of similar age, strain and health. This is useful standardisation for the purpose of minimising confounding variables, and therefore examining the relationship between WIT and IRR. The correlation between IRR and post-transplant function and viability may be tighter than in clinical practice, as IRR will be affected by other donor factors than WIT. Such factors may include donor age, renovascular disease, hypertension (affecting autoregulation) and diabetes mellitus. Therefore, it may be possible to tightly define threshold values for renal viability in animal models that do not translate to clinical practice.

Even with the control of confounding variables, a canine autotransplant model has demonstrated that increasing WIT is associated with decreasing flow during preservation[387]. Longer WIT is also associated with poorer post-transplant survival. However using the cut-off value for 'good flow' defined in the literature, 10% of kidneys with no WIT, all of which functioned immediately, had poor flow. No animals survived after a 90 minute WIT but 50% of the kidneys had 'good flow' during preservation. Once again, the relationship between IRR and viability is established but not found to be tight enough for clinical use.

There are some early clinical references in which kidneys were transplanted irrespective of their flow characteristics[388], either IRR or correlates thereof. The results of these studies suggested that IRR did not predict PNF, DGF or IF in clinical practice.

3.8 Renal Function

Perhaps the most obvious target for the evaluation of post-transplant renal function is the function of the organ prior to implantation. A pre-requisite for functional evaluation is normothermia or near normothermic conditions. Hypothermic kidneys lose function, which can be demonstrated by gradual cooling of perfused kidneys[174], with glomerular filtration ceasing below temperatures of 22^0C and tubular function arrested at 18^0C. Renal function has been measured at temperatures between 25-37^0C in *ex vivo* WP systems and early during WP was found to correlate with the injury sustained prior to perfusion, although the functional values obtained were almost universally substantially sub-physiological[193, 318, 391-395]. The function is often measured in terms of creatinine or inulin clearance[393, 395, 193, 392], or PAH clearance[392], sodium or potassium excretion[193]. Early WP preparations often used perfusates without oxygen carriers, and so *ex vivo* function deteriorated within a few hours[257, 311, 391, 396]. This was not necessarily a problem

when *ex vivo* function was used purely to provide an outcome measure for an experiment. However if WP is used to determine post-transplant viability, then the process of evaluation must not cause deterioration in organ condition. Organs transplanted following warm perfusion with these early systems were found to be non-viable. Therefore the potential application of functional measurement as a pre-transplant predictor of viability could only be realised with the advent of perfusates augmented with oxygen carriers. Initial experience with these was fraught with problems deleterious to graft condition as discussed in chapter 2, however relatively recent advances have permitted WP which resuscitates ischaemically damaged organs, kidney[153, 265, 302, 305, 307, 309, 397] or extra renal[264, 267-269, 321]. Such systems are ideal for functional analysis of organs intended for transplantation. The so-called exsanguinous metabolic support (EMS) system developed by Brasile et al has been used in the functional evaluation of ischaemically-damaged kidneys in a canine model. They initially used PFC emulsion[153, 302, 305, 397] and more recently polymerised haemoglobin as an oxygen carrier[307, 309]. The reason for this switch is not documented in their literature, although a possible explanation is that the PFC emulsion they used is manufactured by Alliance Pharmaceuticals and is currently undergoing phase III clinical trials as a blood substitute. Alliance clearly regards this as a time of extreme commercial sensitivity and would not supply their PFC emulsion for use in the experiments for this MD thesis. They may also have been reluctant to continue to supply Brasile et al for the same reasons. The results of EMS system perfused kidneys do not appear to have been changed by the different oxygen carrier. The *ex vivo* function of kidneys on EMS WP correlates with the WIT suffered and with function post-transplant. WP with adequate metabolic support also permits the measurement of oxygen consumption by the kidney and still allows the measurement of IRR as for CP. These parameters also correlate with WIT with EMS

preserved kidneys and with kidneys undergoing WP on the apparatus used in this thesis. Interestingly, the more recent EMS studies have used lower perfusion pressures than the earlier ones, again without specifying the reason. It may be that lower pressures are better for preserving viability in their experience but a mean arterial pressure maintained below 40mmHg is unlikely to perfuse glomeruli when the normal canine kidney autoregulates blood flow with systolic pressures between 90 and 150mmHg[398]. Perhaps in recognition of this, the most recent publication of viability testing during EMS technology does not rely on the measurement of function but rather a composite of oxygen consumption, vascular resistance and platelet washout (as an index of flushing efficacy)[309]. This also correlates with post-transplant function but although a 'cut-off' value is ascribed to distinguish between well functioning and poorly functioning grafts post-transplantation, only 1 of 9 kidneys had PNF and 3 had composite scores below the designated threshold, so no statistical evaluation of the predictive power of the test could be made.

Renal function on the POPS autologous blood WP preservation system described in chapter 2 has been found to be better for kidneys preserved on that system than for those preserved by CP with UW[265]. By using the system as one of the experimental groups and for the outcome measure of the experiment, the preservation efficacy of the system and the capacity to measure function *ex vivo* were demonstrated together. The correlation of *ex vivo* function with viability was not proven by transplantation in this study. A similar approach was taken by Imber et al in assessing the efficacy of their autologous blood perfusion system for livers, also demonstrating that *ex vivo* function was superior in the WP group than CP with UW group[268]. They also have not yet transplanted their organs to confirm relationship of WP function to viability.

3.9 Summary

There is a pressing need for an accurate and reliable test of organ viability in order to safely expand the donor pool with NHBD in renal transplantation. A large number of different parameters have been proposed as potential indicators of viability, for good theoretical reasons. However when these have been investigated, although many have demonstrated a correlation with viability, no correlation has been tight enough to provide a useful clinical diagnostic tool of viability with acceptable sensitivity and specificity.

Some have advocated the construction of composite viability tests using several parameters[63, 149, 309, 399], in an attempt to address this problem. However none of the suggested composite indices have been reported to improve upon the accuracy of a single parameter.

Statement of Aims

This thesis examines the potential of a warm *ex vivo* perfusion system to predict post-transplant function of kidneys from NHBD, and also the efficacy of the warm perfusion system as a preservation technique. Thus the thesis sets out to determine whether the warm perfusion system may help to address the problems of viability assessment and organ preservation that particularly beset the use of NHBD kidneys for clinical transplantation.

Specifically, this thesis aims to address three questions:

1) Does the function of kidneys measured during warm *ex vivo* perfusion correlate with the duration of warm ischaemic injury suffered by the kidneys prior to machine perfusion?

2) Does the *ex vivo* function during warm perfusion relate to the post-transplant function of the kidney?

3) How does the efficacy of preservation by warm perfusion compare with that achieved by conventional hypothermic techniques for kidneys suffering varying degrees of warm ischaemic insult?

PART TWO

Methods

Chapter 4

Methods

4.1 Overview and Experimental design

The relationship between WIT and *ex vivo* renal function measured during warm perfusion was examined first, using a cadaveric porcine model. Pigs were killed and the kidneys subjected to specified ischaemic insults, then perfused for three hours at 32^0C as described below, and their *ex vivo* function measured by parameters described in detail below.

Then a porcine auto-transplantation model was used to compare the *ex vivo* function measured during warm perfusion with the *in-vivo* function post-transplantation. The same model was used to compare the efficacy of preservation by warm perfusion with that achieved by hypothermic techniques (static storage and perfusion), both for kidneys suffering minimal WIT as per HBD, and for those with substantial WIT as per NHBD.

In this model the left kidney of a pig was harvested after a specified WIT induced by cross clamping the renal artery. The kidney was then preserved by one of the three preservation techniques, during which the kidneys preserved by warm perfusion had their *ex vivo* function measured. All kidneys from all groups were then transplanted back into the donor animal 24 hours later, and at the same operation a right nephrectomy was performed. The renal function was then assessed daily over the next two weeks by the monitoring of serum biochemistry and other methods, as detailed below.

4.2 Cadaveric porcine kidney model

40-50Kg large white male pigs were stunned and killed by intra-cardiac injection of phenobarbitone. Death was confirmed by cardiac and respiratory auscultation, and the

time recorded. They were then left on the abattoir floor in accordance with a specified WIT.

Five minutes before the end of the specified WIT, the kidney retrieval operation for the kidneys was commenced. The anterior abdominal and thoracic walls were excised, and the thoracic and intraperitoneal viscera excised en masse, revealing the retroperitoneal structures very quickly and with excellent access. The kidneys were mobilised by blunt dissection of the surrounding fascia from lateral to the pedicles, and the renal vessels were sharp dissected to the aorta and the inferior vena cava, where they were divided. The ureters were blunt dissected to the pelvic brim and divided.
The kidneys were then transferred to a bowl of ice-cold saline, and the arteries cannulated with Tibbs cannulae, and 500mls ice-cold HOC was infused to each kidney under one metre of gravitational hydrostatic pressure. The kidneys were then sealed in a polythene bag with 200ml of cold Marshall's HOC, and transported back to the laboratory on ice. Once there, the kidneys were established on the warm perfusion apparatus as described below, with the exception that the kidneys were simply weighed and discarded at the end of the three hours perfusion.

4.3 Autotransplant initial preservation

For each preservation group, the initial preservation was the same. From the moment that the kidney was explanted, the renal artery was immediately cannulated in the operating theatre, and perfused with 500ml of University of Wisconsin solution at 4^0C under 1 metre of gravitational hydrostatic pressure (figures 4.1(a) and (b)). The WIT was recorded as the time taken to commence the infusion from the time of the clamping of the renal artery to the nearest minute. A cortical wedge biopsy was taken of

approximately 0.2g, estimated 'freehand'. The kidney was then secured in a polythene bag with 100ml of preservation solution still at 4^0C and stored on ice until transferred to the laboratory, where the preservation method allocated for that organ is employed, 2 hours after explantation. All of this was performed under strict sterile conditions.

4.4 Hypothermic static storage

In this, the kidney remained in the polythene bag as above, on ice, in an insulating polystyrene organ retrieval box. The following day the box was transferred back to the operating theatres in time for the autotransplant to be performed 24 hours after the retrieval. On removing the kidney from the polystyrene box, the presence of residual ice was confirmed around the kidney bag, to ensure that the kidney has remained adequately hypothermic throughout preservation. Immediately prior to transplantation, another wedge cortical biopsy was taken.

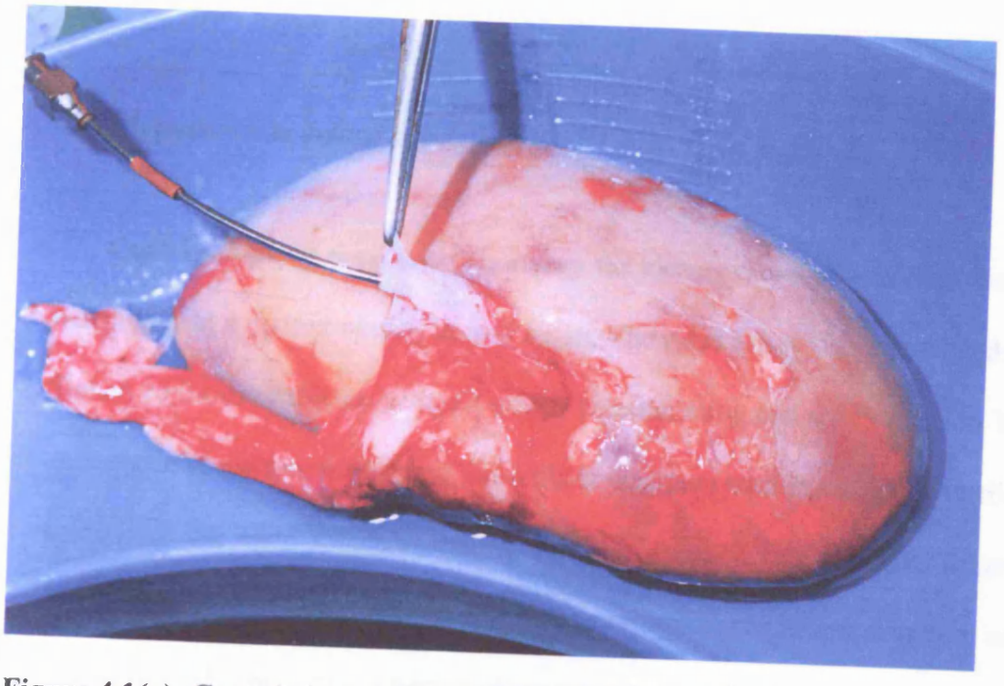


Figure 4.1(a): Cannulation of the renal artery of a freshly harvested kidney for autotransplant.

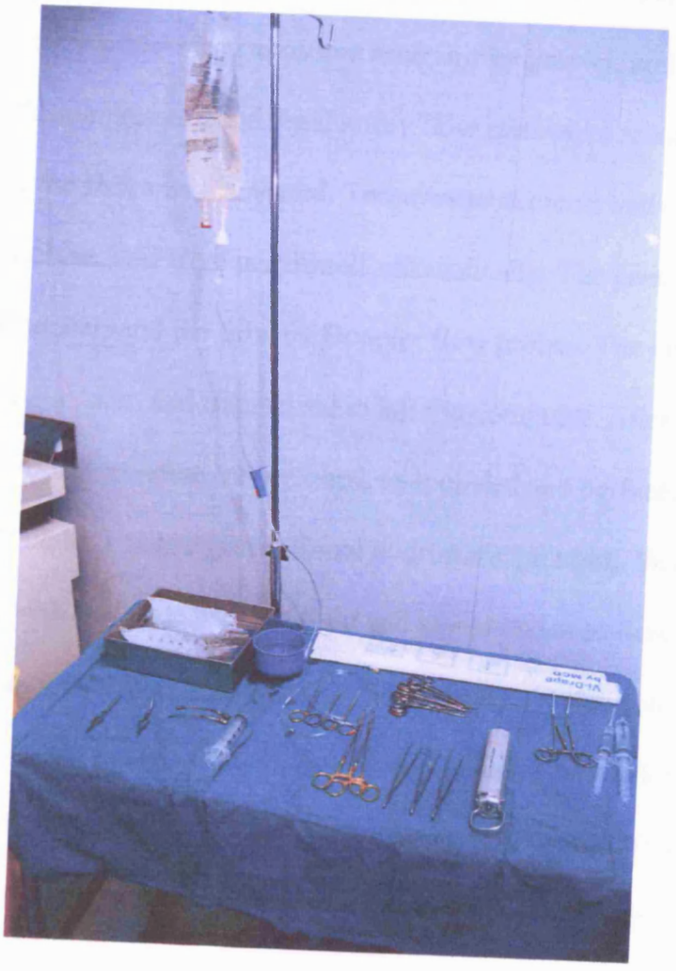


Figure 4.1(b): Retrieval bench for cold flushing of autotransplant kidneys.

4.5 Hypothermic pulsatile perfusion

Once back in the perfusion laboratory the kidney was removed from the bag on ice, weighed, and a further wedge biopsy taken. The renal artery was cannulated with an 8 French Nellerton catheter, secured with a vascular sling and artery forceps. This is atraumatic to the arterial intima. The kidney was then transferred to the sterile mox-100 cassette on the RM3 pulsatile perfusion machine primed with 500ml of Belzer's II machine perfusion solution (figure 4.2). The set up of the perfusion system is described in appendix B. The systolic perfusion pressure was maintained at 60 (+/-5) mmHg, and the perfusate temperature maintained in the range 3-8^{0}C. The renal vein drains freely into the cassette and the perfusate returns to a reservoir by gravity, and so is re-circulated. The mean perfusion pressure and renal artery flow rates were recorded minute by minute, and from these the IRR was calculated. These measurements and calculations are integral to the RM3 machine, and were performed automatically. The pressure was recorded by a solid state transducer, and the flow by Doppler flow probes. The data was stored in the memory of the machine, and transferred to lap-top computer. After 6 hours the kidney was removed from the system, re-weighed, re-biopsied and perfused with 100ml UW solution at 4^{0}C under 1 metre gravitational hydrostatic pressure, then secured in a polythene bag with cold preservation fluid and stored on ice as described above until the time of autotransplantation, exactly as for the hypothermic static storage group. The procedures described for this preservation were all carried out under strict aseptic conditions, and the perfusion cassette and perfusate were sterile. 750mg of cefuroxime were added to the perfusate prior to establishing the kidney on the machine.

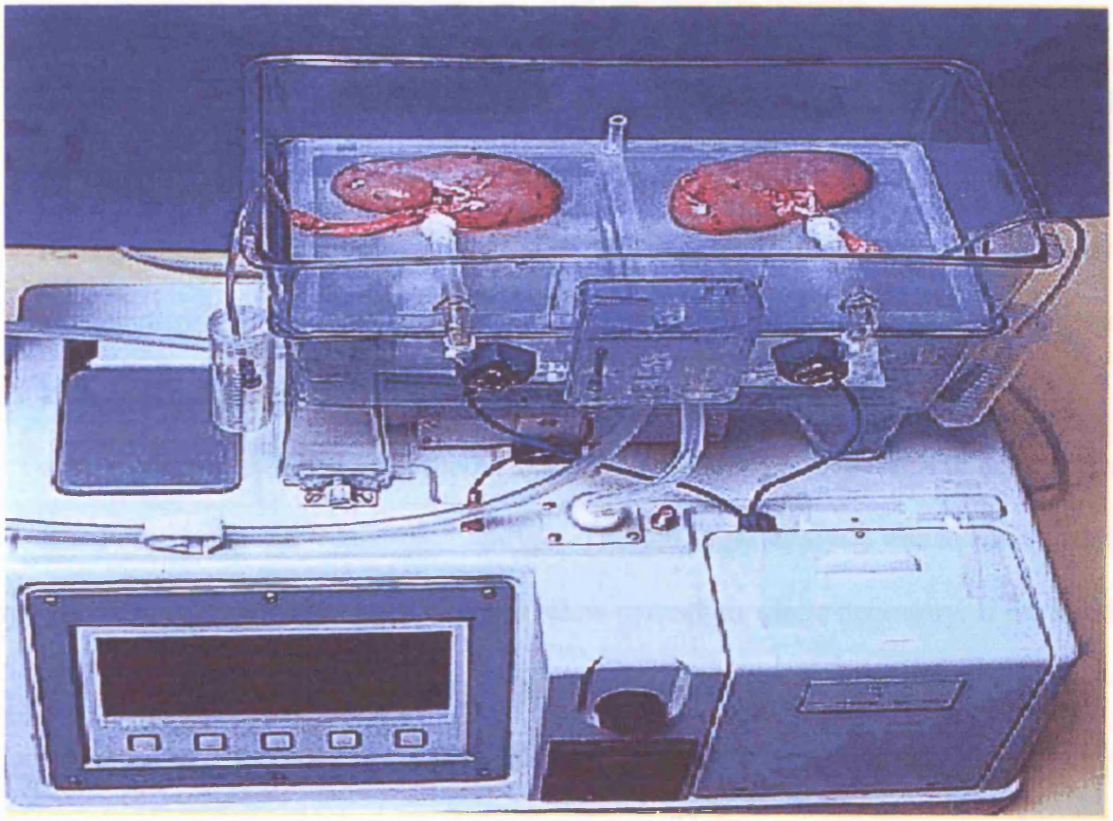


Figure 4.2: The Waters RM3 unit with Mox 100 cassette set up for cold perfusion.

4.6 Normothermic *ex vivo* perfusion

In the perfusion laboratory the kidney was removed from the bag on ice, weighed, and a further wedge biopsy taken. The renal artery was cannulated with an 8 French Nellerton catheter, secured with a vascular sling and artery forceps. Thus to this point kidneys were treated in an identical manner as for hypothermic perfusion. The ureter was also cannulated with a 6 French paediatric urinary catheter. The kidney was then transferred to the perfusion apparatus. The perfusion apparatus consisted of a modified RM3/Mox-100 system (below). The systolic perfusion pressure was set at 100 (+/-10) mmHg and the temperature maintained at 32 (+/-2) ^{0}C. The mean pressure and flows were recorded by the RM3 in exactly the same way as for the hypothermic perfusion kidneys. In addition, the urine output was recorded and collected for biochemical analysis. Arterial and venous perfusate samples were taken at the start of perfusion and hourly thereafter for 'blood gas' analysis. The blood gas analysis was to ensure adequate oxygenation and pH homeostasis, and allow correction where necessary. If the arterial pO_2 was less than 60kPa then the flow rate of the equilibrating gas (see below) was increased by 1 litre/minute. If the perfusate pH was less than 7.35 then 10mmol $NaHCO_3$ was added to the venous reservoir of the system. The venous samples were also sent for biochemical analysis (see chapter 6). After 3 hours the kidney was removed from the apparatus and flushed with cold UW solution, re-weighed and biopsied, and treated in exactly the same way as the hypothermic perfusion group kidneys until transplantation.

4.7 Warm perfusion apparatus

The RM3/Mox-100 system was adapted to re-route perfusate away from the counter-current cooling unit, and instead through a Capiox SX 10 paediatric heat-exchanger and oxygenator unit (figure 4.3 (a) and (b)). The warm perfusion circuit is therefore based on the concept of a cardiopulmonary bypass circuit, designed for a single organ instead of a whole body. The renal venous system drains into a reservoir, from whence perfusate is pumped through a heat exchanger to achieve thermoregulation, thence through a hollow fibre oxygenator to achieve gas exchange and pH homeostasis. The gas passed through the oxygenator is 95% oxygen, and 5% carbon dioxide, the latter for pH homeostasis, in conjunction with the bicarbonate buffer in the perfusate. The gas flow rate is set at between 0.5 and 1 litre per minute, and varied according to p_AO_2 (as above). From there the oxygenated and warm perfusate enters the arterial chamber, where the temperature and pressure are recorded, and through and arterial limb perfuses the cannulated renal artery. The perfusate is thus re-circulated. The modification to the system increases the priming volume slightly compared with the hypothermic perfusion system, and therefore the system is primed with 700 ml of perfusate.

4.8 Warm Perfusate

This consists of tissue culture medium designed to support vascular endothelial cell culture, emulsified with perfluorodecalin (immiscible liquids), for oxygen carriage, and with albumin for oncotic pressure to balance the transudative force of perfusion pressure therefore reduce oedema. Tracer creatinine is also added for measurement of creatinine clearance. Antibiotics are added to reduce the risk of sepsis to the kidney and subsequently the pig post-transplant. The precise composition of the perfusate, per litre is

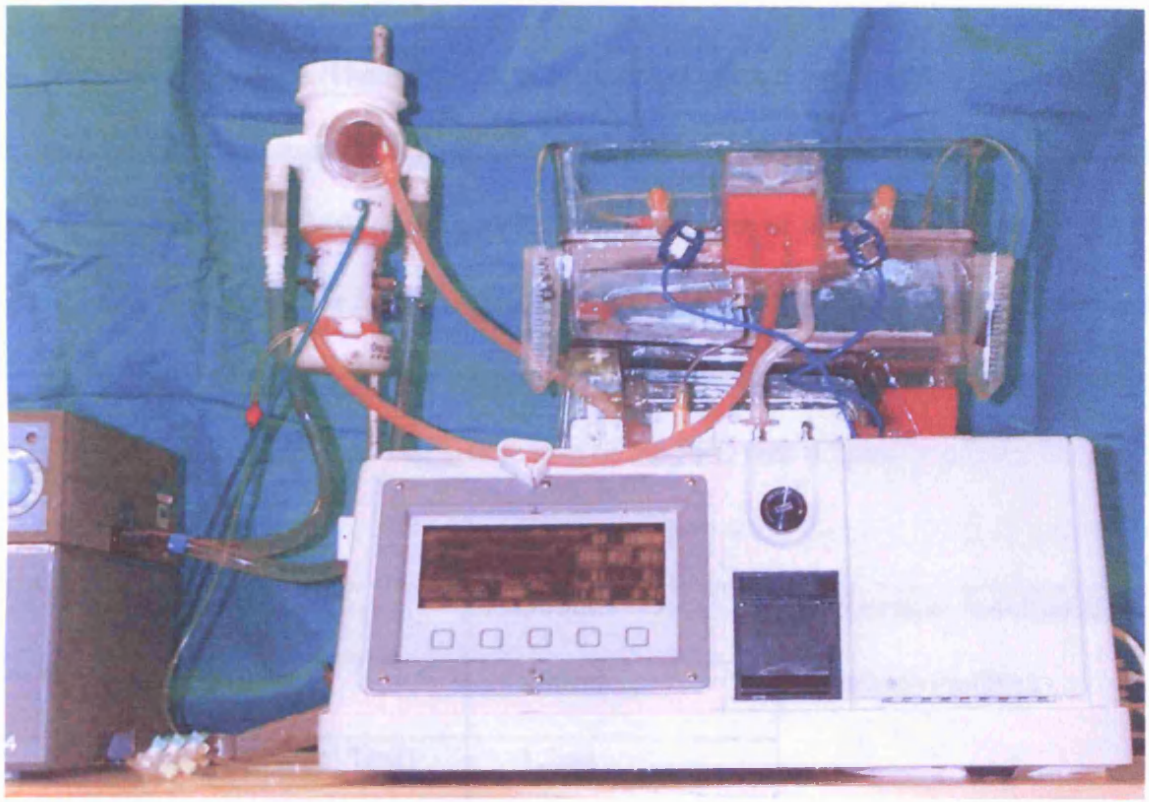


Figure 4.3 (a): The Waters hypothermic perfusion system adapted to incorporate waterbath and oxygenator for WP.

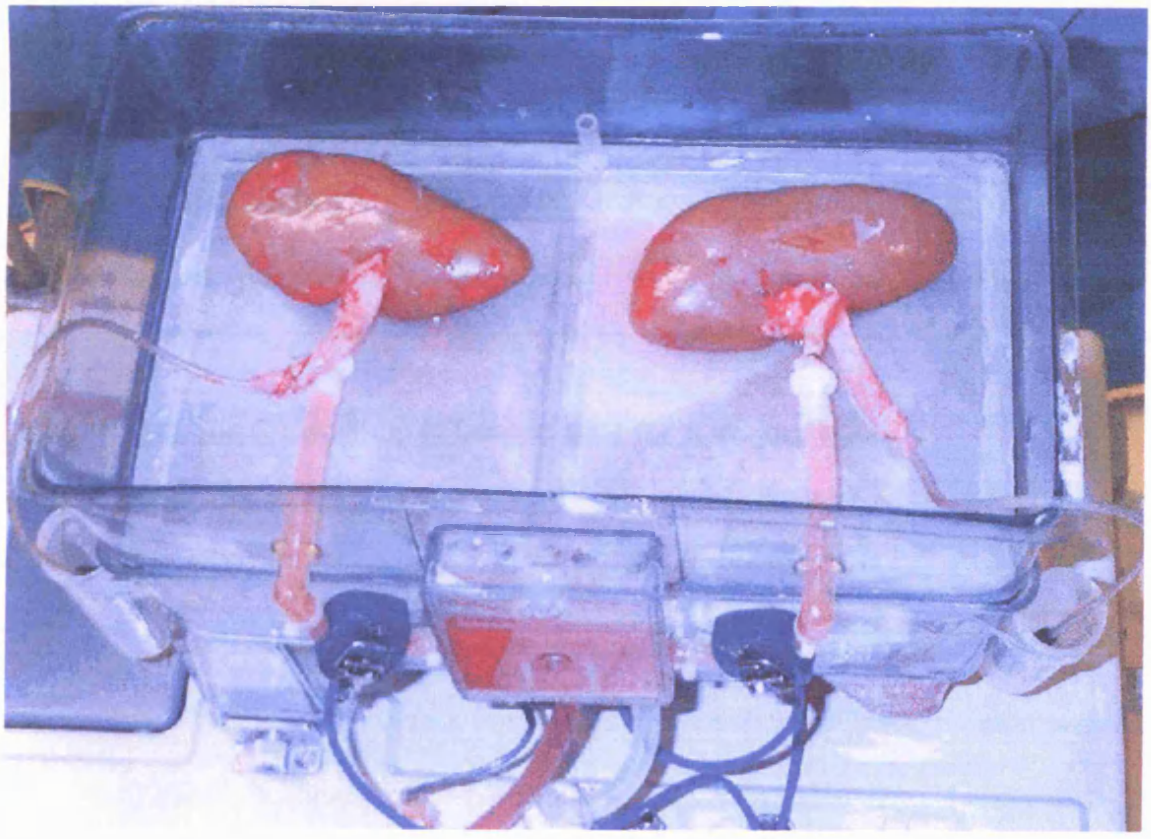


Figure 4.3 (b): View from above of WP apparatus, showing cannulation of the renal arteries and ureters.

detailed in table 4.1. The emulsion process is performed under sterile conditions, and 0.4 micron filters are used to further reduce the chances of contamination. The emulsion is performed with the basic culture medium at a strength of 40%PFD w/v, this is then 'diluted' with the other ingredients to result in a final PFD proportion of 12% w/v. This is necessary so that the protein components of the medium are not denatured in the emulsion process. The mean particle size of the PFD was 0.2μm.

RPMI 1640	500ml	Vascular endothelial culture medium
Foetal Bovine Serum	200ml	
Glutathione (100mmol/litre)	1.2ml	
Soluble Insulin (100u/ml)	0.2ml	
Bovine albumin (culture grade)	60g	Oncotic pressure
Streptomycin	40mg	Antibiotics
Benzyl penicillin	100mg	
PFD 40%w/v in RPMI	300ml	Oxygen carriage
Creatinine (anhydrous)	0.05g	Creatinine clearance

Table 4.1: The composition of the perfusate for warm perfusion.

4.9 Auto-transplant model

4.9.1 Animals and husbandry

Female large white pigs weighing 30 to 55 Kg were housed in the research facility for a minimum of 2 weeks prior to any procedure. These came from recognised breeding establishments and were clear of common porcine pathogens on screening. They were fed a standardised diet. They had access to water ad libitum at all times. They were individually housed and allowed out for exercise and socialisation twice a day except on operative days or when unwell.

4.9.2 Anaesthetic

Animals were starved from midnight of the day prior to surgery. 1.5 mg/Kg azaperone im was used as a premedication 20 minutes prior to inducing general anaesthesia. An ear vein was cannulated and 10 to 40ml of propofol was used to induce anaesthesia, permitting orotracheal intubation with size 7 or 7.5 human ET tubes. The airway was secured by inflating the cuff with 5 ml air. Deep general anaesthesia was established on isofluorane, in the range of 2-5% inspired fraction being required. 0.5mg of atropine and 0.05mcg/kg buprenorphine were given intramuscularly once intubated. No neuromuscular blocking agents were used, and the animals ventilated spontaneously throughout. During surgery, an FiO_2 of 40% maintained oxygen saturations of 95-100% as measured by infra-red saturation monitor on one of the ears. In general, a respiratory rate of 20-30, and a pulse rate of 80-100 were consistent with deep anaesthesia, sufficient for the surgery to proceed. Once 'permanent' iv access was obtained, 625 mg of Augmentin and 1.5 litres of warm 0.9% saline was infused per operation, and any significant blood loss was replaced by haemaccel.

4.9.3 Line Placement

The animal was placed in the left lateral position, and the right side to the dorsum of the neck was shaved, washed with soap, and prepared with aqueous povidone iodine solution. The area was squared off with sterile drapes. The surgeon scrubbed, and operated wearing sterile gown and gloves. A transverse incision was made centred on the midpoint between the anterior tuberosity of the humerus and the angle of the mandible (figure 4.4(a)). The incision was through skin and platysma, deep to which lay the external jugular vein (figure 4.4(b)). In pigs this is generally larger than the internal jugular vein. The vein was dissected and controlled between ligatures. It was ligated in continuity cephalad with 3/0 vicryl. A 40 cm double lumen Vascath was tunnelled from a small incision in the midline at the dorsum of the neck through loose fascia deep to platysma, and the end brought out into the lateral wound (figure 4.4(c)). Both lumens were flushed with heparinised saline. A small venotomy was then made in the external jugular vein, and the catheter tip introduced approximately 5 cm into the vein (figure 4.4(d)). The ease of aspiration and flushing of both lumens was tested with a 20 ml syringe of hepsal, and the tip position adjusted until both were satisfactory. The vein was then tied down onto the catheter with 3/0 vicryl. Haemostasis was assured with monopolar diathermy, and the platysma was closed with 2/0 vicryl, and the skin with 2/0 prolene sutures (figure 4.4(e)). The lines were then locked with the intra-luminal volumes of 1:5000 heparin. This was always removed and replaced before and after any blood sampling or intravenous administration to prevent systemic bleeding and line thrombosis. The lines were always handled using aseptic technique.

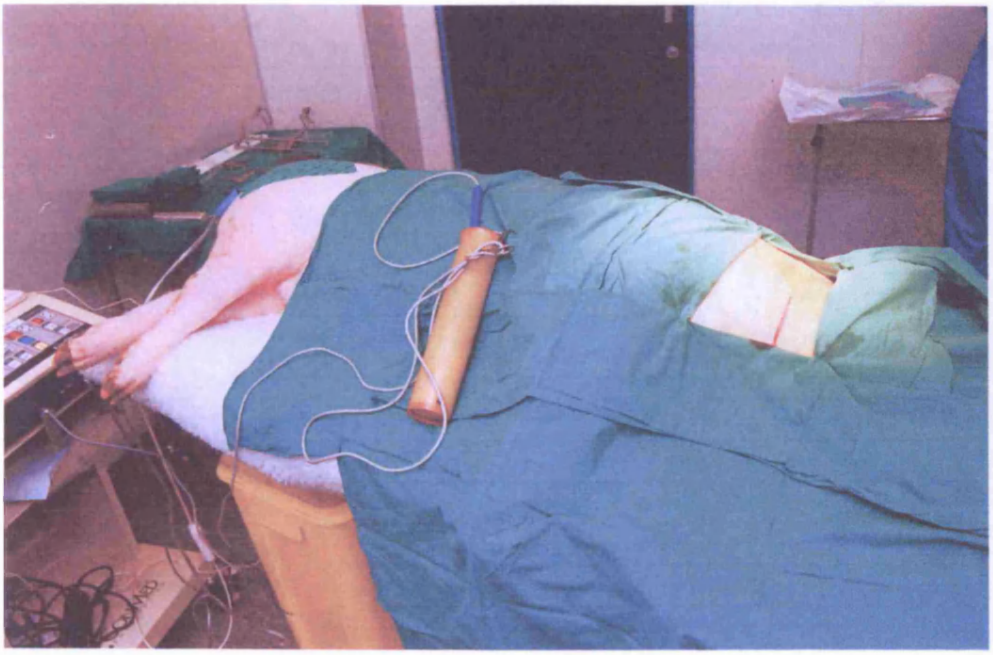


Figure 4.4 (a): Postion of pig and incision for insertion of permacath.

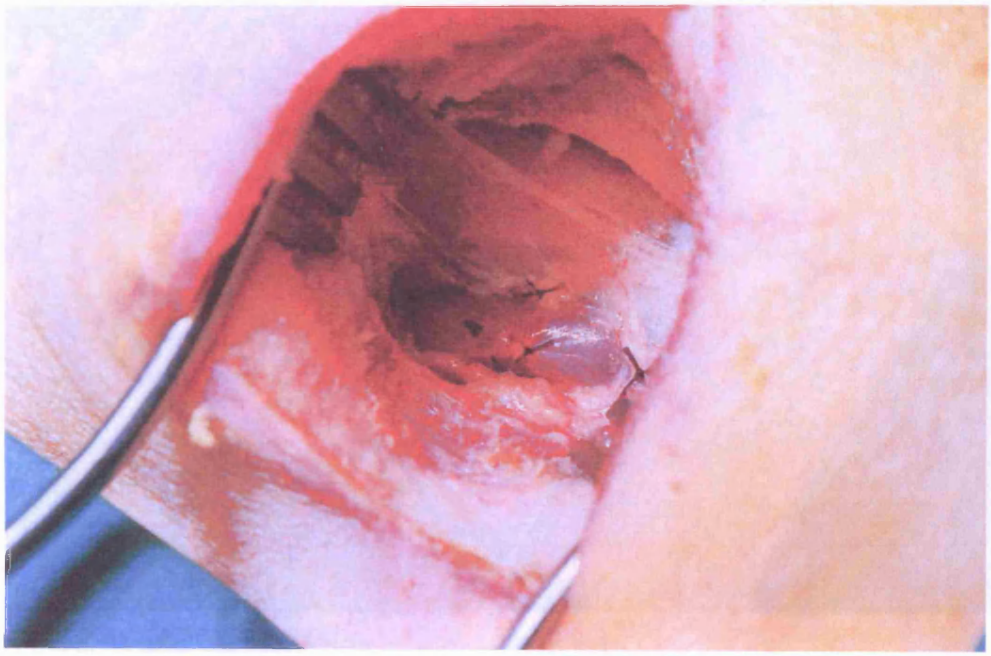


Figure 4.4 (b): The external jugular vein dissected and small tributaries divided.

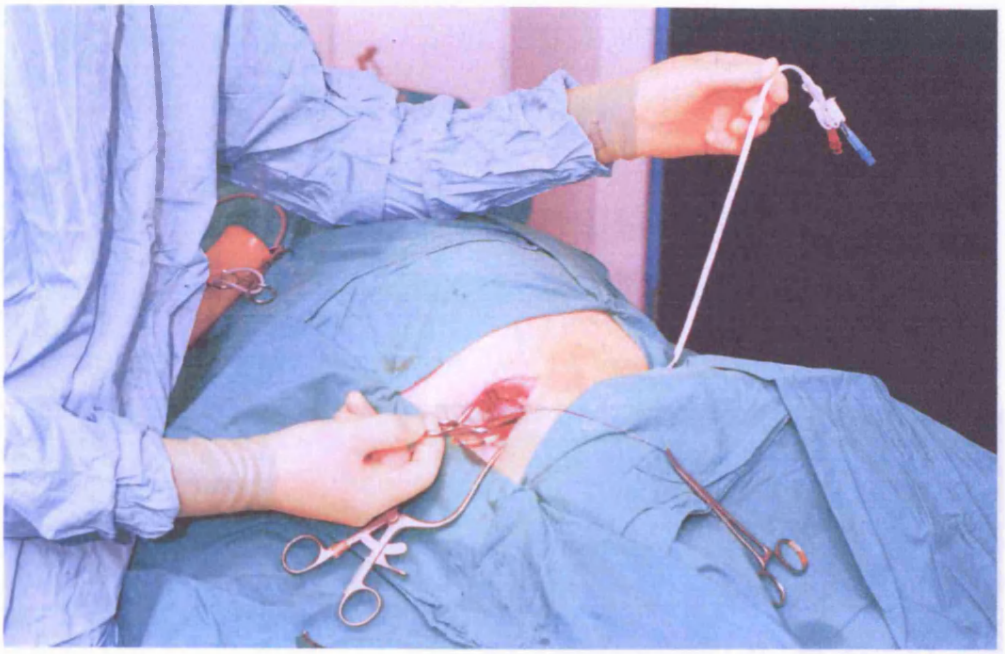


Figure 4.4 (c): The permacath tunnelled from the dorsum of the neck to the EJV.

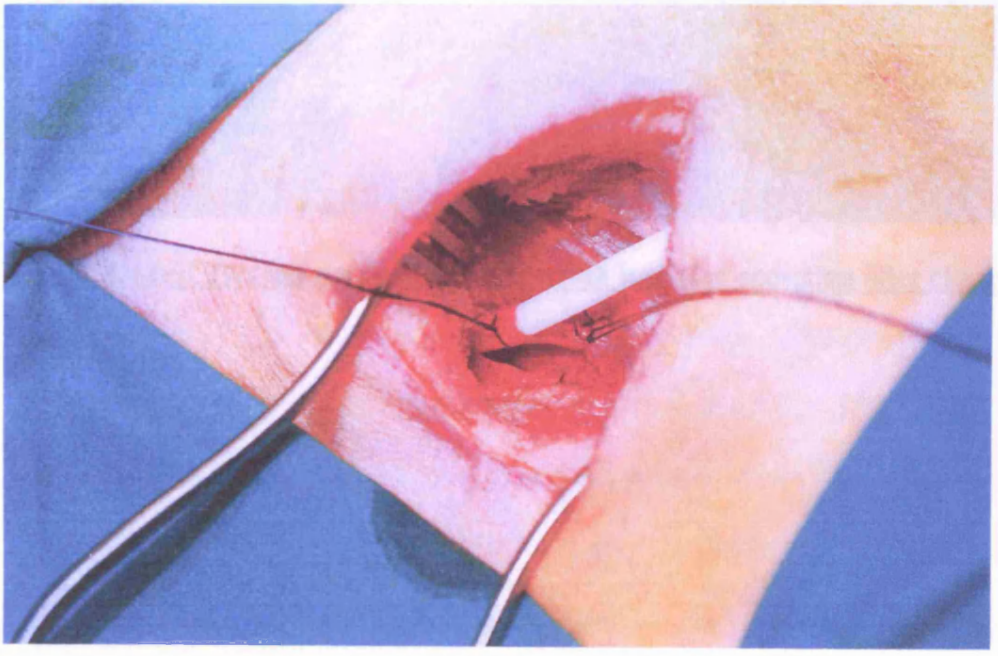


Figure 4.4 (d): The permacath in the vein, and the vein ligated proximally.

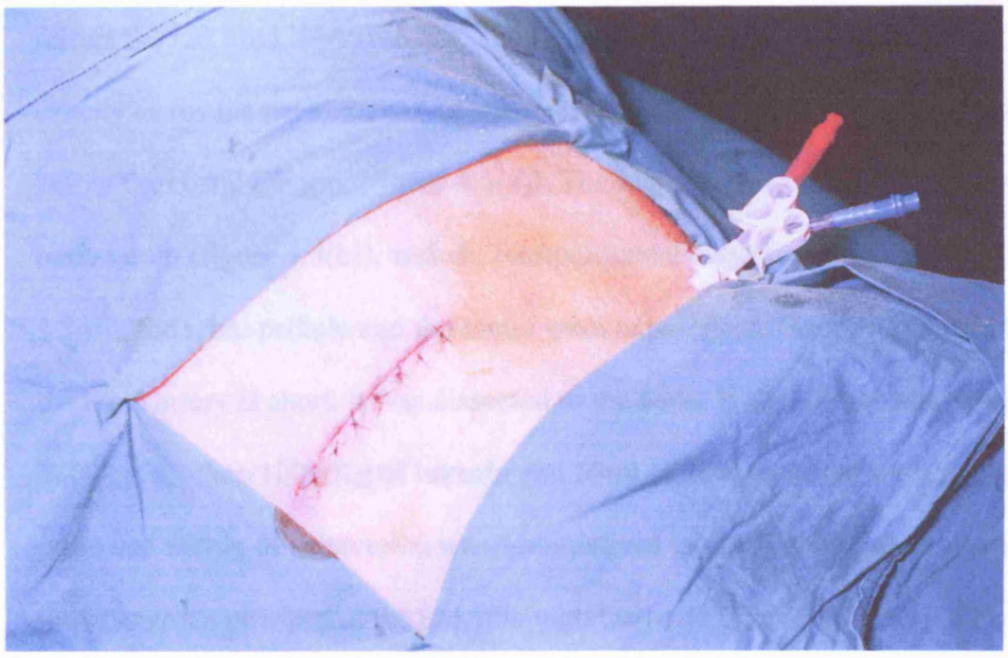


Figure 4.4 (e): The end result of the permacath insertion operation after closure.

4.9.4 Nephrectomy

The pig was then re-positioned whilst still under general anaesthesia, such that the thorax was in the right lateral position, and the pelvis is supine. This position was achieved by laying the animal on the right hand side, then using a crepe bandage to retract the left hind leg to the left. The anterior and left lateral abdomen was prepared exactly as for the neck above. An subcostal incision was made one finger's breadth below the costal margin, (figure 4.5(a)). The incision was muscle cutting down to the peritoneum (figure 4.5(b)), and via retroperitoneal dissection the left kidney (figure 4.5(c)), the renal pedicle and the ureter were exposed and dissected (figure 4.5(d)). As the renal artery is short, it was dissected to the aorta. If the animal was in a thirty minute WIT group, then 100u/Kg of heparin and 50ml of 20% mannitol were given iv at this point and 600μg of papaverine was administered topically to the renal artery, and then the artery was clamped at the junction with the aorta. The rest of the pedicle and ureter were then dissected. If the animal was in a zero WIT group, then the dissection was completed prior to administration of drugs and cross clamping. In this way the total operative times were similar irrespective of WIT assigned. In either case, a wedge biopsy of the renal cortex was taken immediately prior to the arterial clamping. There was only one animal in the study with two left renal arteries. The lower polar artery of this kidney was anastomosed end to side with the main renal artery with 8/0 prolene. On flushing this kidney, it appeared that approximately 20% of the renal cortex was supplied by the polar artery. This kidney was transplanted subsequently in the same way as all the others. The 'golden triangle' between pedicle, ureter and aorta was preserved to maintain ureteric blood supply. The renal vein was dissected to the level of the adrenal vein, which always afforded 0.5-1cm longer vein than artery. Particular care was taken to

control and ligate large posterior lumbar branches of the renal vein during dissection. The ureter was dissected to the pelvic brim, where it was ligated with 3/0 vicryl and divided. Ten minutes prior to the expected time of explantation, 1 litre of ice-cold Marshall's hyperosmolar citrate solution was poured into a sterile kidney preparation dish, and kept cold with sterile frozen 250ml saline packs (still in polythene bags). 500ml of UW at 4^0C was run through a giving set, to the end of which was attached a small Tibbs' cannula for renal artery perfusion. At the time of explantation the renal vein was clamped proximally, divided and the organ removed, and perfused as described in Chapter 4. The renal vessel stumps were then double tied with 2/0 vicryl, and where the stumps were short and potentially insecure, oversewn with 4/0 prolene. Haemostasis was assured, and mass closure of the muscle layer undertaken with loop nylon, the skin was closed with 2/0 prolene. The isoflourane was stopped, and when the pig recovered its cough reflex the oxygen was stopped and the animal extubated. Once it was clear that the animal was protecting its airway, it was recovered in a clean single pen. The post-operative analgesia regimen was 0.5 mcg of buprenorphine iv 8 hourly for 72 hours post the nephrectomy operation. Pulse and temperature were monitored 3 times per day for the duration of the animals survival. On both post-operative days a further 1.5 litres of 0.9% saline were given in 3 divided infusions to prevent dehydration whilst the animals may not have been drinking normally.

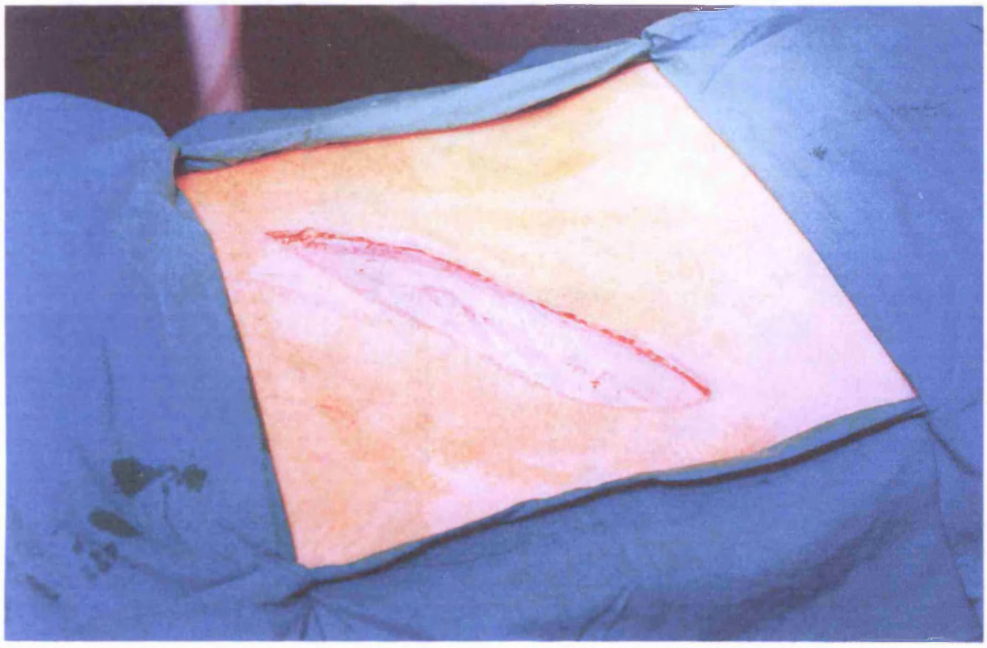


Figure 4.5 (a): Subcostal incision for nephrectomy operation.

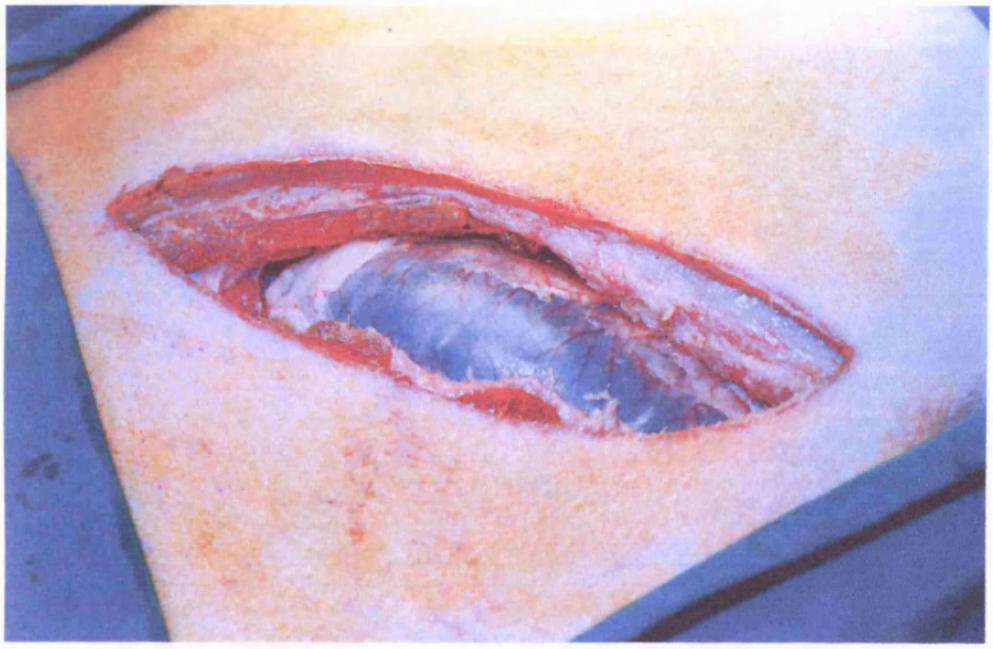


Figure 4.5 (b): Incision deepened to peritoneum.

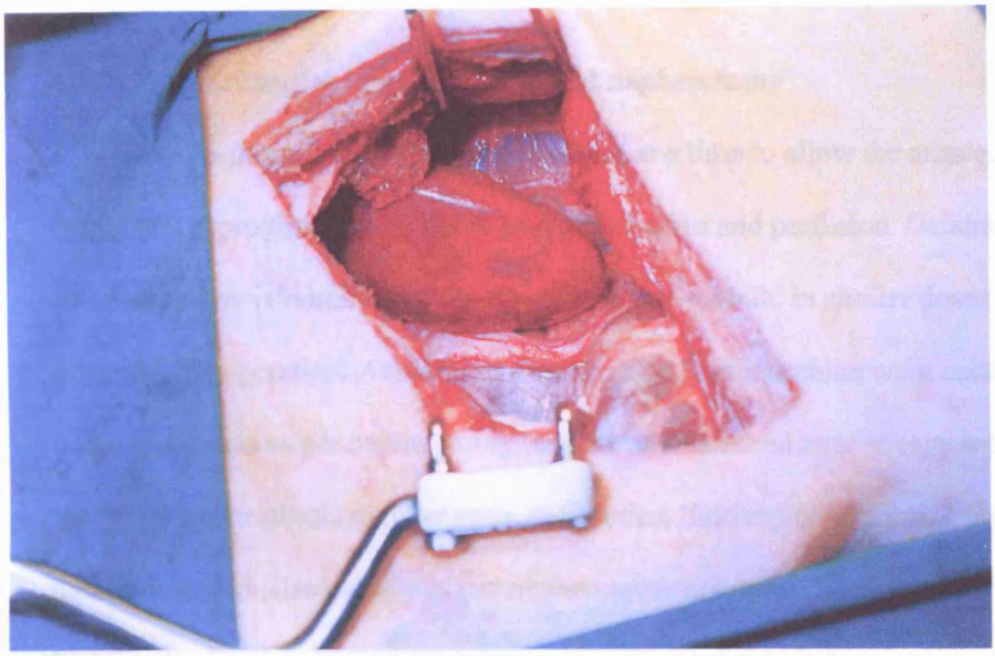


Figure 4.5 (c): Retroperitoneal dissection to expose kidney.

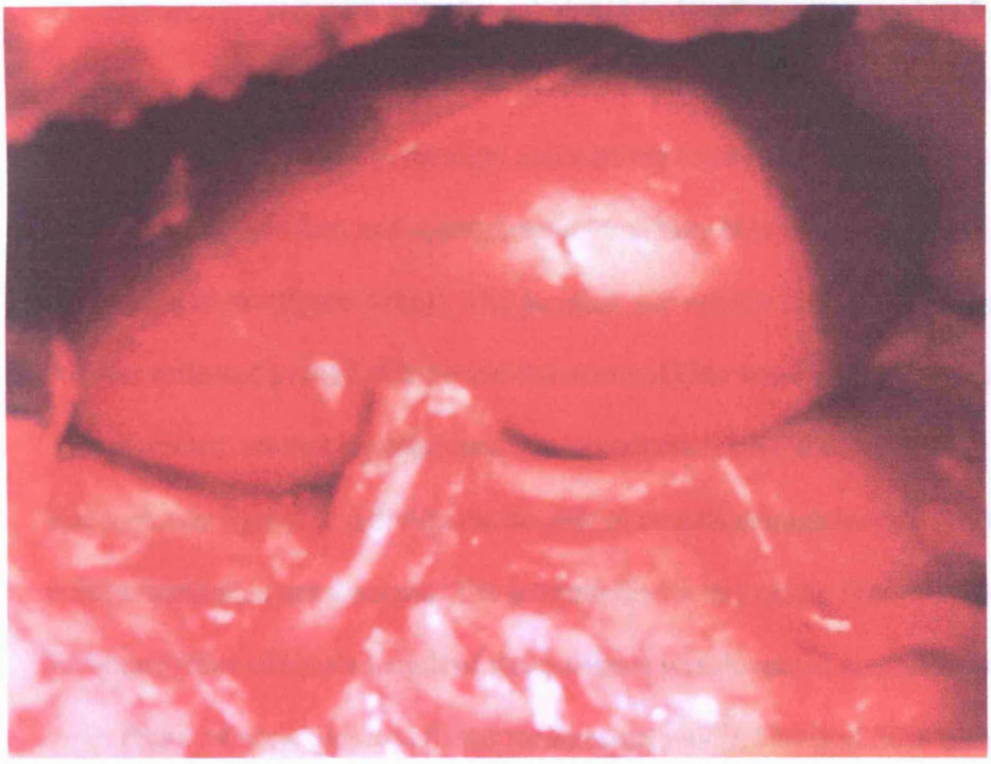


Figure 4.5 (d): Pedicle dissected to expose renal vein, artery and ureter from left to right.

4.9.5 Transplant and contralateral nephrectomy

The auto-transplant operation was begun at a time to allow the anastomoses to be completed approximately 24 hours post explanation and perfusion. General anaesthesia was induced by iv administration of propofol per vascath, in similar doses to the nephrectomy operation. Azaperone, atropine and buprenorphine were used in the same doses and routes as per nephrectomy, as part of a balanced anaesthesia. Intubation and isofluorane anaesthesia maintenance, antibiotics, fluid replacement and physiological monitoring were also exactly as per nephrectomy operation. The animal was placed so that the chest was in the left lateral position and the right hind leg retracted by crepe bandage so that the pelvis was supine. In this way the peritoneum tended to fall away from the implantation site during the retroperitoneal dissection, facilitating access.

The anterior and right anterolateral abdominal wall was prepared as for the other surgical procedures. An incision was made from the midline just above the symphysis pubis, extending laterally to the right and curving cephalad to finish 4 finger's breadth lateral to the nipple line, and approximately half way between the hind leg skin fold and the costal margin (figure 4.6(a)). The incision was muscle cutting to the peritoneum, and this was reflected to the left to reveal the external iliac vessels (figure 4.6(b)). These were dissected, arterial branches and venous tributaries ligated and divided. The internal iliac vein was ligated and divided to be able to control enough length of external iliac vein for transplant anastomosis. The internal iliac artery origin is higher and was not dissected. Once adequate length of these vessels was dissected and controlled between slings (figure 4.6(c)) (approx. 8 cm) for transplantation, 50mls of 20% mannitol was given iv, then the external iliac vein was controlled between vascular clamps. A venotomy was made with a number 11 scalpel blade so that the total length approximated the diameter of the left renal vein. The opened isolated segment of vein was flushed with

hepsal. Four 6/0 prolene stay sutures were used to quarter the venotomy (figure 4.6(d)), then the kidney was taken out of ice, a wedge biopsy taken, and the time recorded. The venous anastomosis was performed with continuous 6/0 prolene (figure 4.6(e)). The renal vein was occluded by a vascular clamp, and the clamps on the external iliac vein were released, proximal then distal, and the anastomotic integrity confirmed (figure 4.6(f)). The external iliac artery was then isolated between vascular clamps and the renal artery was manoevered to assess whether it would lie better proximal or distal to the vein, most often distal was a better orientation. An arteriotomy was then fashioned in the same manner as for the venotomy, and the anastomosis was performed in the same manner (figure 4.6(g)). The renal vein clamp was released, and then the distal arterial clamp, finally the proximal arterial clamp. The time to release of arterial clamps was recorded, and the difference between this and the time out of ice recorded as the anastomosis time. The reperfused kidney is shown in figure 4.6(h). The bladder, by now full as a result of the effect of the mannitol, was identified, and a neoureterocystotomy was performed by the onlay technique with continuous 4/0 PDS, over an 8cm 4.7 French double J stent (figures 4.6(i) and (j)). Through the upper end of the incision, the right kidney was bluntly dissected by hand until the pedicle was isolated and then the pedicle and ureter were clamped. The renal pedicle and ureter were divided and the kidney removed. The pedicle was double tied with 2/0 vicryl and the ureter ligated with 3/0 vicryl. Haemostasis was assured. 30 minutes post-anastomosis a further wedge biopsy of the transplant was taken. The lie of the kidney without kinking of the renal vessels was confirmed prior to the mass closure of the muscle layer with loop nylon (figure 4.6(k)). The skin was closed with 2/0 prolene (figure 4.6 (l). The animal was recovered and post-operative observations and fluid administration were identical to the nephrectomy operation.

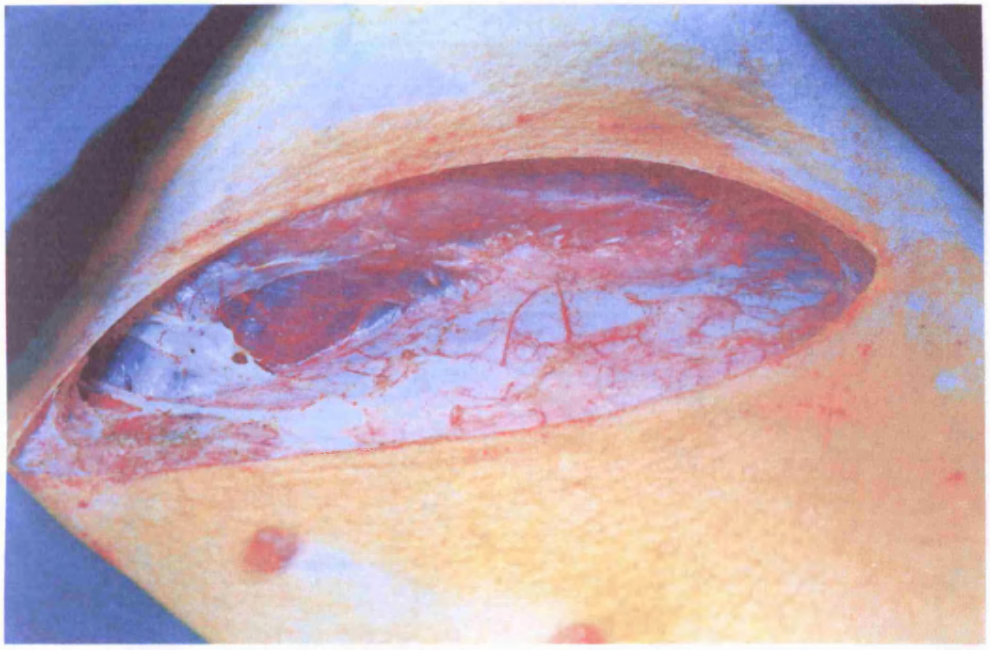


Figure 4.6 (a): Groin incision for autotransplant.

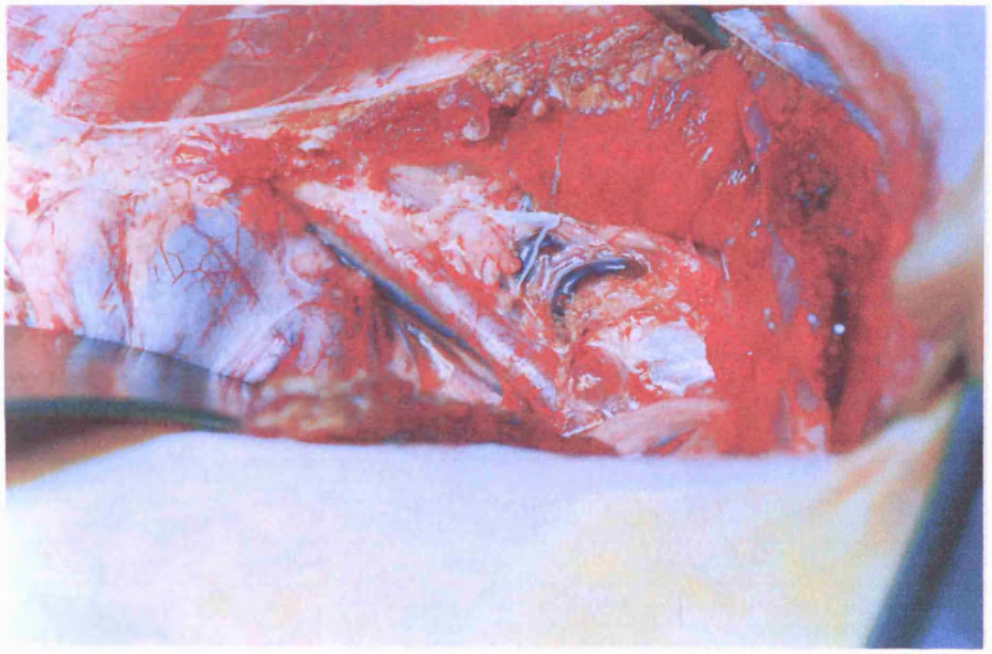


Figure 4.6 (b): Right iliac vessels exposed by retroperitoneal dissection.

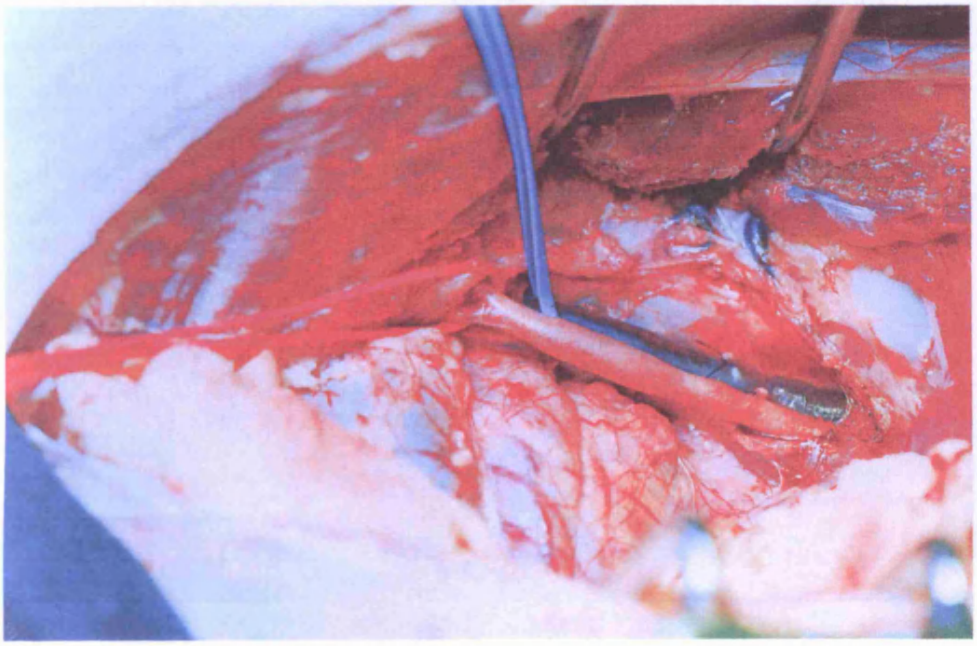


Figure 4.6 (c): Vessels dissected and controlled with branches/tributaries ligated and divided.

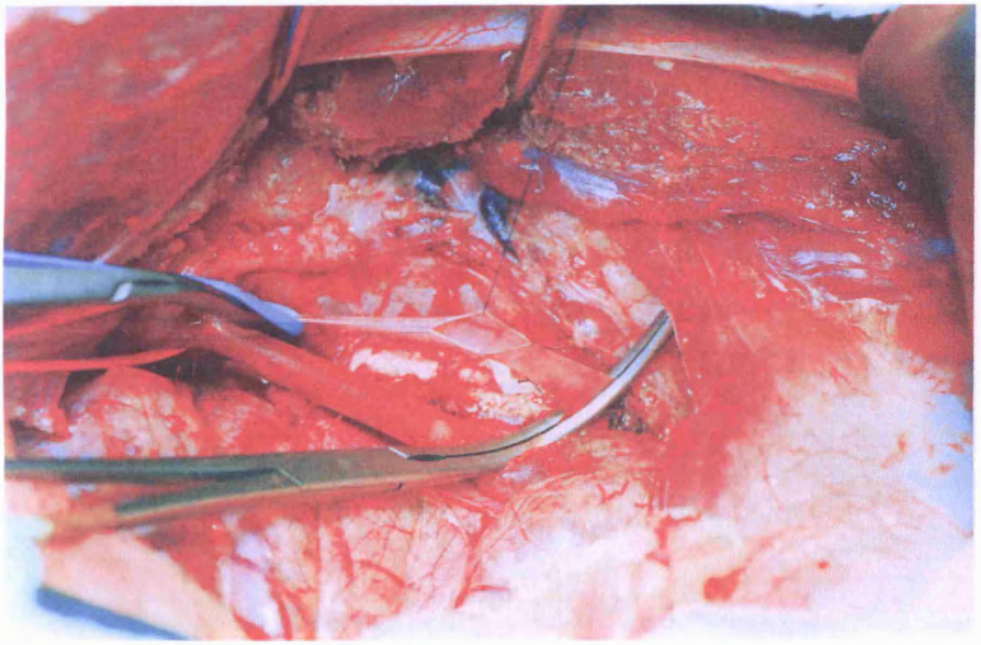


Figure 4.6 (d): Renal vein clamped and quartered with 6/0 prolene sutures.

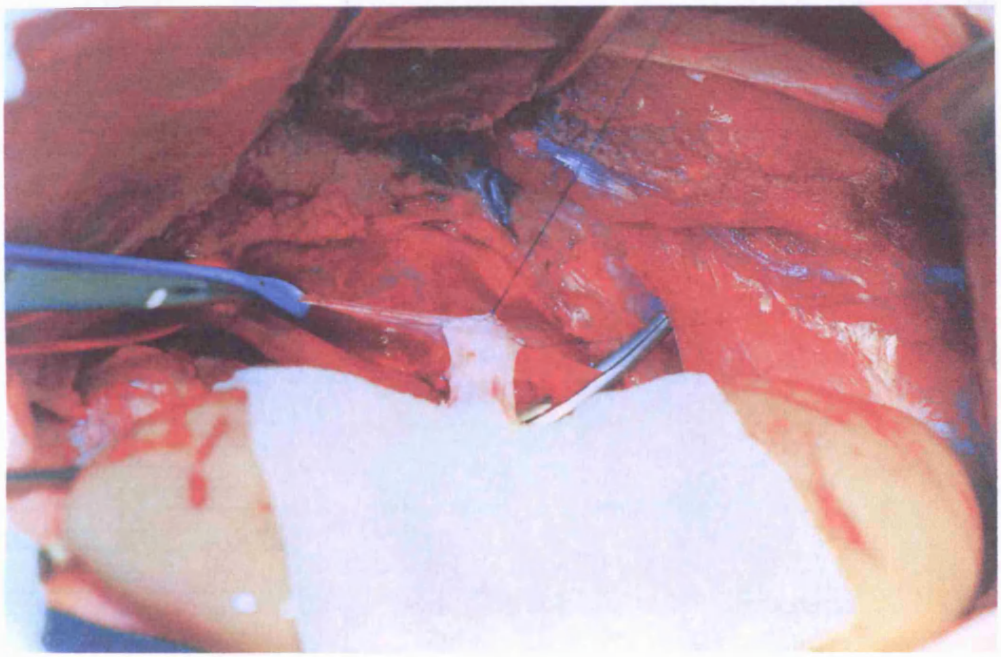


Figure 4.6 (e): The venous anastomosis with 6/0 prolene.

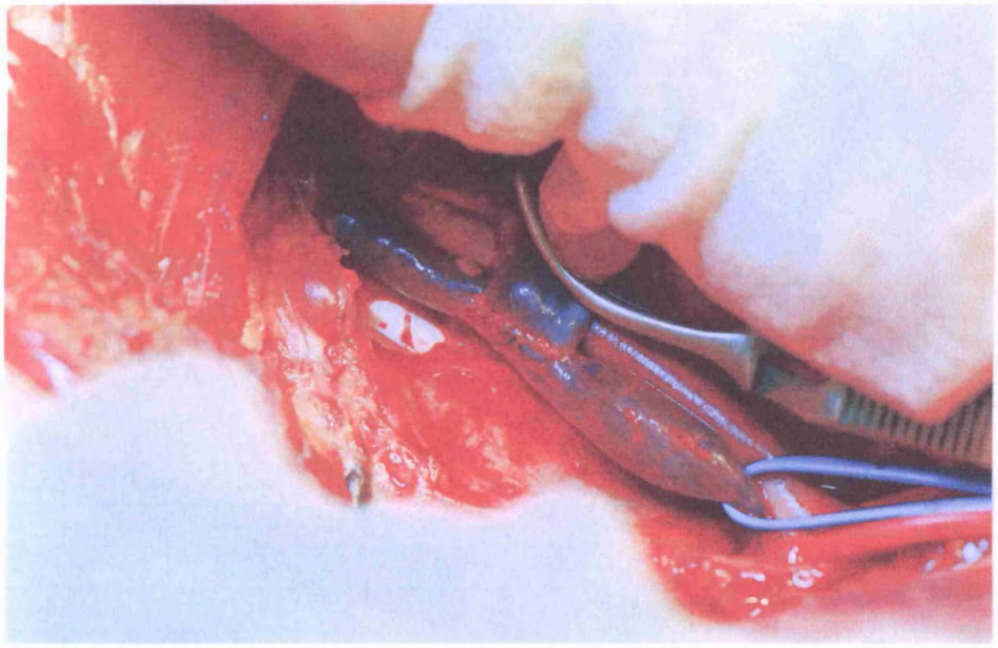


Figure 4.6 (f): The venous anastomosis is completed and the integrity tested, with a bulldog clip on the renal vein to prevent retrograde perfusion and re-warming.

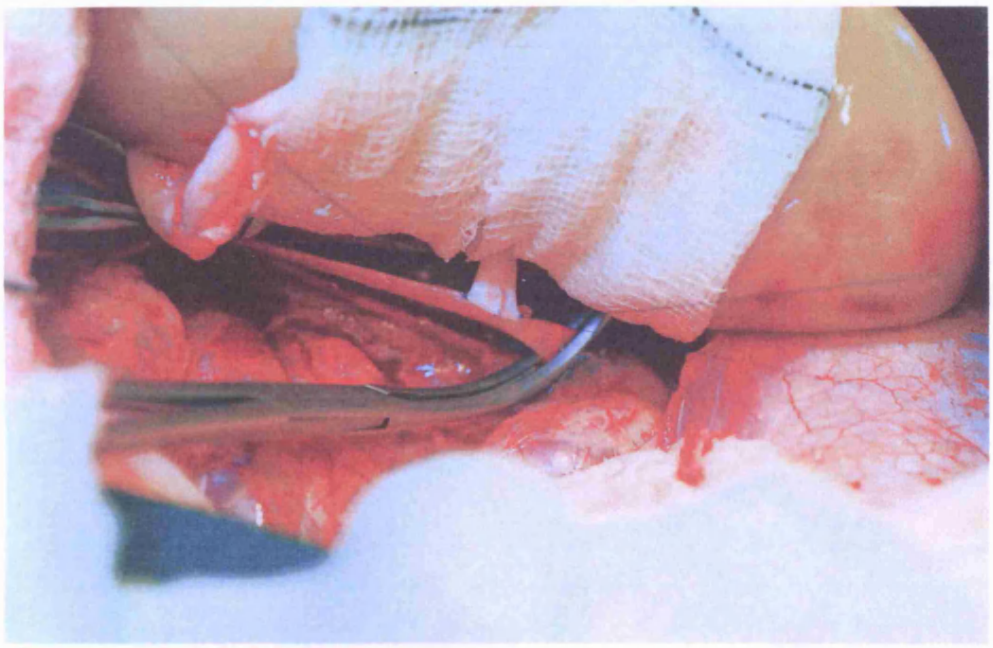


Figure 4.6 (g): The arterial anastomosis is fashioned in the same manner as for the venous.

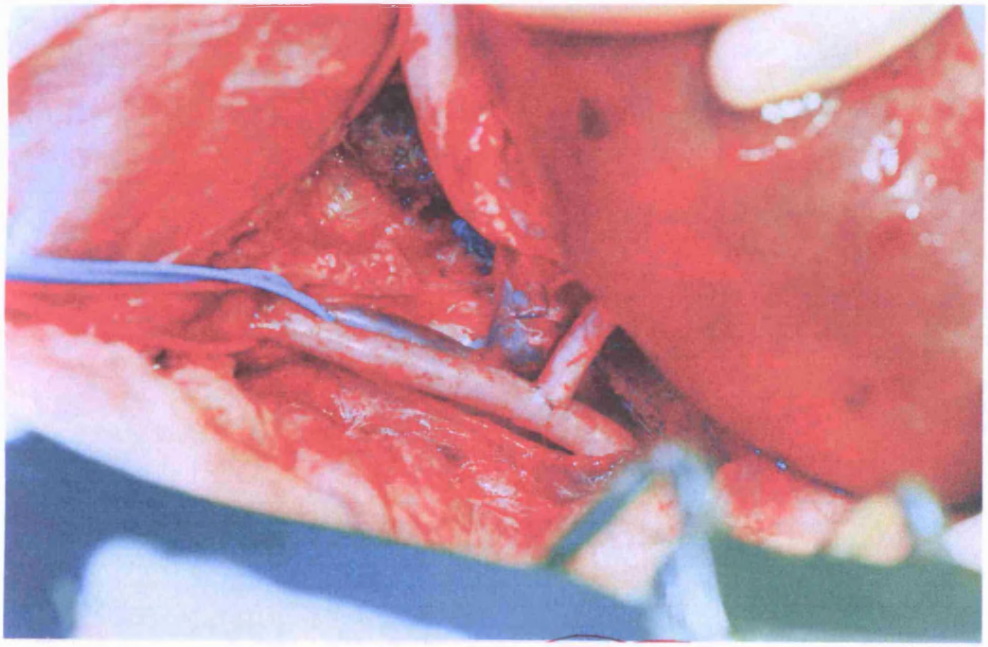


Figure 4.6 (h): Both anastomoses are complete, and the clamps are removed, reperfusing the kidney.

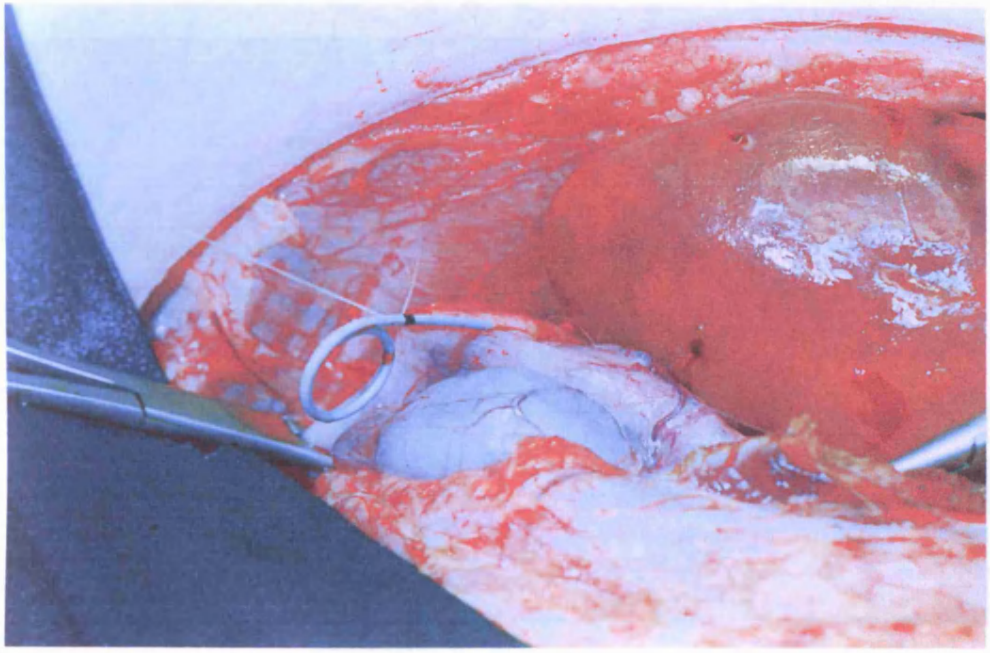


Figure 4.6 (i): Part of the dome of the bladder is controlled by a vascular clamp, and an 8Fr double J stent is inserted in the ureter.

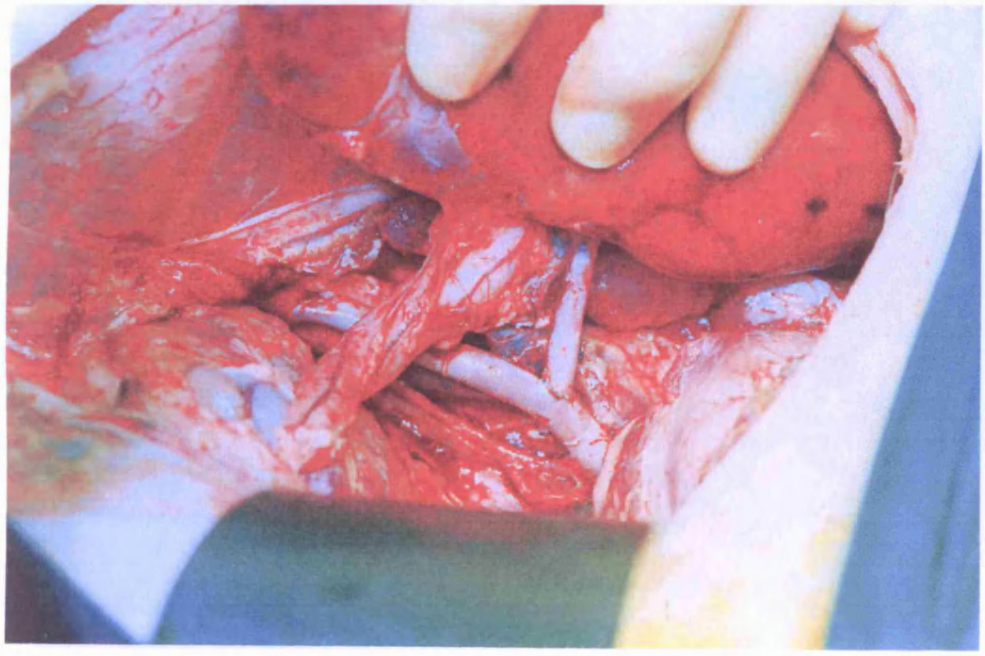


Figure 4.6 (j): All 3 anastomoses are complete and the kidney is well perfused, the ureter is distended with urine.

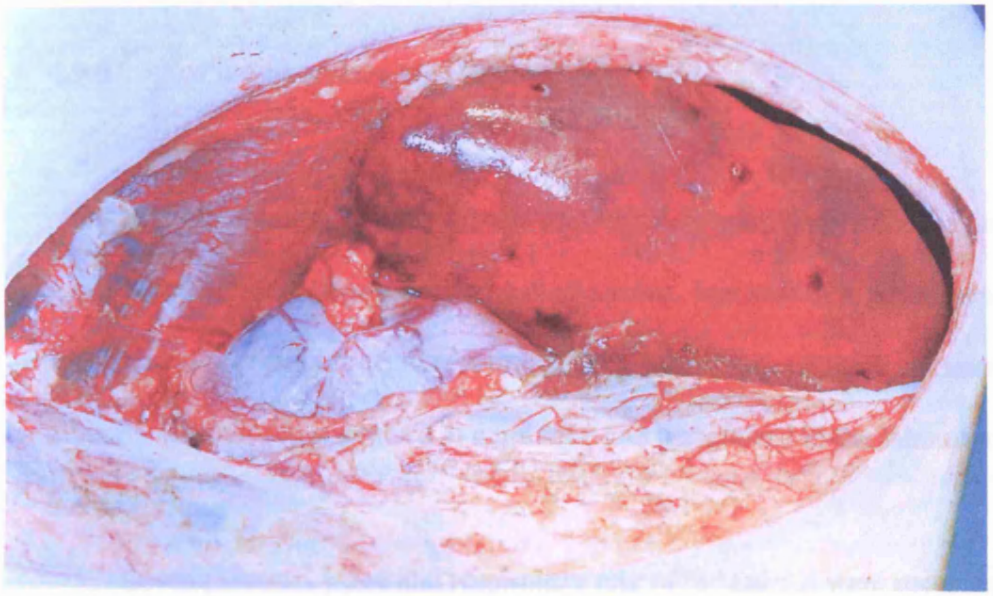


Figure 4.6 (k): The lie of the kidney is arranged so as not to kink any of the vessels or place anastomoses under tension. A short tunnel has been fashioned for the ureter.

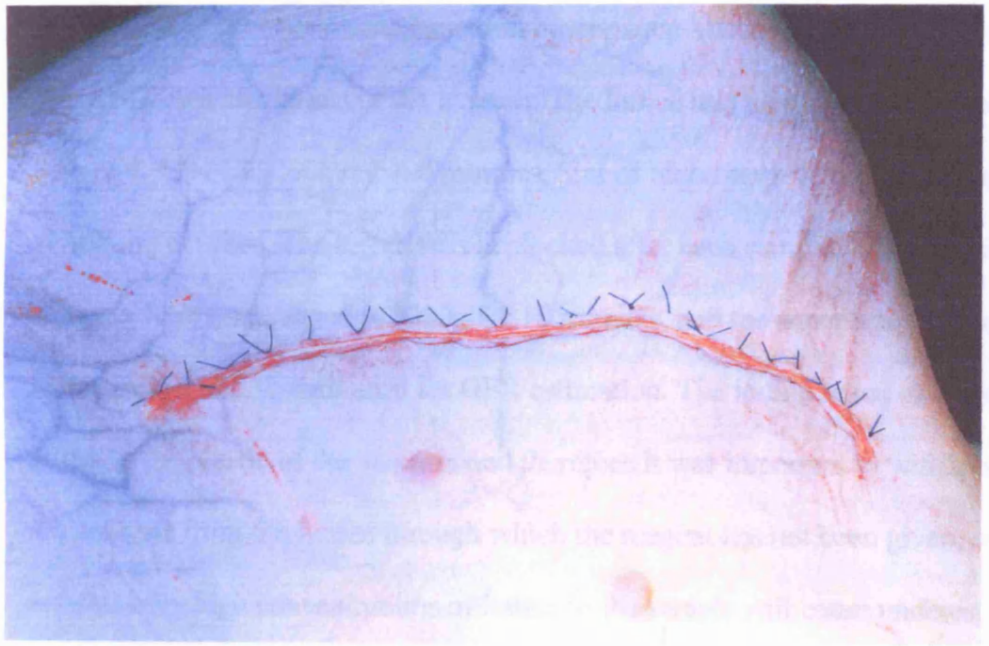


Figure 4.6 (l): The transplant (and contralateral nephrectomy wound is closed.

4.9.6 Post-operative monitoring

Daily blood samples were withdrawn from the central line, after the heplock and 5 ml of blood had first been aspirated and discarded. 5ml was then withdrawn for biochemical analysis of serum creatinine, urea, sodium, potassium, calcium, and albumin. The blood was stored in a gel tube and transferred to the laboratory for same day analysis.

The temperature, pulse and respiratory rate of the animal were recorded three times per day. The wounds were inspected for signs of infection, dehiscence or herniation. Mobility was assessed daily post-operatively, and when moving freely animals were exercised as per pre-operative regimen.

On the 7^{th} day post-transplantation omnopaque 300mg in 20ml 0.9% saline was injected down one lumen of the vascath. The lumen and time of injection were recorded. After 90, 135, 180, 240 and 300 minutes, 5ml of blood were removed as above, and stored in gel tubes. The lumen was heplocked after each sample. The same day the samples were centrifuged at 3000g for 10 minutes, and the supernatant serum aspirated and stored at -80^0C until used for GFR estimation. The iodine based omnopaque reagent sticks to the plastic of the vascath, and therefore it was important to withdraw samples for analysis from the lumen through which the reagent has not been given, or artefactually high concentrations of iodine in the sample will cause underestimation of the GFR (see below).

4.9.7 Termination

On the 14th day post-transplant, the animal was anaesthetised with propofol per central line, and the right-sided abdominal incision was re-opened, and a renal cortical wedge biopsy was taken by direct cut down onto the kidney. The biopsy was treated as below. The animal was then terminated with a massive overdose of phenobarbitone iv. The vascular and ureteric anastomoses were then dissected, and the kidney was removed en bloc with a cuff of bladder and sections of the external iliac vessels. The patencies of the anastomoses were confirmed. The presence of the ureteric stent was also confirmed. The kidney was then bi-valved in the coronal plane from cortex to pelvis, and any pelviceal dilatation or frank cortical necrosis noted.

In the event of the pig attaining the criteria for early termination, the above procedure were performed at any time prior to day 14. The circumstances under which an early termination was indicated were animal distress in excess of the severity limit permitted in the Home Office licence. The principal indicators of unacceptable animal suffering were vomiting, immobility and crying. Anorexia was a common transient finding, and did not warrant termination on its own. Biochemical parameters of renal function alone were not used to make decisions regarding early termination.

When an animal died unexpectedly, the same surgical procedure was performed on the body. A post-mortem examination of the intraperitoneal and intrathoracic organs was also performed to exclude causes of death other than renal, principally infection remote to the transplant. Signs of generalised bleeding diathesis were also sought, as uraemia causing platelet dysfunction was deemed a renal cause of death.

4.9.8 Biopsies

The wedge biopsies were immediately fixed in 10% formaldehyde. The fixed formaldehyde samples were blocked in paraffin between 5 and 7 days post collection, preventing variation between sample in tissue shrinkage. Biopsies were taken in situ and after upon cooling at the donor nephrectomy operation, pre- and 30 minutes post-transplantation, and at termination.

4.10 Biochemistry

The biochemical analysis was performed on an automated Abbott Aroset analyser machine, which measured the blood, perfusate and urine concentrations of sodium, potassium, urea, creatinine, protein and glucose.

Blood gas analyses on arterial and venous perfusate samples were performed on a radiometer ABL 5000 machine, with low pH values corrected with additional sodium bicarbonate. The oxygen consumption in ml/min was calculated by multiplying the difference between arterial and venous partial pressures of oxygen by the solubility of oxygen in perfluorodecalin at standard pressure and 290^0C Kelvin multiplied by the percentage of perfluorodecalin in the perfusate, multiplied by the perfusate flow rate in ml/min through the kidney at the time. The oxygen consumption was then adjusted per 100g of kidney.

$$O_2 \text{ consumption (ml/min)} = (p_aO_2 - p_vO_2) \times 0.001356 \times \text{flow (ml/min)}$$

(0.001356 is the product of the solubility of oxygen in perfluorodecalin (ml/ml/kPa) and the percentage of perfluorodecalin in the perfusate).

4.11 Glomerular Filtration Rates

The GFR serum samples taken at intervals following iohexol administration were analysed by gamma scatter counter, which calculated the concentration of iohexol in the serum by the degree of scatter. The decay of iohexol concentration over time was plotted on a log linear scale ($ln_{concentration}$ against time), and the intercept with the concentration axis used to derive the glomerular filtration rates in ml/min.

4.12 Histology

4 μm sections of formalin preserved biopies were stained with H&E. These were then grouped into in-situ, post WIT, pre-transplantation and 30 minutes post re-perfusion, and terminal samples. They were submitted to Professor Peter Furness for his expert assessment of renal ischaemic damage, for the purposes of which he was blinded to the experimental groups. The state of the tubules was assessed for the in situ, post WIT, pre-transplantation and 30 minute post-reperfusion groups, and given a numerical score of 0 to 3, with 0 representing undamaged tubules, and 3 representing severe damage from which recovery seemed most unlikely. In the slides of the terminal biopsies the tubules were also assessed in the same manner, and in addition the degree of lymphocyte infiltration and the state of the interstitium graded also on a scale of 0 to 3.

4.13 Statistics

Categorical data was analysed by Chi squared test. Continuous non-parametric data were analysed by Kruskal Wallis (KW) test, and where comparisons between individual groups are required the Mann Whitney U (MW U) test was used. For parametric data, ANOVA was used to detect differences between several groups, and the student t-test to differentiate between individual groups. Correlations were evaluated by Spearman's rank correlation. For paired data, Wilcoxon test was used. Significance was determined at $p<0.05$ level throughout.

PART 3

Results and Discussion

Chapter 5

Ex-vivo function versus warm ischaemic time – cadaver experiments

Contents **Page Number**

5.1 Ischaemic time and kidney weight data

10 kidneys were retrieved as quickly as possible after death, with the aim of perfusion within 10 minutes. 6 kidneys were retrieved with a target warm ischaemic time of 30 minutes, and 6 kidneys were assigned to the 60 minute WIT group. The actual WIT for each group are displayed in table 5.1, with the CIT and kidney weights before and after perfusion. Neither the CIT nor the starting weights were different between the groups (p=0.3 and 0.15 respectively, KW test). The <10 minute group did not significantly change weight during perfusion (p=0.12, Wilcoxon), however there was significant weight gain in the 30 and 60 minute groups (p=0.03, both groups, Wilcoxon), despite the weight changes not varying significantly between groups (p=0.15, KW).

WIT Group (minute)	Actual WIT (min:sec)	CIT (minute)	Kidney weight pre-perfusion (g)	Kidney weight post-perfusion (g)	Change in weight (%)
<10 (n=10)	7:05 (6:26 – 7:55)	122 (117-129)	117 (103 – 130)	122 (105 – 154)	5 (0 – 29)
30 (n=6)	30:00 (29:30 – 30:20)	120 (119-125)	120 (105 – 130)	128 (126 – 157)	19 (5 – 24)
60 (n=6)	60:00 (59:00-60:00)	123 (120-125)	130 (128 - 135)	169 (128 - 170)	27 (14–31)

Table 5.1: The WIT, pre- and post-perfusion weights of kidneys undergoing WP

5.2 Intrarenal vascular resistance

The IRR from the start of perfusion increased in each group to reach a peak within a few minutes, then fell steadily thereafter until reaching a plateau, finally exhibiting a late gradual rise towards three hours of perfusion, at which time the perfusion was ended. The groups differed most at the beginning of the perfusion, during the rise and fall from the peak value. The IRR from each group tended towards similar values as they tended to plateau (figure 5.1).

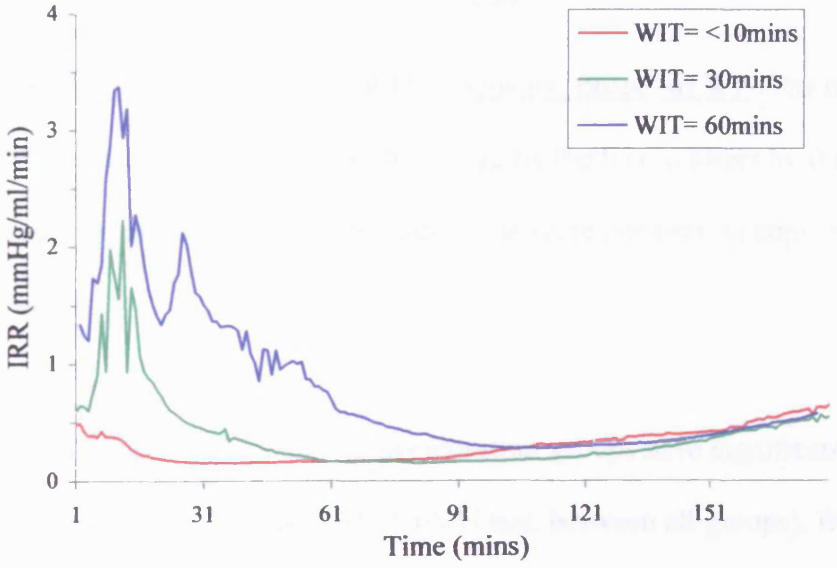


Figure 5.1: The mean trends of IRR over time during WP by WIT group

The IRR has therefore been analysed over three separate perfusion 'phases', the initial 'peak phase' for the first 60 minutes, a 'plateau phase' during the period 90-120 minutes, and the 'late phase' from 150-180 minutes to examine the period of the late rise in IRR (figure 5.2).

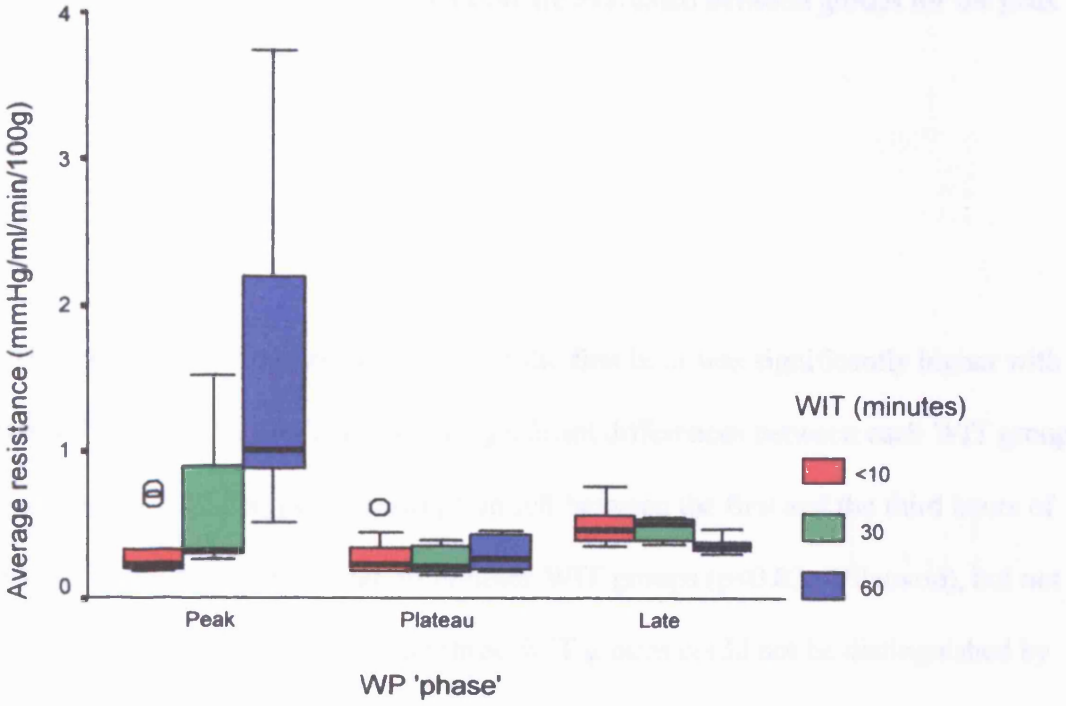


Figure 5.2: IRR between the WIT groups by 'phase' of WP. The median is represented by the heavy bars, the interquartile range by the box, outliers by the whiskers and extreme values are plotted separately. The same convention applies to all subsequent box-plots.

During the peak phase, all preservation groups have significantly different resistances (p=0.003 KW, and <0.05 U test, between all groups). By contrast there were no differences in resistance between preservation groups in the plateau or late phases of WP (p=0.6 and 0.9 respectively, KW).

The resistance tended to fall in all groups between the peak and plateau phase, although only reaching significance in the 60 minute WIT group (p=0.03, Wilcoxon, p=0.6 in the <10 minute group, p=0.1 in the 30 minute group). The late rise in resistance towards the end of perfusion, although modest, was statistically significant for all groups (p=0.03, Wilcoxon, all groups).

Ex-vivo renal metabolism and function are evaluated between groups for the peak phase and the late phase.

5.3 Renal metabolism

The oxygen consumption at the end of the first hour was significantly higher with shorter WIT ($p=0.002$, KW), and with significant differences between each WIT group ($p<0.03$) (table 5.2). Oxygen consumption fell between the first and the third hours of perfusion for the <10 minute and 30 minutes WIT groups ($p<0.03$, Wilcoxon), but not for the 60 minute group ($p=0.6$). The three WIT groups could not be distinguished by oxygen consumption after 3 hours of WP ($p=0.7$, Wilcoxon), see figure 5.3.

	WIT (minutes)			
	Time (hours)	<10	30	60
O_2 Consumption (ml/min/100g)	1	18.4 (16.9-21.1)	14.5 (11.4-16.5)	10.1 (7.5-11.0)
	3	10.0 (8.8-12.8)	10.5 (9.4-11.8)	10.5 (9.6-10.9)

Table 5.2: Oxygen consumption at the end of the first and third hours ofWP

Values are median (interquartile range)

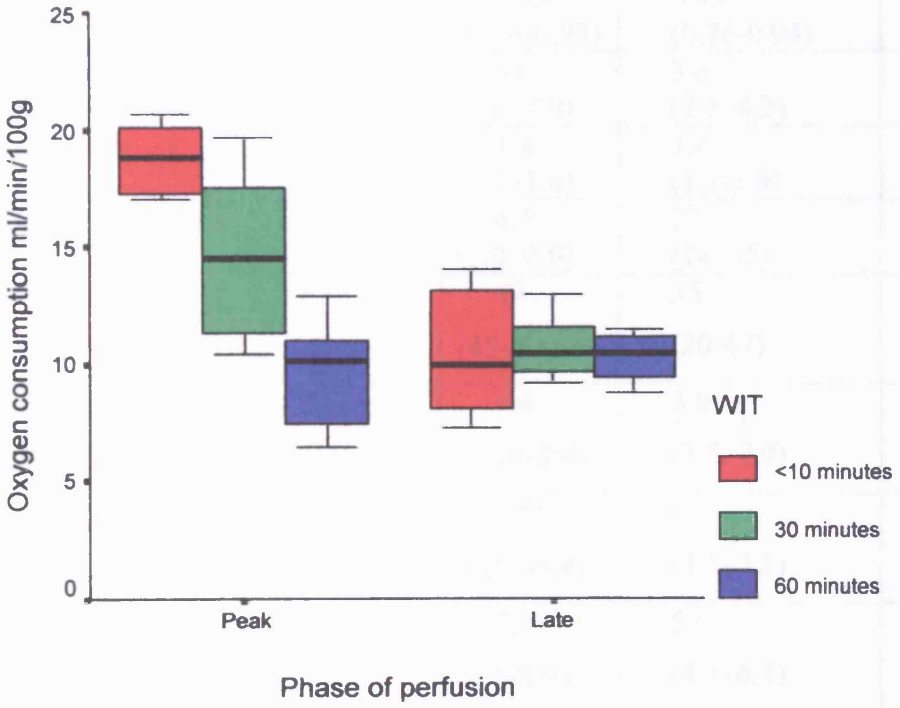


Figure 5.3: The relationship of oxygen consumption to warm ischaemic time and by phase of perfusion

5.4 Renal function

The data for renal function are summarised in table 5.3 below.

		Warm Ischaemic time (mins)		
	Time (hours)	<10	30	60
Urine :Perfusate $[Na^+]$ ratio	1	0.67 (0.30-0.82)	0.90 (0.85-0.98)	1.01 (0.79-1.04)
	3	0.94 (0.76-0.98)	0.93 (0.76-0.94)	0.95 (0.93-0.97)
Urine :Perfusate $[K^+]$ ratio	1	5.0 (3.6–7.0)	3.6 (2.5–4.2)	2.0 (1.6–5.3)
	3	1.4 (1.1–1.6)	1.4 (1.1-1.9)	1.1 (0.92–1.4)
Proteinuria (g/L)	1	4.5 (3.0–8.0)	22 (14–35)	46 (9.0–56)
	3	47 (45–55)	35 (20-47)	53 (48–56)
Glycosuria (mmol/L)	1	1.4 (0.50–2.4)	3.8 (3.5–7.0)	5.3 (1.7–7.3)
	3	6.05 (0.50-6.4)	6.7 (4.1–7.8)	4.8 (4.5–6.4)
Urine:Perfusate [Creatinine] ratio	1	2.4 (1.1-8.9)	5.5 (4.1–6.5)	1.8 (1.4–1.9)
	3	1.5 (1.1-2.4)	2.4 (1.6–2.6)	1.1 (1.0-1.2)

Table 5.3: *ex vivo* renal function parameters at the end of the peak and late phases of WP by the different WIT groups. Values are median (interquartile range). [substance] = concentration of substance.

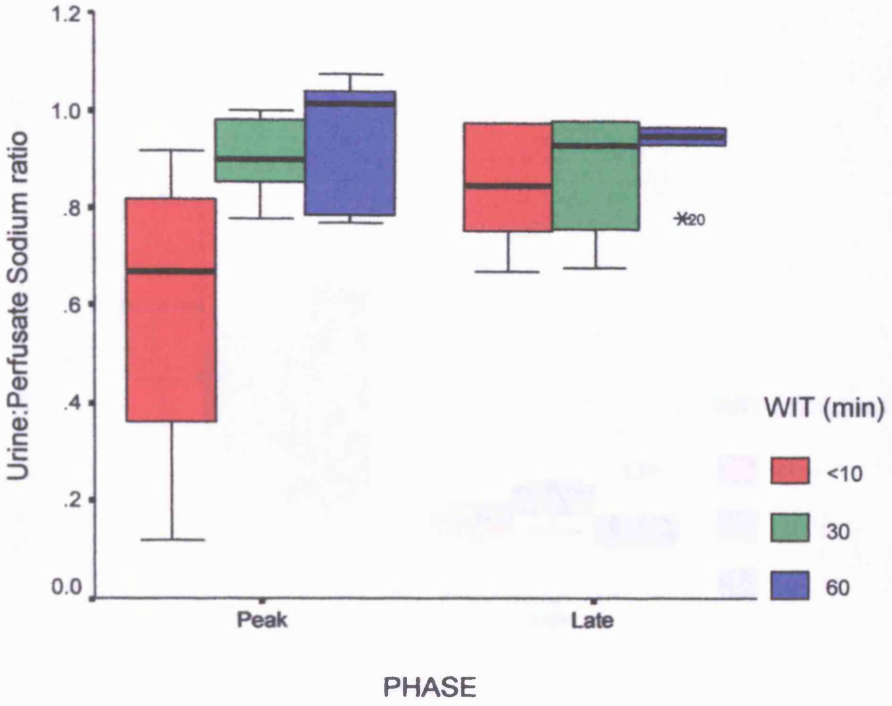


Figure 5.4: The ratio of urine to perfusate sodium ion concentrations between WIT groups, after the peak and late phases of WP.

The amount of sodium loss during the first hour of perfusion increased with WIT ($p=0.01$, KW), with less sodium lost in the <10minute WIT group than in the 30 or 60 minute groups ($p<0.02$ for both, U-test). The ratio was not different between the 30 and 60 minute groups ($p=0.3$, U-test). Sodium loss showed a trend towards increasing by the end of three hours of warm perfusion for the <10 minute group, but was unchanged in the 30 and 60 minute WIT groups ($p=0.07$, $p=0.5$ and $p=0.5$ respectively, Wilcoxon). After 3 hours WP there were no significant differences between WIT groups for sodium loss in the urine ($p=0.7$, KW).

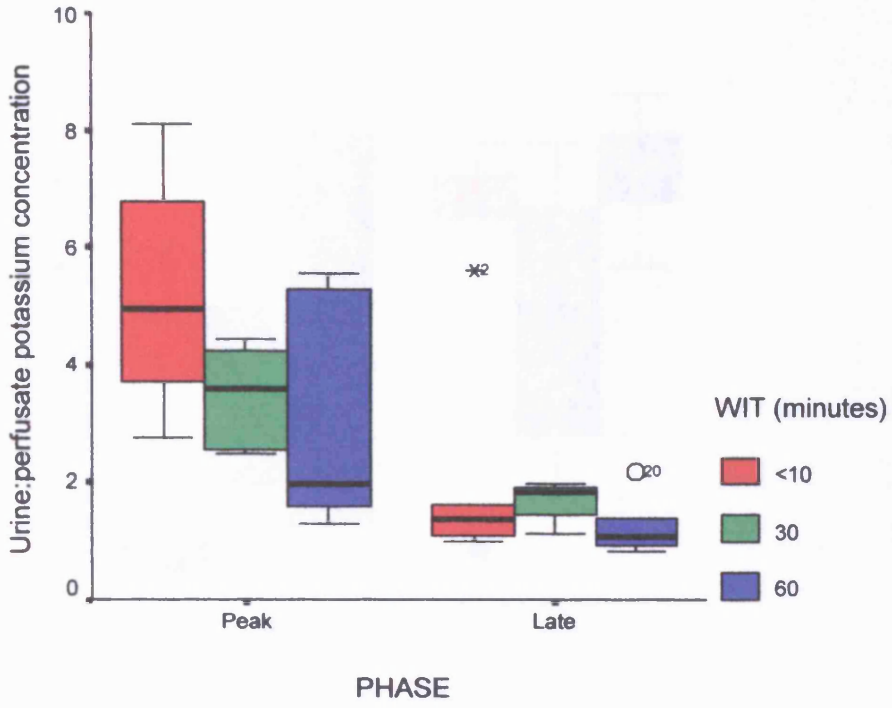


Figure 5.5: Ratio of urine to perfusate concentrations of potassium between WIT groups at the end of the peak and late phases of WP.

The ability of the kidney to excrete potassium in the urine during the peak phase of WP tended to fall with increasing WIT, but this did not quite achieve significance when compared between all three groups (p=0.06, KW). Potassium excretion fell significantly by the late phase of WP for all WIT groups (p<0.03, all groups, Wilcoxon). There was no difference between WIT groups in the ability to concentrate potassium after the late WP phase (p=0.13,KW).

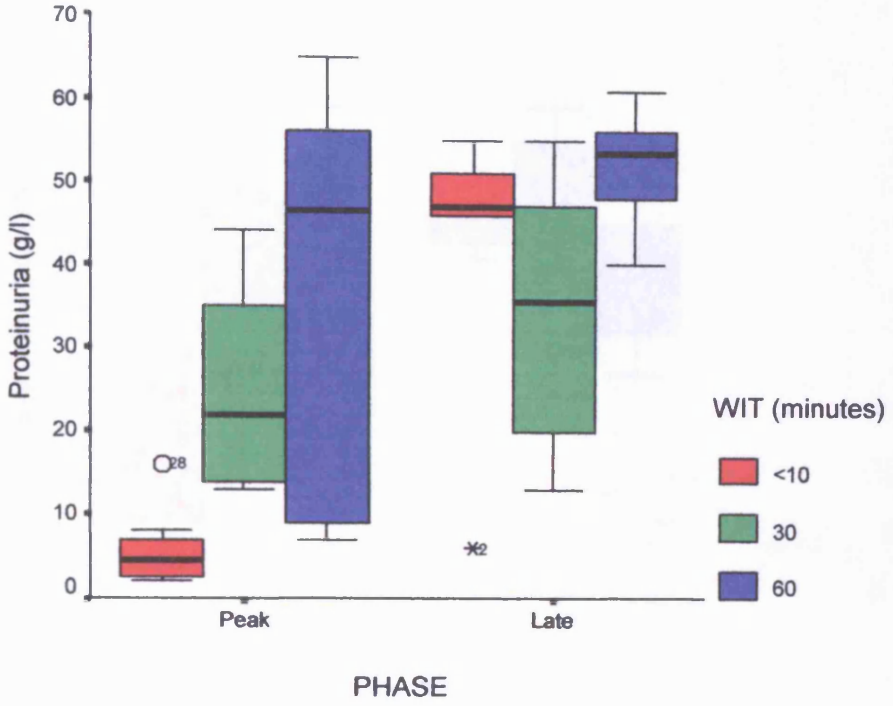


Figure 5.6: Proteinuria between WIT groups during the peak and late phases of WP

During the peak phase, proteinuria was heavier with increasing WIT (p=0.03, KW), with significantly less proteinuria in the <10 minute WIT group than the 30 or 60 minute groups (p<0.04 for both groups, U-test). There was no difference between the 30 and 60 minute groups (p=0.3 U-test). Proteinuria became heavier by the late phase of WP for all groups, significantly so for the <10 and 30 minute groups, but not for 60 (p=0.03, 0.05 and 0.2 respectively, Wilcoxon). There was no significant difference in proteinuria between all groups during the late WP phase (p=0.06, KW).

The proteinuria was strikingly heavy after even 30 minutes WIT, and suggests that significant damage occurs to the glomeruli early during the course of warm ischaemia.

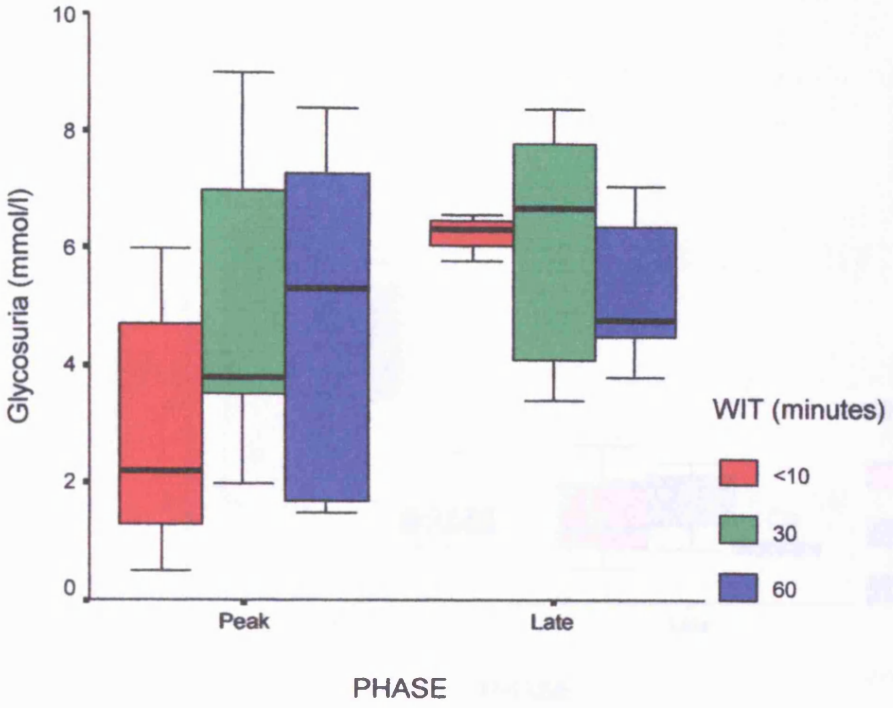


Figure 5.7: Glycosuria between WIT groups during the peak and late phases of WP

During *ex vivo* WP the relationship of glycosuria to WIT group and phase of perfusion closely mirrored that of proteinuria to the same factors. During the early phase the <10 minute group had significantly less glycosuria than the 30 or 60 minute groups, which did not significantly differ from each other ($p=0.002$ all groups, KW, $p<0.005$ for <10 versus 30 and 60, and 0.9 for 30 versus 60, U-test). Glycosuria for the <10 and 30 minute groups tended to increase by the late phase of WP, but the same was not true for the 60 minute group ($p=0.07$, 0.07 and 0.9, Wilcoxon). There was no difference in glycosuria between groups during the late phase of WP ($p=0.5$, KW).

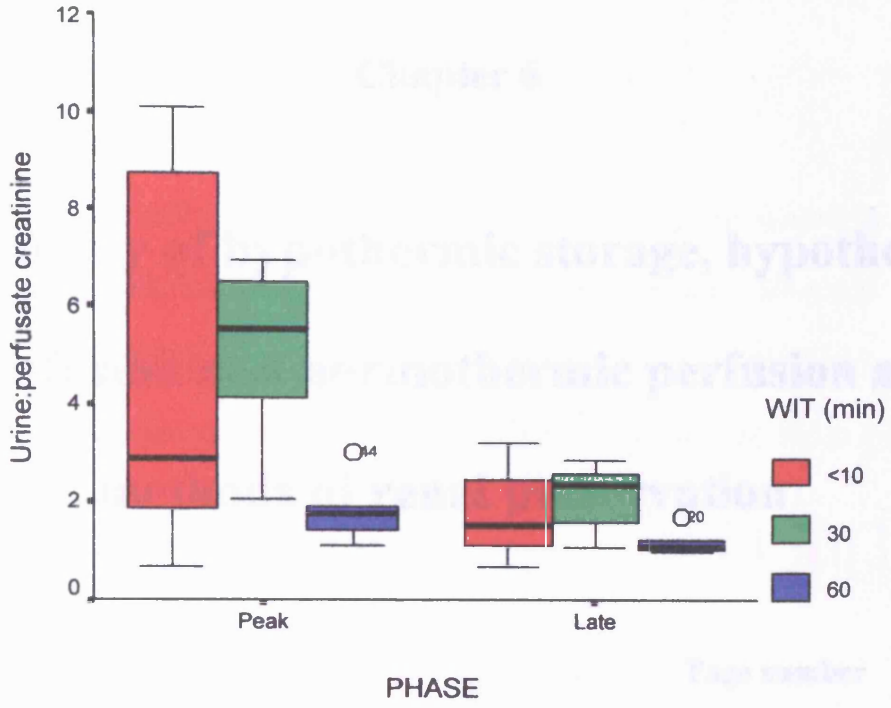


Figure 5.8: The ratio of urinary to perfusate creatinine concentrations between WIT groups for the peak and late phases of WP

The creatinine ratio did not differ in the early phase between <10 and 30 minutes WIT, but fell between 30 and 60 minutes WIT (p=0.7 and 0.006, U-test, all groups p=0.04, KW). The ratio for all groups fell by the late phase of perfusion, significantly so for the 30 and 60 minute groups (p<0.03, both groups, Wilcoxon), but not for <10 minutes WIT, (p=0.2, Wilcoxon). There were no differences between WIT groups during the late phase of WP (p=0.1, KW).

Chapter 6

The efficacy of hypothermic storage, hypothermic perfusion and normothermic perfusion as methods of renal preservation

Contents **Page number**

6.1 Technical success rate

Thirty of forty-nine renal auto-transplants were deemed suitable for analysis in that the surgery was technically successful and there were no post-operative complications other than those directly attributable to renal failure. A detailed list of the 49 animals, the causes of death and the reasons for exclusion where appropriate, is provided in appendix C.

6.2 Ischaemic times and animal and kidney weight data

The WIT, CIT and anastomosis times for the auto-transplanted kidneys are given in table 6.1. The animal and kidney weights, both pre-and post-perfusion where appropriate, are also included.

PM	Pig Wt (Kg)	WIT (i) (min)	WIT (a) (min)	CIT (hr)	AT (min)	Weight (pre)	Wt (post)	% change
CS (n=5)	51 (8)	0	2 (0)	24 (5.1)	27 (7)	100 (32)	N/A	N/A
CP (n=6)	69 (27)	0	2 (0)	23.9 (3)	27.5 (8)	125.5 (67)	194.5 (66)	56 (27)
WP (n=4)	49 (22)	0	2 (0)	22.3 (3.3)	29 (3)	99 (43)	139 (54)	40 (22)
CS (n=5)	37 (21)	30	30 (0)	23.3 (2.3)	28 (9)	103 (38)	N/A	N/A
CP (n=5)	31 (27)	30	30 (0)	23.2 (5.9)	32 (7)	76 (52)	118 (93)	71 (34)
WP (n=5)	41 (34)	30	30 (0)	24.2 (2.5)	32 (9)	105 (28)	141 (19)	38 (32)

Table 6.1: Animal weights (wt), WIT, intended (i) and actual (a), CIT and anastomosis times (AT), kidney weights pre and post perfusion (in grams), according to preservation method (PM) and WIT. Values are median (range).

The kidney weights were not evenly distributed between the groups ($p=0.004$, KW). Those in the CP group with minimal WIT were significantly heavier than those in all other groups ($p<0.02$, all groups, U-test), whereas kidneys in the CP group with 30 minutes WIT were significantly lighter than all other groups ($p<0.05$, all groups, U-test). There were no other differences between groups, ($p>0.2$, all comparisons, U-test). All groups with perfusion preservation demonstrated significant weight gain by the end of perfusion compared with the pre-perfusion weight ($p<0.05$, all perfusion groups, Wilcoxon). The weight gain differed between groups ($p=0.01$, KW), and was significantly greater in the CP preserved kidneys than in those preserved by WP ($p<0.04$, all comparisons, U-test), irrespective of WIT. In fact WIT had no effect on weight gain within a preservation group, either for CP or WP ($p=0.4$ and 0.2 respectively, U-test).

Pigs in the CP with minimal WIT group were significantly heavier than those in all other groups ($p=0.02$, KW all groups, $p=0.05$ versus groups 1,3 and 6, and 0.009 versus groups 4 and 5, U-test). There were no significant differences between groups for cold ischaemic time or anastomosis times ($p=0.5$ and 0.1 respectively, KW). There were no differences in actual WIT between groups with the same intended WIT, ($p=1$, both intended WITs, KW).

6.3 Animal survival

The number of animals surviving 14 days post-transplant in each group is summarised in table 6.2, and figure 6.1. Those which did not survive had no other cause of death identifiable on post-mortem and had either very high recorded serum potassium levels, or a bleeding diathesis associated with a high urea level. All other causes of death were excluded from analysis (see appendix C).

There were no significant differences in survival between preservation methods at either 0 or 30 minutes WIT, ($p=0.9$ and 1 respectively, Chi squared).

	WIT (minutes)	
Preservation	0	30
CS	4/5	1/5
CP	5/6	1/5
WP	3/4	1/5

Table 6.2: Number of autotransplant animals surviving out of the total performed for each group.

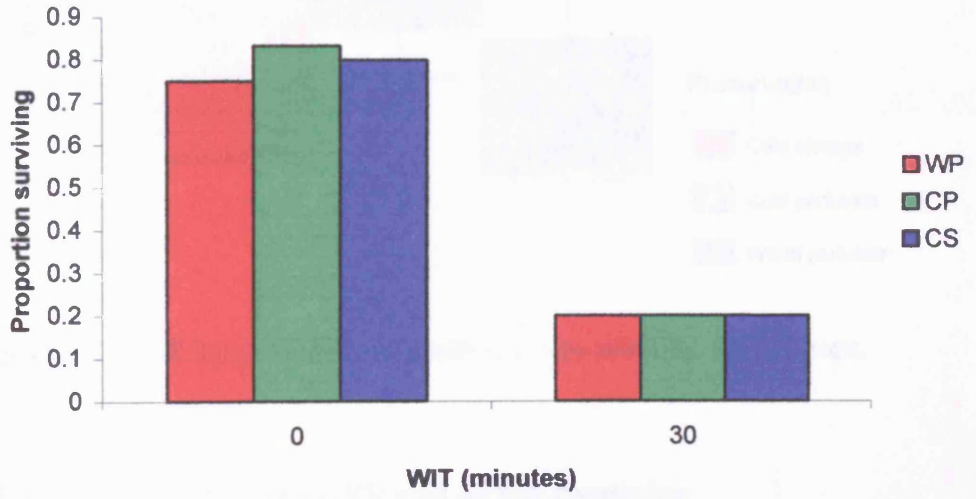


Figure 6.1: Proportion of auto-transplanted animals surviving by preservation method and WIT.

6.4 Glomerular filtration rates

The renalyser GFR did not differ significantly between preservation methods in the minimal WIT groups (p=0.2, KW, figure 6.2). There were inadequate numbers to compare GFR measurements in the 30 minute WIT groups, (2 animals in the CS and CP

groups, none in the WP group). Apart from animals dying of renal failure before the 7th post-operative day, the other reasons for not measuring GFR were one line of the vascath being blocked (1 animal) and GFR samples being haemolysed and therefore un-analysable (2 animals).

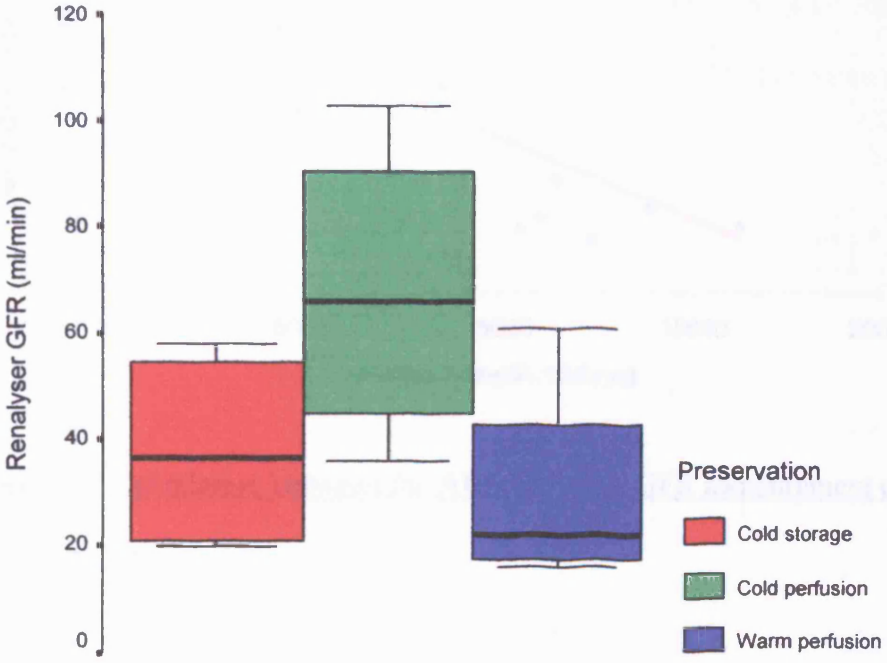


Figure 6.2: GFR by preservation method in the minimal WIT groups.

6.5 Correlation between GFR and serum creatinine

In total there were 15 GFR measurements made, and these were analysed for their relationship to serum creatinine. The renalyser GFR measurements correlated well with the area under the curve for serum creatinine (AUC_{cr}), ($p=0.02$, Spearman's rank, $R=0.64$), validating the use of AUC_{cr} as an index of post-transplant renal function (see figure 6.3). Similarly, and importantly for animals surviving to day 7 but not day 14, GFR measurements correlated with the serum creatinine on day 7 post-transplantation ($p=0.009$, Spearman's rank, $R=0.62$, figure 6.4). Subsequent analyses of renal function

post-transplant use AUC_{cr} rather than day 7 creatinine on the basis of the marginally higher R value.

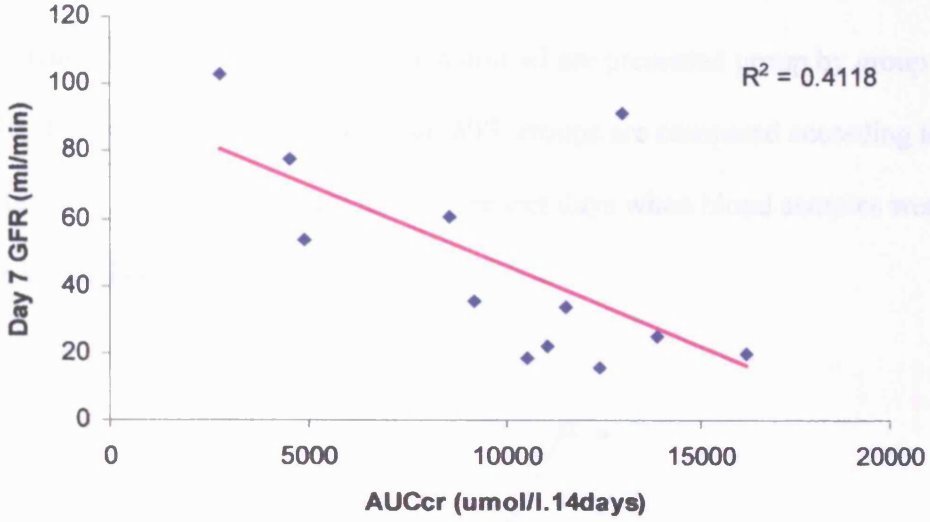


Figure 6.3: Correlation between the AUC_{cr} and the GFR measurement on day 7 post-transplantation.

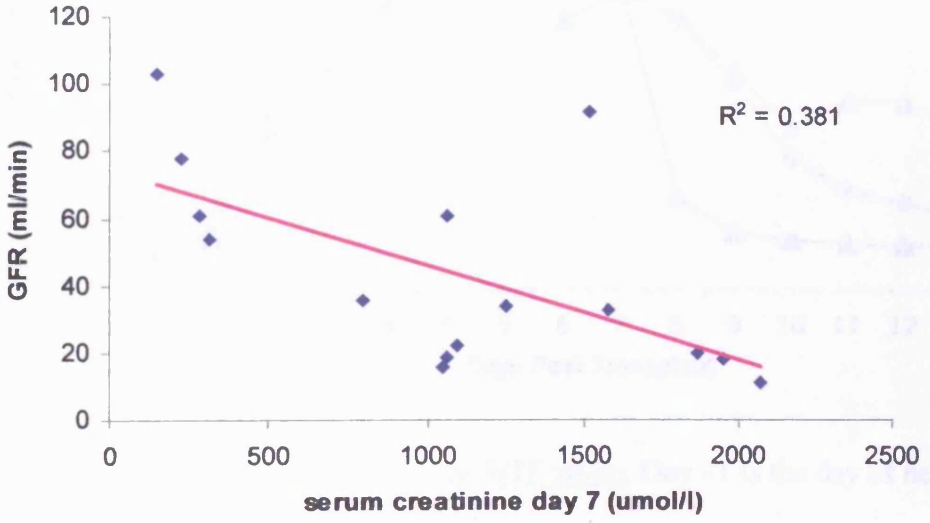


Figure 6.4: Correlation between serum creatinine and the GFR, both as measured on day 7 post-transplantation.

6.6 Individual animal renal function by group

Daily serum creatinines for each animal are presented group by group in figures 6.5-6.10. The renal function of minimal WIT groups are compared according to AUC_{cr}'s in figure 6.11. Gaps in the trend lines represent days when blood samples were not possible due to blocked lines.

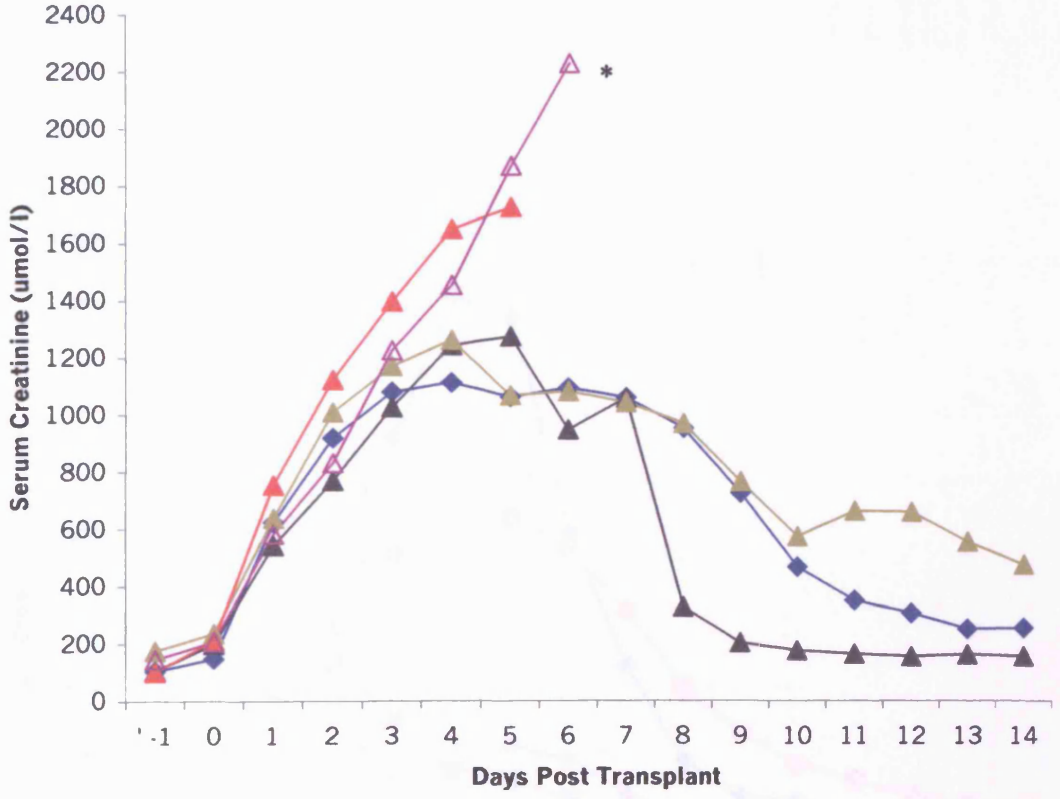


Figure 6.5: Cold storage with minimal WIT group. Day –1 is the day of nephrectomy, day 0 the day of transplantation. * denotes an animal dying of renal failure. The same conventions are used on figures 6.6-6.10.

▲ denotes an animal who survived the 14 days follow-up period, but managed to remove her vascath on day 6. Due to an oversight no blood sample was taken for biochemical analysis at the time of termination.

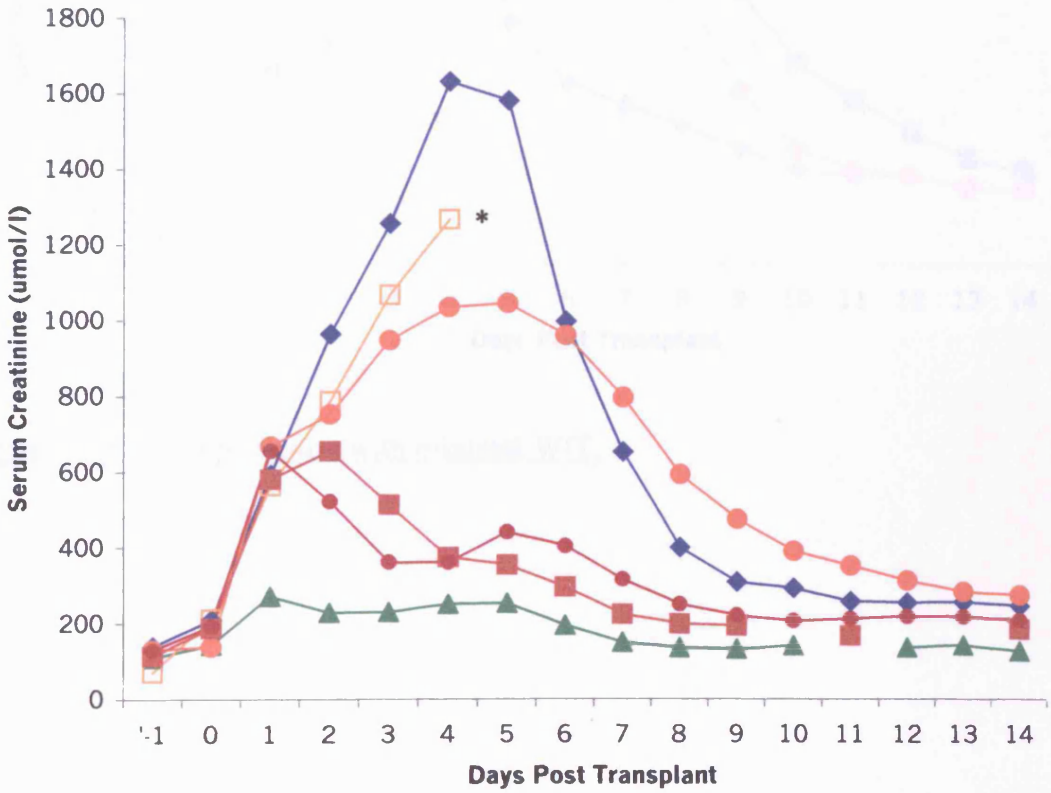


Figure 6.6: Cold perfusion with minimal WIT.

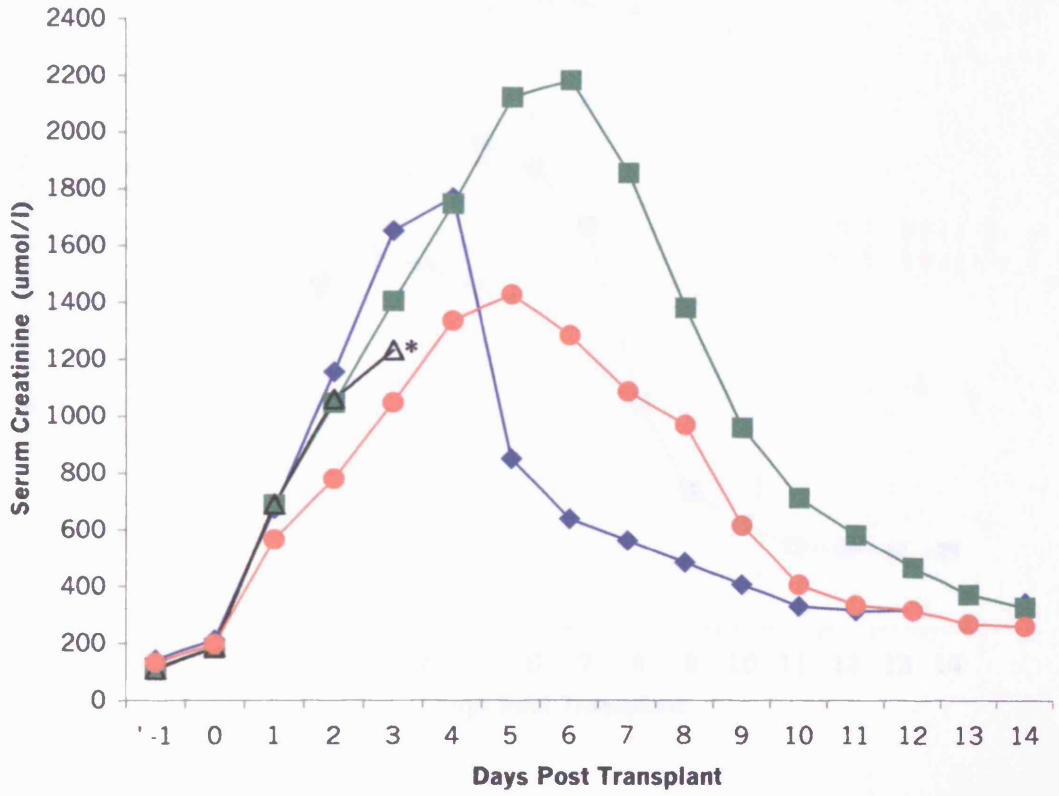


Figure 6.7: Warm perfusion with minimal WIT.

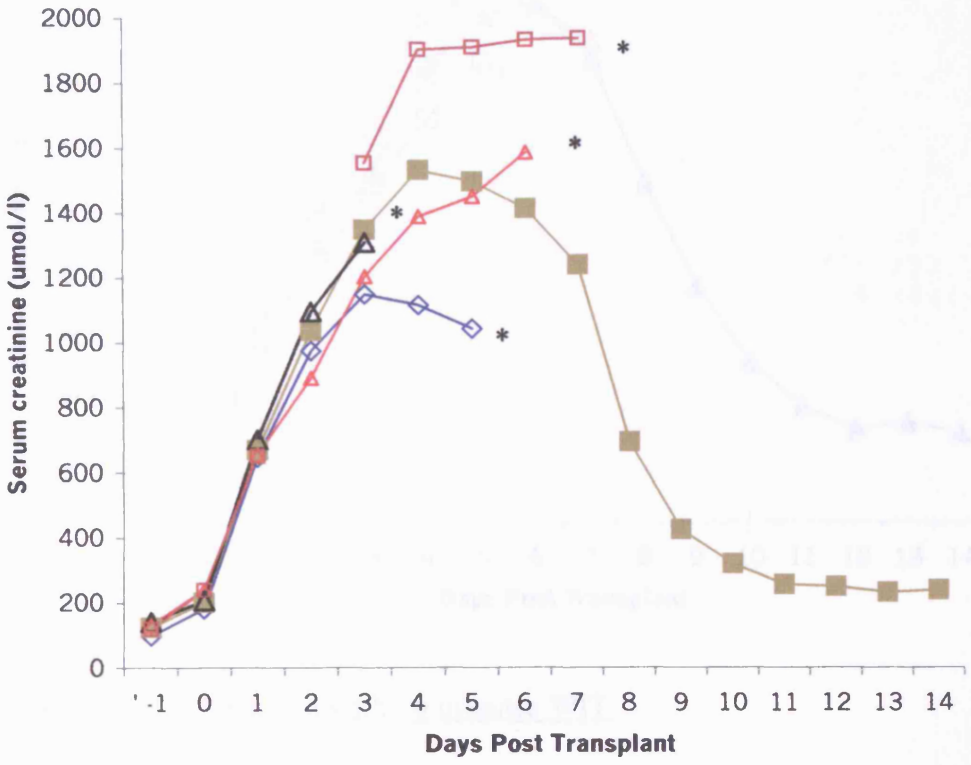


Figure 6.8: Cold Storage with 30 minutes WIT.

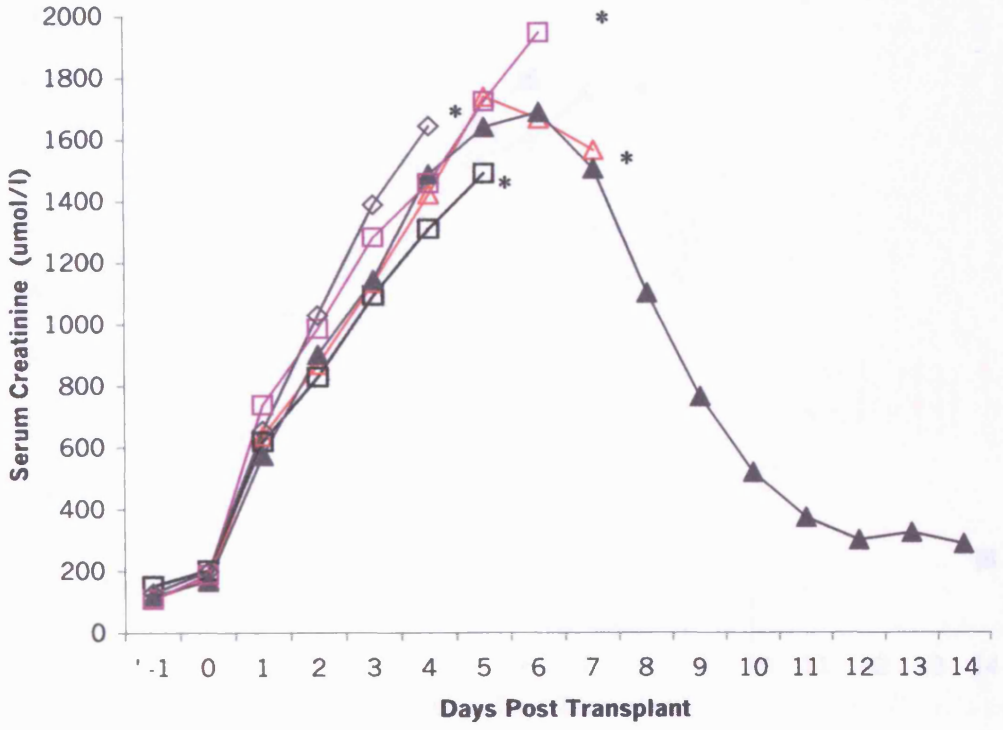


Figure 6.9: Cold Perfusion with 30 minutes WIT.

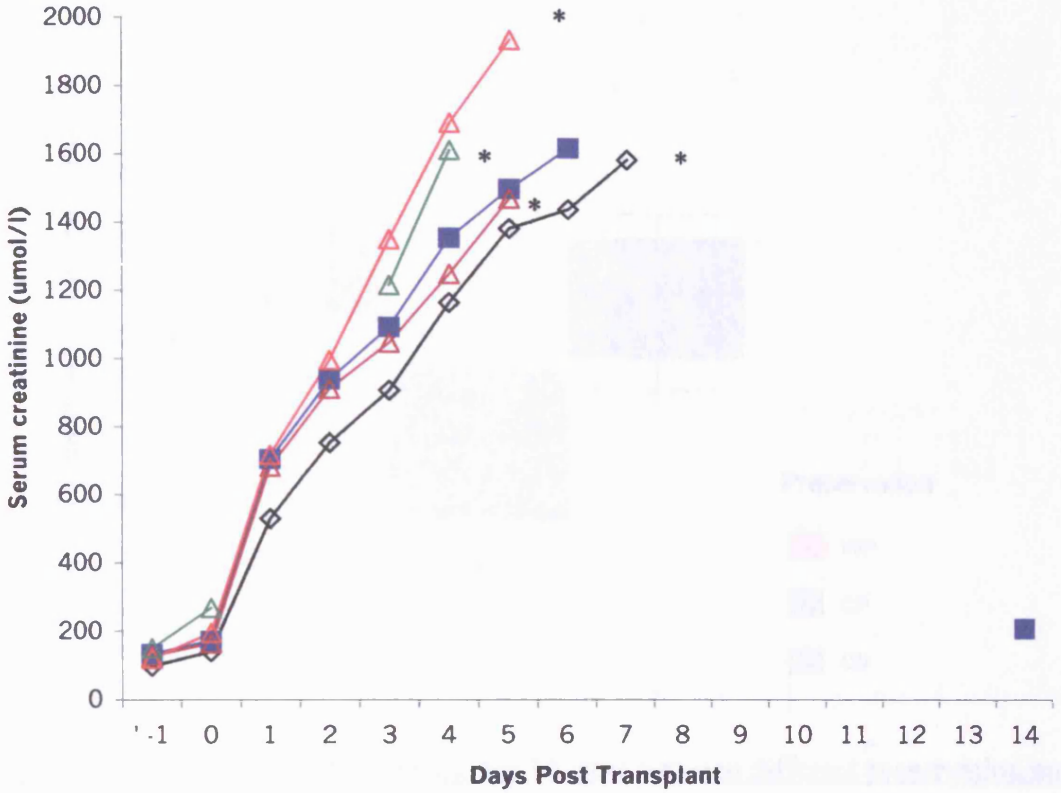


Figure 6.10: Warm perfusion with 30 minutes WIT.

6.7 Renal function by preservation method

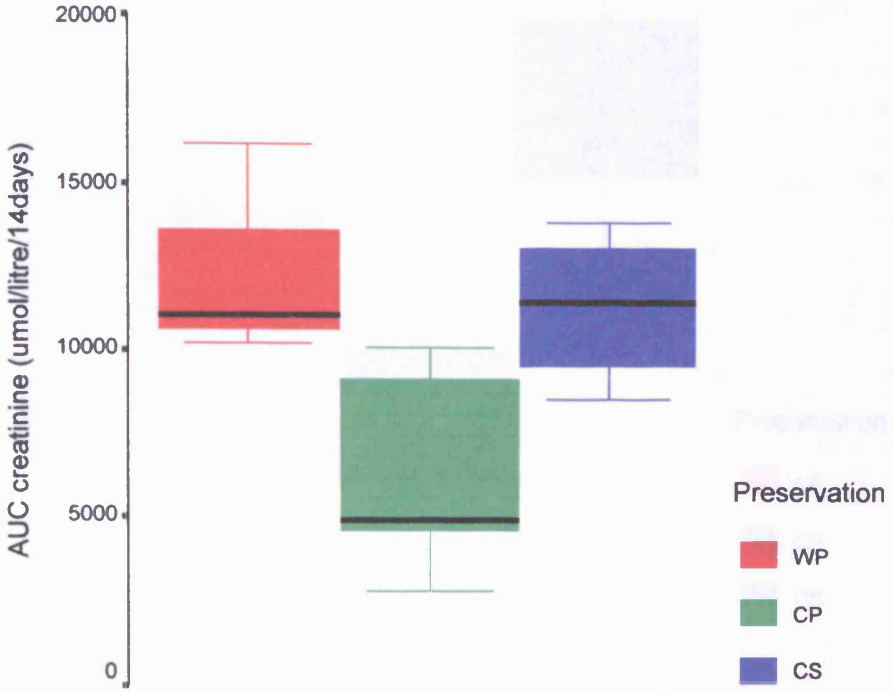


Figure 6.11: Comparison of AUC_{cr} for 14 days between different preservation methods in minimal WIT groups.

The AUC_{cr} is significantly different between preservation methods for minimal WIT transplants (p=0.04, KW). CP animals have better renal function by this measure than CS and WP animals (p=0.04 and 0.06 respectively, MW-U). There was no difference in AUC_{cr} between CS and WP (p=0.9, U-test). AUC_{cr} cannot be compared between preservation methods for the 30 minute WIT groups because there is only one case per group.

The daily serum urea values for individual animals were used to calculate the areas under the curves for serum urea (AUC_{ur}) over the two weeks post transplant. The AUC_{ur} are presented by preservation method for the minimal WIT groups in figure 6.12.

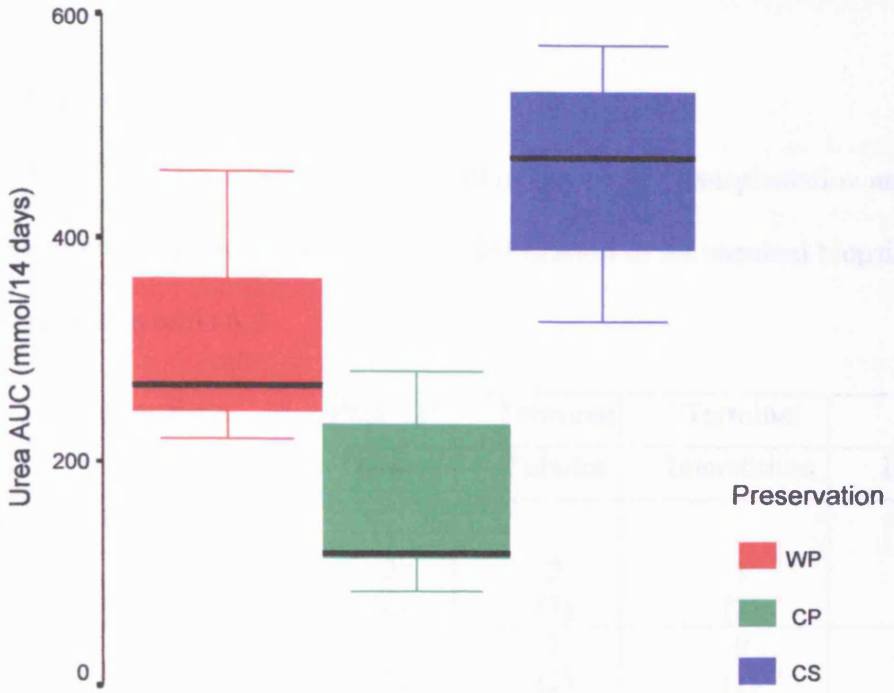


Figure 6.12: AUC_{ur} by preservation method for minimal WIT groups.

The AUC_{ur} was not the same for all preservation methods (p=0.03, KW). The AUC_{ur} was significantly higher in the CS group than the CP group (p=0.02, U-test) there were no significant differences between CP and WP (p=0.2, U-test) or between WP and CS (p=0.9, U-test).

6.8 Histology

Histological scoring of tubular injury 30 minutes after transplantation and to the tubules, interstitium and degree of lymphocytic infiltration in the terminal biopsies are summarised in table 6.3.

Biopsy time		Post –Tx	Terminal	Terminal	Terminal
Site		Tubules	Tubules	Interstitium	Lymphocytes
Preservation	WIT				
CS	0	2 (0)	2 (1)	1 (2)	1 (1)
CP	0	1.5 (1)	1 (2)	0 (1)	1 (2)
WP	0	1 (0)	2 (2)	2 (3)	2 (2)
CS	30	2 (2)	1.5 (2)	2.5 (2)	0.5 (1)
CP	30	2 (0)	3 (1)	0 (2)	1 (1)
WP	30	2 (0)	3 (4)	2 (2)	2 (2)
Significance		0.01	0.2	0.02	0.07

Table 6.3: Histology of kidneys 30 minutes after reperfusion and at termination. Values are median (range). Statistical analysis is by KW.

KW revealed a significant differences between groups for the reperfusion tubular score and the terminal interstitial score, and for these the MW U test was used to further delineate which individual experimental groups differed from each other.

For minimal WIT kidneys, there was significantly less tubular damage 30 minutes after re-perfusion in the WP group than in the CS group ($p=0.03$, MW U), and the CP group scores lay between the other 2 groups, being significantly different from neither.

There was no difference between preservation groups in kidneys that had undergone 30 minutes WIT.

There was no difference between preservation methods in the terminal histology for kidneys with minimal WIT, but in the 30 minute WIT group, the interstitium was significantly less damaged in CP preserved kidneys than for CS. CP was also probably superior to WP, although the variance of the latter rendered the observed difference insignificant.

Chapter 7

Viability assessment of kidneys prior to transplantation

Contents **Page Numbers**

7.1 Survival

Three of four animals in the minimal WIT group with WP preservation survived to 14 days, compared to only one of five in the 30 minute WIT group with WP. This difference does not achieve statistical significance (p=0.17, chi-square), due to the small numbers, particularly in the minimal WIT group (see discussion of methods).

7.2 *Ex-vivo* Function, resistance and metabolism

The *ex vivo* function measured during WP also varied according to WIT, for each parameter measured, figures 7.1-7.5, as did intra-renal vascular resistance, figure 7.6 and oxygen consumption, figure 7.7 and table 7.1.

WP Parameter	WIT (min) 0	WIT (min) 30	P value (U)
Creatinine concentration (U:P)	11.2 (15.7)	1.6 (0.2)	0.008
Sodium concentration (U:P)	0.36 (0.5)	0.69 (0.14)	0.008
Potassium concentration (U:P)	7.9 (10.6)	3.1 (2.2)	0.008
Glycosuria: $[glucose]_{perfusate}$	0.34 (0.7)	1.2 (0.2)	0.02
Proteinuria: $[protein]_{perfusate}$	0.51 (0.41)	1.3 (0.1)	0.02
IRR (mmHg/ml/min/100g)	12.7 (5.7)	19.0 (15.7)	0.02
O_2 consumption (ml/min/100g)	20.6 (12.4)	8.1 (4.9)	0.02

Table 7.1: *ex vivo* renal functional parameters, IRR and oxygen consumption by WIT during the first hour of WP. (U:P): urine to perfusate ratio, $[]_{perfusate}$: concentration of a substance in perfusate, P value (U): statistical significance by the Mann Whitney U-test. Values are median (range).

Figure 7.1: The urinary to perfusate creatinine concentration ratio during the first hour WP by WIT. The middle bar represents the median, the box the interquartile range, the whiskers the outliers, and extremes are labelled individually. This convention is applied to all box-plots in chapter 7.

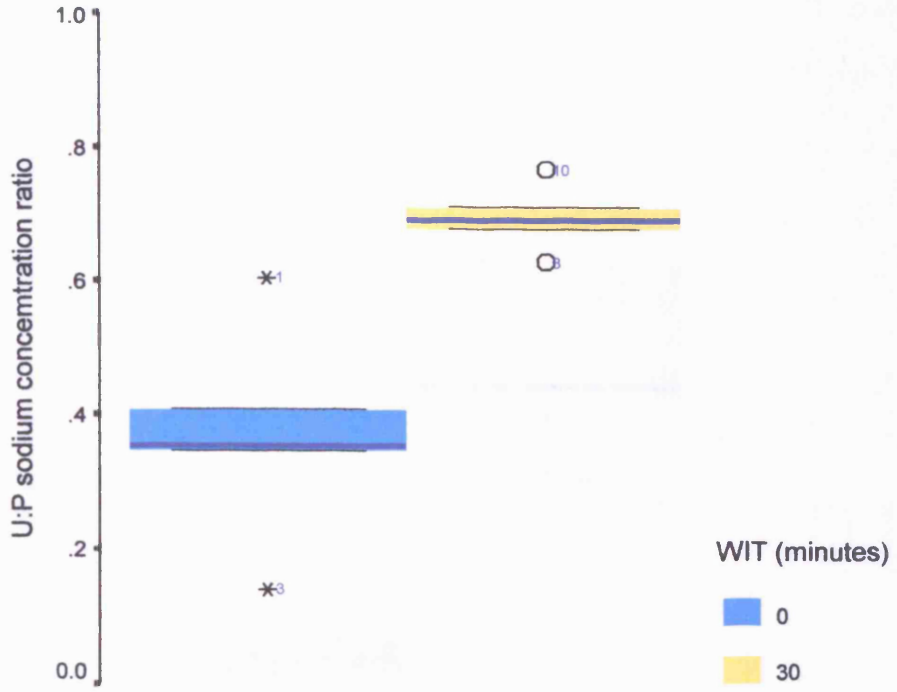


Figure 7.2: The urinary to perfusate sodium concentration ratios during the first hour of WP.

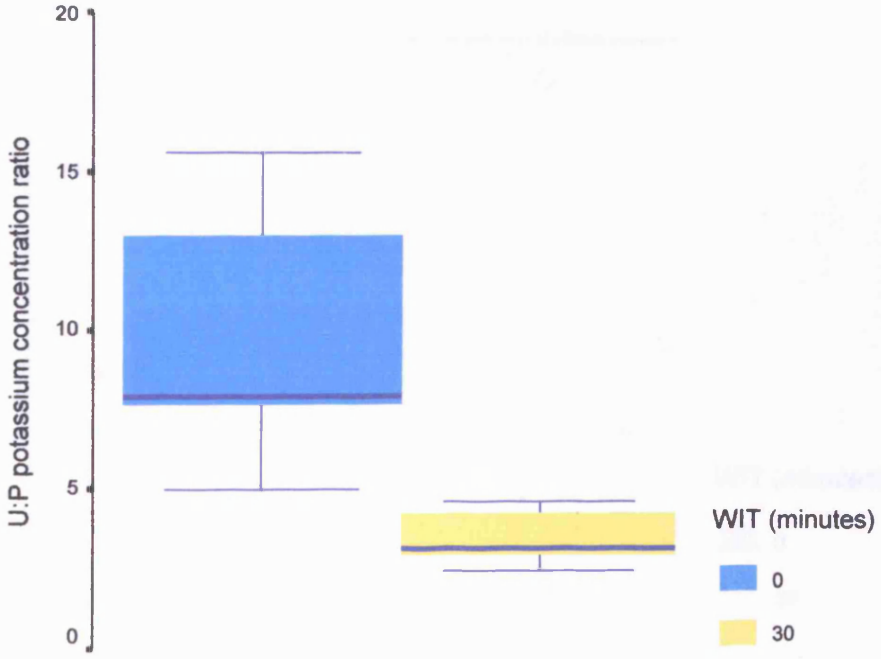


Figure 7.3: Urinary to perfusate potassium concentration ratios during the first hour of WP.

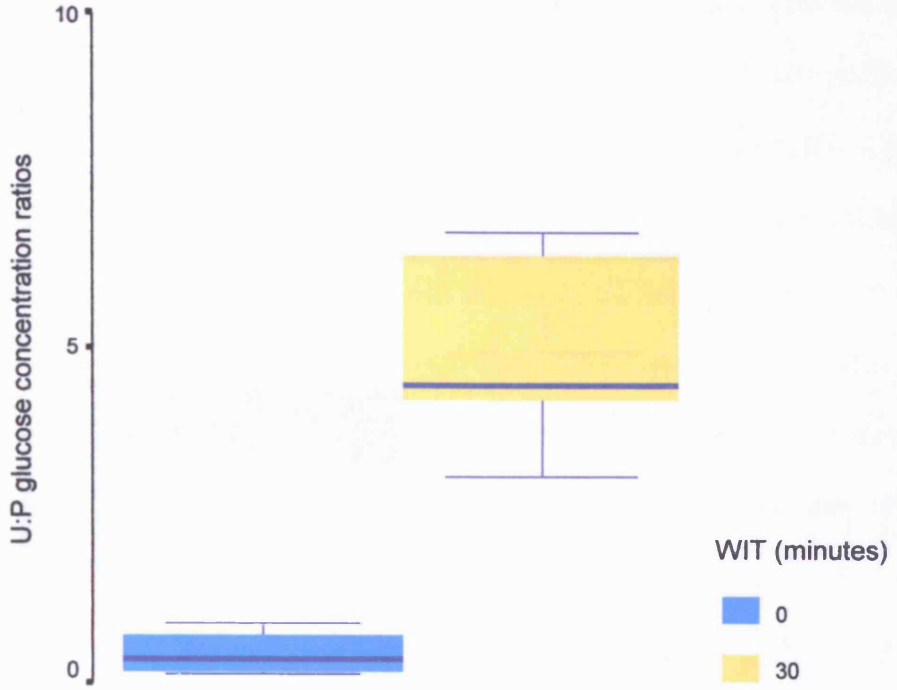


Figure 7.4: Urine to perfusate glucose concentration ratios in the first hour of WP.

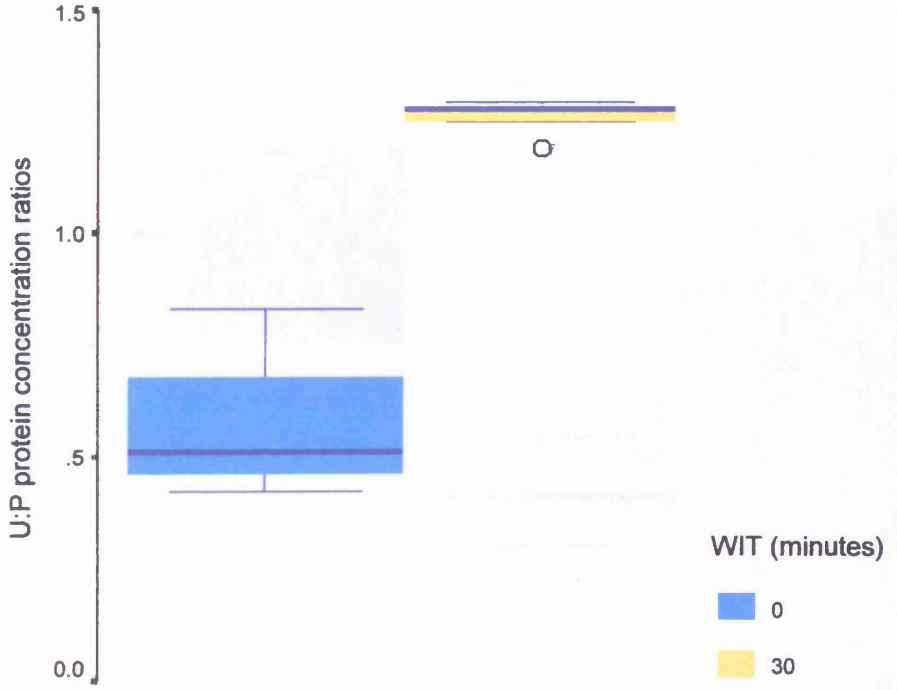


Figure 7.5: Urine to perfusate protein concentration ratios in the first hour of WP.

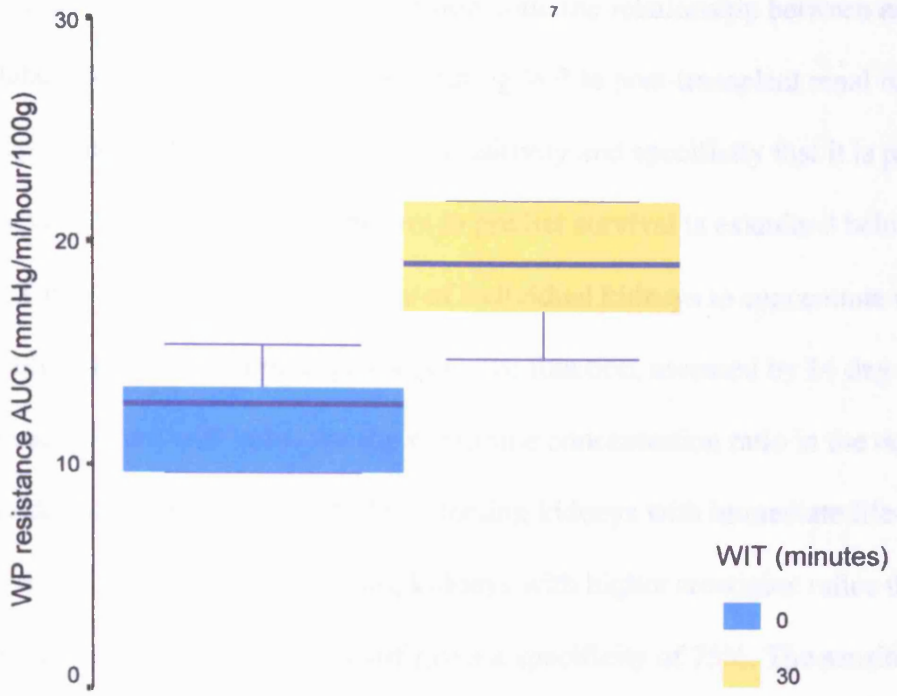


Figure 7.6: AUC_{IRR} during the first hour of WP.

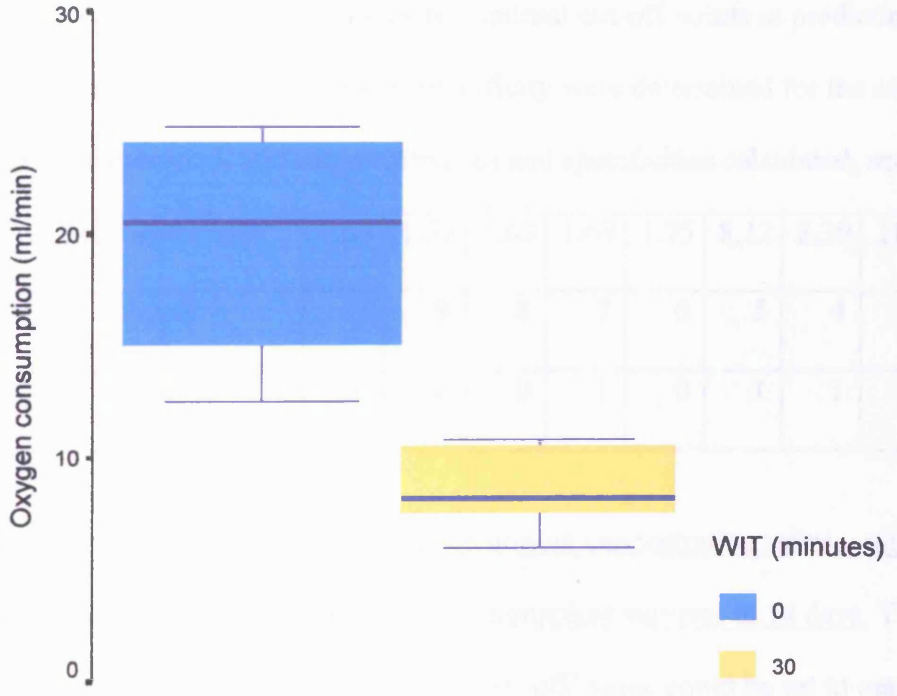


Figure 7.7: Oxygen consumption at the end of the first hour of WP.

The results presented above demonstrate the relationship between *ex vivo* function, metabolism and vascular dynamics during WP to post-transplant renal function on a group basis, according to WIT. The sensitivity and specificity that it is possible to achieve by each of these parameters to predict survival is examined below.

Table 7.2 compares the ability of individual kidneys to concentrate urinary creatinine *ex vivo* with their post-operative function, assessed by 14 day animal survival. Choosing a 'cut-off' value for the creatinine concentration ratio in the range of 1.76-8.11 provides maximum specificity for selecting kidneys with immediate life-sustaining function. 3 of 4 animals receiving kidneys with higher creatinine ratios than that range survived. Therefore such a cut-off gives a specificity of 75%. The sensitivity is also 75%, as 3 of the 4 kidneys that were capable of immediate life supporting function had ratios below that range.

In the same manner, ranges for optimal cut-off points in predicting post-transplant survival with maximum specificity were determined for the other *ex vivo* parameters recorded, and the sensitivities and specificities calculated, see tables 7.3-7.8.

Creatinine ratio	1.55	1.57	1.63	1.69	1.75	8.12	8.39	11.24	23.82
Rank	10	9	8	7	6	5	4	3	1
Survival	0	0	0	1	0	1	1	1	0

Table 7.2: *Ex vivo* urine to perfusate creatinine concentration ratios, values and ranks from best (1) to worst (10), against post-transplant survival to 14 days. The arrow indicates the range of ratios in which a 'cut-off' value could be set to maximise the sensitivity and specificity of this parameter for predicting survival. This annotation is consistent for tables 7.3-7.9.

Sodium ratio	0.35	0.36	0.41	0.61	0.63	0.68	0.69	0.71	0.77
Rank	1	2	3	4	5	6	7	8	9
Survival	1	1	0	1	0	1	0	0	0

Table 7.3: *Ex vivo* urine to perfusate sodium concentration ratios.

Potassium ratio	2.36	3.88	3.08	4.25	4.56	5.0	7.63	7.89	15.6
Rank	9	8	7	6	5	4	3	2	1
Survival	0	1	0	0	0	1	1	1	0

Table 7.4: *Ex vivo* urine to perfusate potassium concentration ratios.

Glucose ratio	0.13	0.52	0.86	1.17	1.22	1.23	1.24	1.37
Rank	1	2	3	4	5	6	7	8
Survival	0	1	1	0	0	1	0	0

Table 7.5: *Ex vivo* urine to perfusate glucose concentration ratios. (Note: the glucose data is not available for one surviving animal due to an error in the biochemistry analyser)

Protein ratio	0.42	0.5	0.83	1.19	1.25	1.28	1.29	1.3
Rank	1	2	3	4	5	6	7	8
Survival	0	1	1	1	0	0	0	0

Table 7.6: *Ex vivo* urine to perfusate protein concentration ratios. (Note: the proteinuria data is not available for one surviving animal due to an error in the biochemistry analyser)

AUC_{IRR} (mmHg/ml/minute/100g)	9.65	12.75	13.44	14.74	15.36	16.95	19.07	21.9	30.46
Rank	1	2	3	4	5	6	7	8	9
Survival	1	0	1	0	1	0	0	0	1

Table 7.7: *Ex vivo* AUR_{IRR} during the first hour of WP.

Oxygen consumption (ml/minute/100g)	5.90	7.50	8.10	10.50	10.80	12.50	17.60	23.50	24.90
Rank	9	8	7	6	5	4	3	2	1
Survival	1	0	0	0	0	1	1	0	1

Table 7.8: *Ex vivo* oxygen consumption at the end of the first hour of WP.

With cut-off points in the ranges indicated by the arrows in the tables above, the specificities and sensitivities for predicting post-transplant survival are shown in table 7.9, along with the specificity and sensitivity that would achieved just by knowing the WIT.

It is possible to alter the position of the arrows (i.e. the cut-off ranges) to improve the sensitivity to 100% for the sodium and creatinine ratios, at the expense of reducing the specificity to 60%. For other parameters, similar alterations in the cut-off range to improve sensitivity will cause greater lowering in the specificity.

Parameter	'Cut-off' range	Specificity (%)	Sensitivity (%)
WIT (min)	1-29	75	75
Creatinine ratio	1.76-8.11	75	75
Sodium ratio	0.621-0.629	75	75
Potassium ratio	4.57-4.99	75	75
Glucose ratio	0.87-1.16	67*	67*
Protein ratio	1.20-1.24	75	100*
Resistance	15.37-16.94	60	75
Oxygen consumption	10.8-12.5	75	75

Table 7.9: The sensitivity and specificity of *ex vivo* parameters for the prediction of post-transplant function. Units are as for graphs 10.3-10.8. * these values may be less reliable than others due to the loss of data for analysis.

7.3 Cold perfusion viability prediction

The survival to 2 weeks post-transplant in the minimal WIT group of CP animals was 4/5 compared with 1/5 for the 30 minute WIT group. This difference is not significant (p=0.06, Chi-square), but shows a trend toward significance that may have been revealed with larger experimental groups.

The AUC_{IRR} in the minimal WIT group of CP preserved kidneys at 33.3 (13.0) mmHg/ml/min/100g was significantly lower than the 30 minute WIT group at 59.1 (25.0) mmHg/ml/min/100g (p=0.008, U-test). Values are median (range), see figure 7.9.

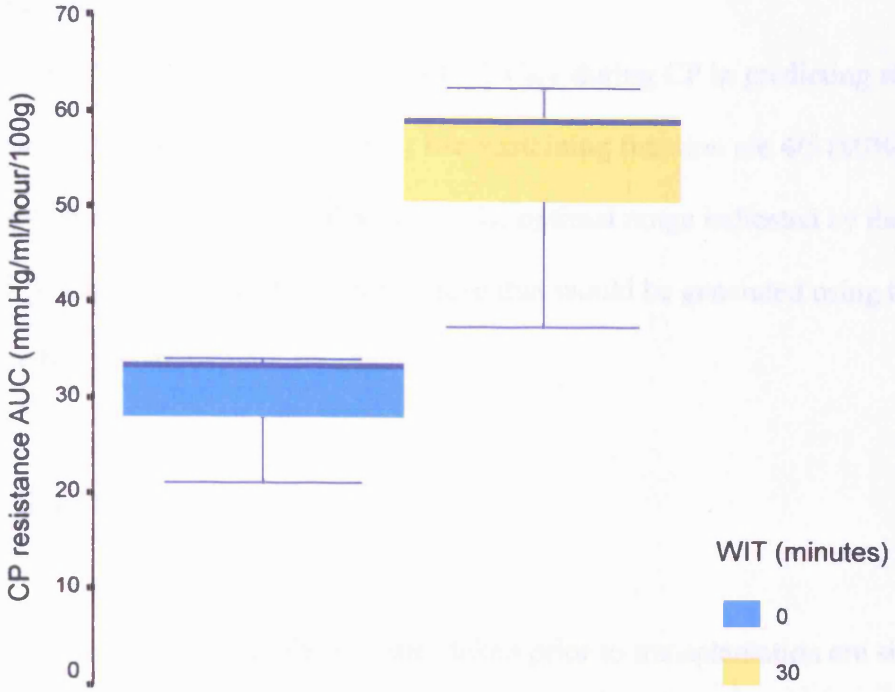


Figure 7.9: The AUC_{IRR} during the first hour of CP for kidneys with minimal and 30 minutes WIT.

Two-week post-transplant survival against the AUC_{IRR} for individual kidneys and animals is set out in table 10.10

AUR_{IRR}	21.2	27.9	33.3	33.3	34.1	37.6	50.7	59.1	59.6	62.6
Rank	1	2	3	4	5	6	7	8	9	10
Survival	1	1	1	0	1	0	0	0	1	0

Table 7.10: AUC_{IRR} during the first hour of cold perfusion against survival to 2 weeks post-transplant.

The specificity and sensitivity of AUC_{IRR} during CP in predicting which kidneys are immediately capable of supporting life-sustaining function are 4/5 (80%) and 4/5 (80%) respectively, using a 'cut-off' value in the optimal range indicated by the arrow of 34.2-37.5. These values are the same as those that would be generated using the known WIT to predict post-transplant function.

7.4 Histology

The results of all the biopsies taken prior to transplantation are shown in table 7.11. There were no differences in the scores given to the condition of the renal tubules in any of the pre-transplant biopsies, irrespective of preservation method or WIT. Examples of the spectrum of renal histology are given in figure 7.10.

Biopsy time		In Situ	Post WIT	Pre-Tx
Site		Tubules	Tubules	Tubules
Preservation	WIT			
CS	0	2 (1)	2 (0)	1 (1)
CP	0	2 (1)	1 (1)	1 (1)
WP	0	2 (2)	2 (1)	1 (1)
CS	30	1 (2)	2 (1)	1 (1)
CP	30	2 (2)	1 (2)	2 (0)
WP	30	2 (1)	2 (1)	1 (2)
Significance		0.6	0.2	0.3

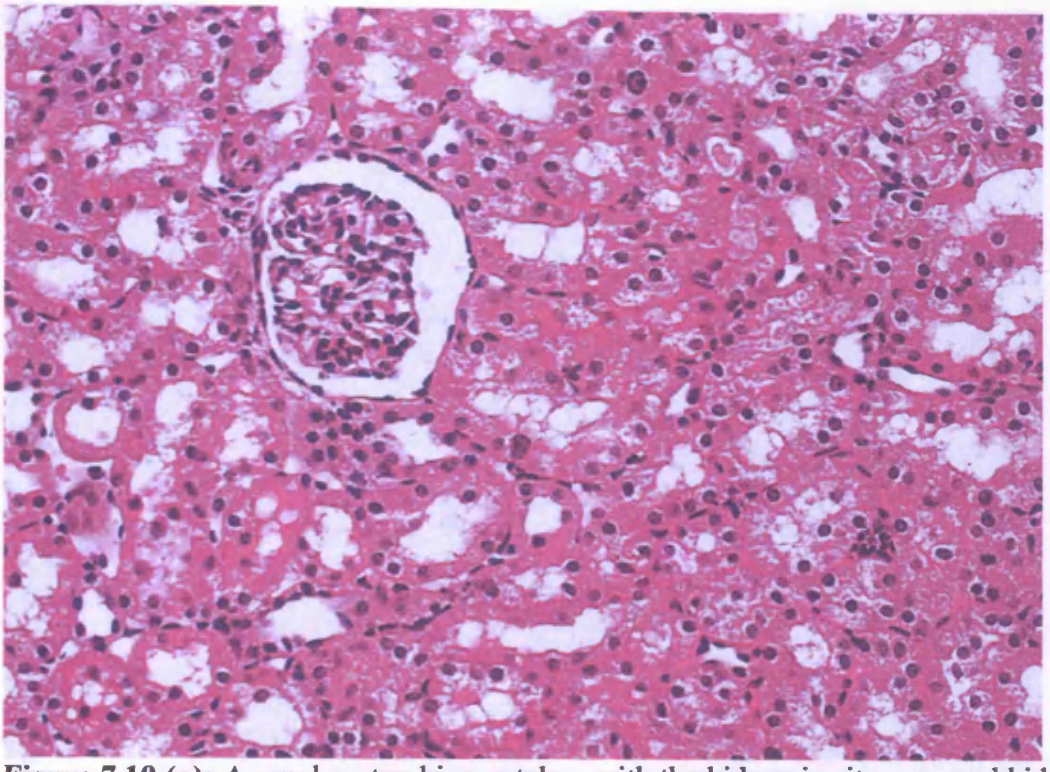


Figure 7.10 (a): A renal cortex biopsy taken with the kidney in situ – normal kidney

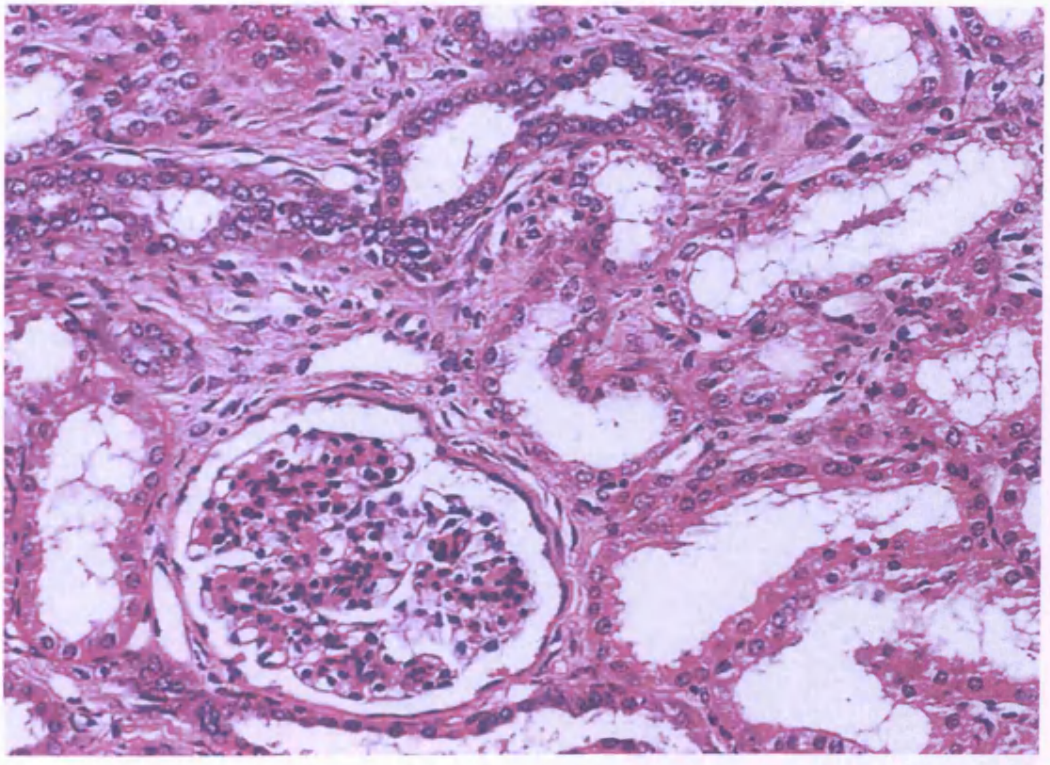


Figure 7.10 (b): A terminal biopsy showing severe ATN. However the tubular architecture is intact and the glomerulus shows only mild tuft collapse. The tubular epithelial cell nuclei are enlarged with open dispersed chromatin, indicating synthetic activity typical of epithelial regeneration, even though mitotic figures are not readily identifiable in this field.

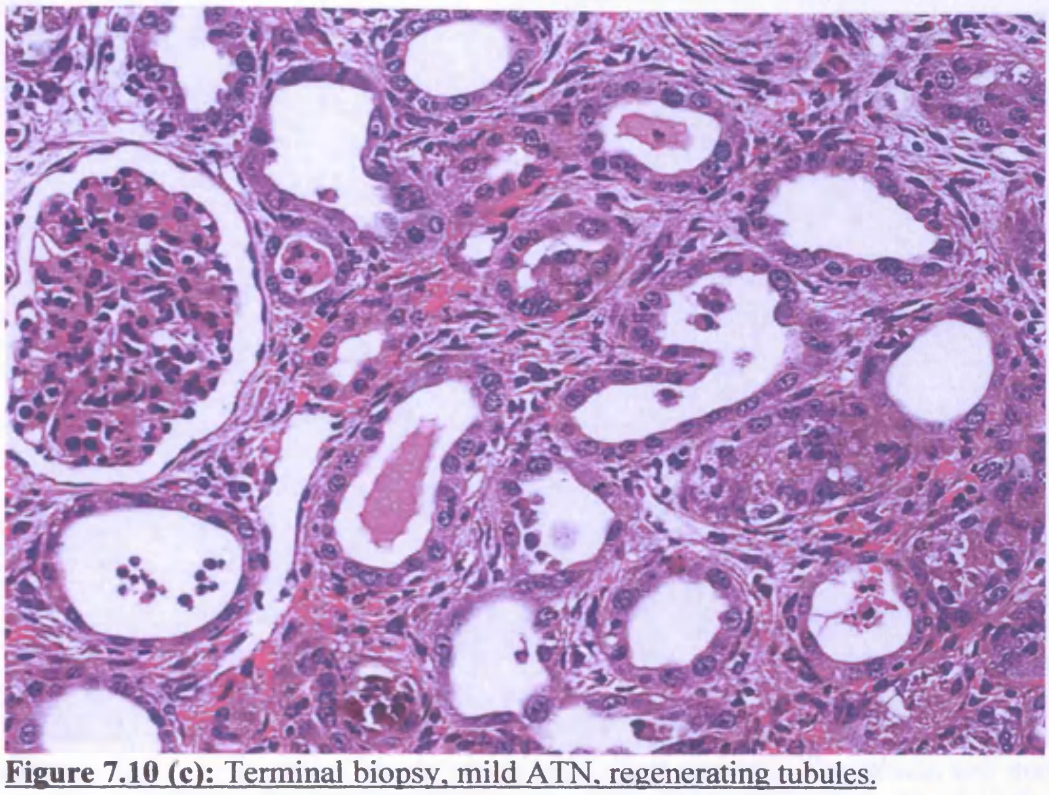


Figure 7.10 (c): Terminal biopsy, mild ATN, regenerating tubules.

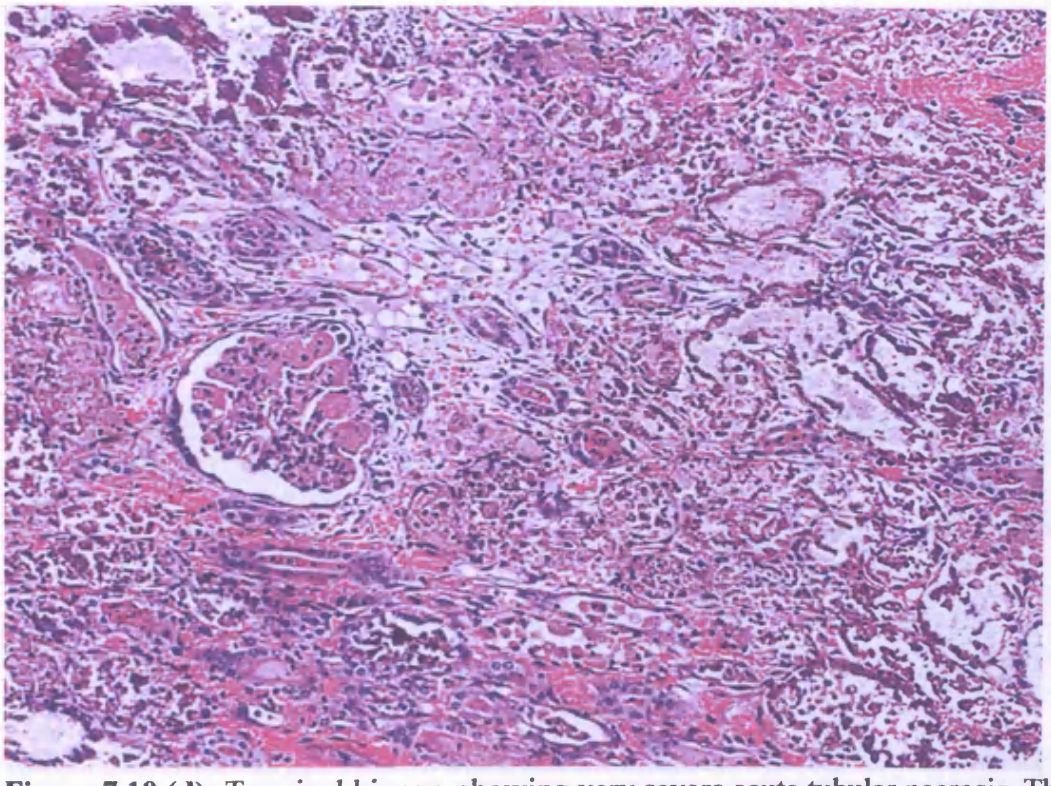


Figure 7.10 (d): Terminal biopsy, showing very severe acute tubular necrosis. There is probably also some focal destruction of tubular architecture in this field, indicating an element of true cortical necrosis. The presence of capillary loop thrombosis in the glomerulus (to the left of centre) is also an adverse prognostic feature suggesting that recovery of function is unlikely.

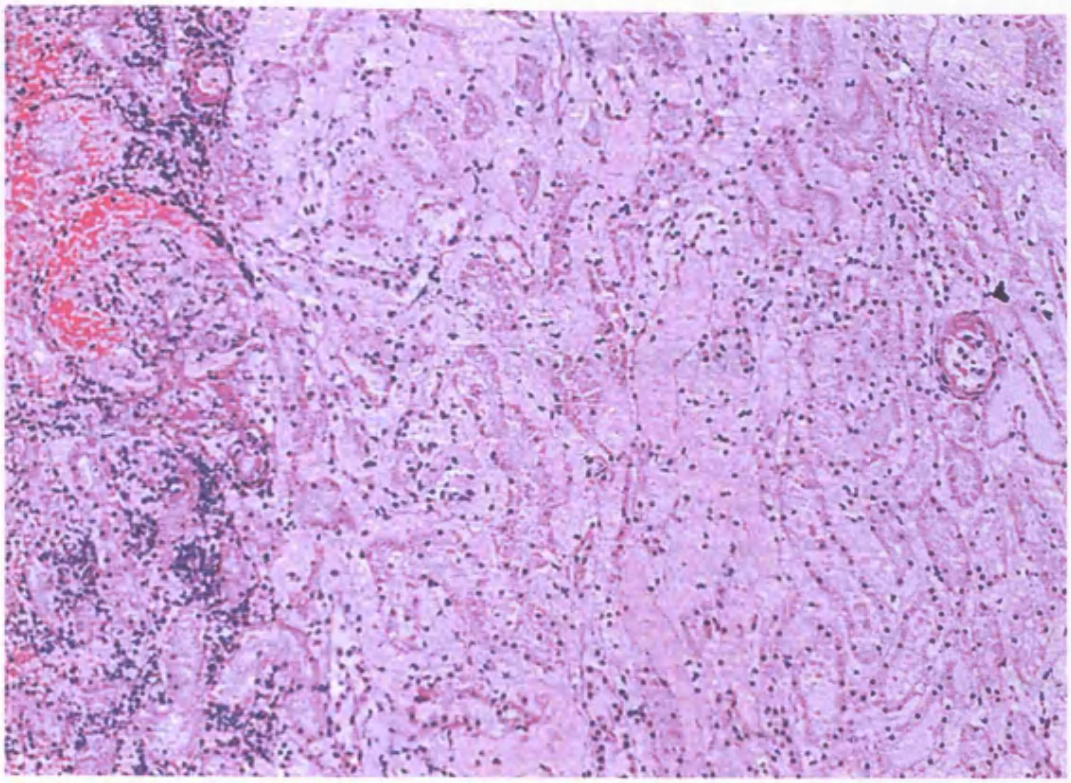


Figure 7.10 (e): Terminal biopsy showing cortical necrosis. The tubular cell nuclei are all pyknotic, and there is early disruption of tubular architecture. On the left of the image there is an inflammatory cell infiltrate in the intersitium, idicating a vital reaction at the boundary between viable and necrotic tissue.

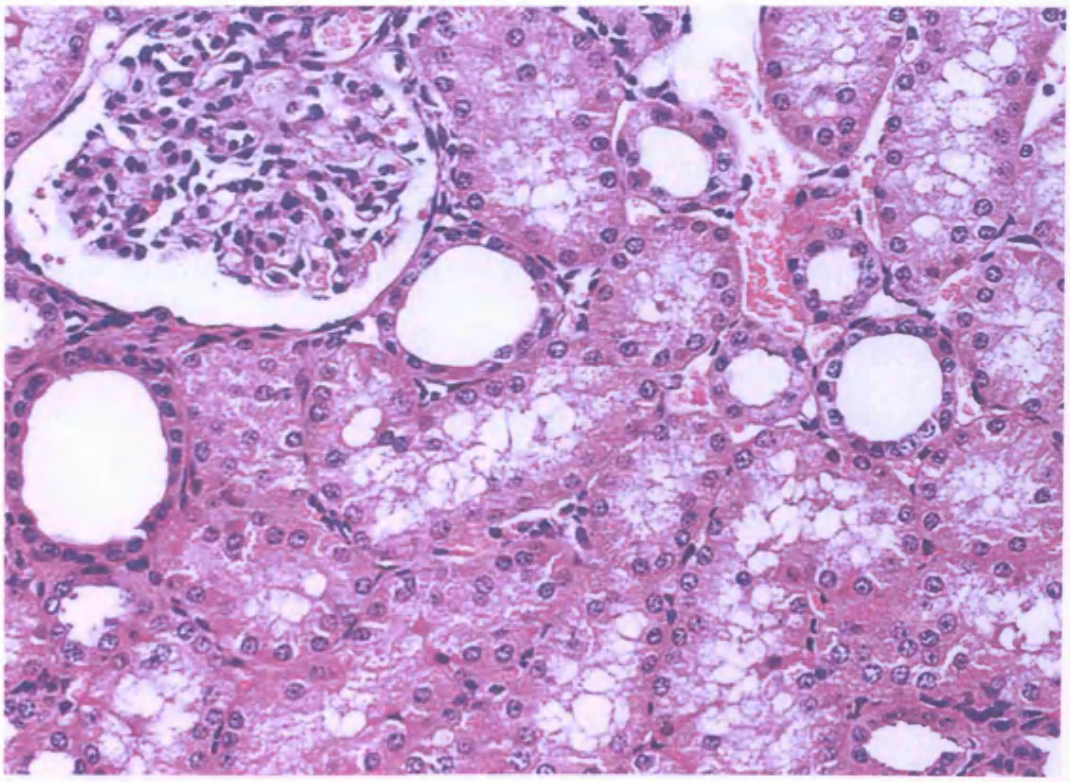


Figure 7.10 (f): Terminal biopsy, near normal kidney.

Chapter 8

Discussion

8.1 Summary of results

In a cadaveric porcine model of NHBD kidneys, renal function, metabolism and vascular tone measured during *ex-vivo* WP correlated with the WIT the kidneys were subjected to prior to retrieval.

This relationship between *ex-vivo* parameters and WIT was confirmed in a porcine renal autotransplant model using minimal WIT analogous to that in clinical retrieval operations from HBD, and 30 minutes WIT, analogous to the WIT estimated in clinical NHBD series. The maximum specificity of any *ex-vivo* parameter was 75% for predicting immediate life-supporting function post-transplantation. The maximum sensitivity of any parameter was 100%, although this may be artificially high due to loss of a data point for this parameter (proteinuria) for a surviving animal. For any parameter with a complete data set the maximum sensitivity is 75%. These values of sensitivity and specificity are identical to the predictive value of knowing the WIT alone. This is inevitable since there is no overlap in the values recorded for any *ex-vivo* parameter between different WIT groups, (see raw data, appendix C). The single exception to this is that the lowest IRR for the 30 minute WIT group is lower than the highest IRR in the minimal WIT group AUC_{IRR}, but this actually decreases the specificity of AUC_{IRR} compared with knowing the WIT alone from 60 to 50%.

The AUC_{IRR} during CP also varies with WIT, and predicts post-transplant function with a specificity and sensitivity of 80%, once again reflecting the rates of post-transplant function according to WIT group, as there is no overlap in AUC_{IRR} between WIT groups.

The oxygen consumption of kidneys during *ex-vivo* WP was in the physiological range, supporting the conjecture that it is possible to provide enough oxygen for and an environment in which aerobic metabolism may be adequately supported. This notion is also supported by the autotransplant experiments comparing the efficacy of WP with the conventionally accepted hypothermic preservation techniques of CS and CP. It is interesting that although the oxygen consumption differed according to WIT, this seems to have been entirely dependent on the flow rate of perfusate, as the tissue extraction of oxygen (as assessed by the arterio-venous difference in perfusate oxygen tension) does not differ with WIT. This suggests that effective oxygen delivery to the tissues may be facilitated by interventions to reduce the renal vascular tone. There were no differences in post-operative survival between the preservation methods, neither for kidneys subjected to minimal nor 30 minutes WIT. Post-operative renal function in the minimal WIT groups, as assessed by the AUC_{cr}, was better in the CP group than the CS or the WP group, but WP was no different to CS.

Pre-transplant histology did not differ with WIT or preservation method at any point prior to transplantation. Post-transplantation, the tubules in the warm perfusion group were more nearly normal than for hypothermic preservation. However, at termination the CP groups had least interstitial expansion, which may be a correlate of subsequent fibrosis.

Therefore this thesis has demonstrated that WP permits an *ex-vivo* functional assessment to be made which correlates with WIT and with post-transplant renal function. However *ex-vivo* function does not appear to predict post-transplant outcome more accurately than knowledge of the WIT the kidneys have suffered. No deleterious effects of WP have been demonstrated, compared with CS. On some of

the parameters measured, there is a trend towards demonstrating that CP may be superior to CS and WP for kidneys with minimal WIT.

8.2 Strengths and limitations of this study

The principal strengths of this study are the use of young healthy animals so that their renal function should not be affected by other variables that may confound results (systemic hypertension, diabetes, etc), and therefore that the only impairment of renal function ought to be caused by warm ischaemic injury inflicted, and subsequent preservation, which is tightly controlled and precisely recorded. Such clear attribution of differences between groups is not possible in clinical trials where donor and recipient factors affect post-transplant outcome.

Also, the use of a transplant model after the viability test has been employed is the only way to truly relate the test to post-transplant function, rather than just surrogate variables, such as the WIT inflicted on the kidneys, which was the end-point used in the initial cadaveric experiments. Although it may useful to relate a viability test to the degree of ischaemia, it does not take into account the reperfusion injury that will be caused by transplantation.

The use of an auto-transplant model is also useful for elimination of confounding variables, specifically acute rejection and the toxicity of immunosuppressive agents.

The WIT groups used range from the clinical correlates of HBD renal donor WIT damage to typical (30 minutes) and extreme (60minutes) WIT for uncontrolled NHBD, but not beyond the limits of WIT from which it is known experimentally that renal function may recover. Therefore the results may be relevant to current clinical practice.

Finally CS and CP represent the currently accepted methods of renal preservation, and in this study they were performed using the best available preservation solutions, and CP under well recognised perfusion conditions, therefore the results in terms of efficacy of preservation method could be confidently interpreted against the gold standards.

The principal limitations of this study are that the pigs were not dialysed post-transplantation, and so the auto-transplant model used was in effect a test of IF, not of PNF, as the model was unable to distinguish between DGF and PNF. This was not the intention when the experiment was planned, and the literature suggests that 30 minutes WIT should not prevent immediate life-supporting renal function, therefore the option to delay the contralateral nephrectomy was not included in the Home Office animal licence application. The original plan was to use AUC_{cr} to compare post-transplant renal function to *ex-vivo* function. In the literature, serum creatinine is reported to have normalised within a week post-transplantation with 30 minute WIT kidneys, so delaying the contralateral nephrectomy would have obscured the renal function of the transplanted kidney during the recovery phase.

The sizes of pigs differed between experimental groups. At the beginning of the study, it was intended to use Large White females of 35-45Kg only, in order to prevent animal and kidney size being potentially confounding variables. However the Foot and Mouth crisis, and the restrictions on livestock movements that this caused completely halted experimental work for some months, and when it was possible to resume there were very few animals available. In order to finish the work for the thesis within the time available it was necessary to accept animals both larger and smaller than those intended. In order to compensate for this degree of variability as best as possible, the *ex-vivo* IRR and oxygen consumption have been calculated per

100g of kidney weight. The group with larger kidneys than all other groups (CP, minimal WIT) also has larger animals, so the larger kidneys have to support a larger body mass. Although crude, this relationship will tend to offset any differences in post-transplant function caused by different masses of functional renal tissue.

The number of animals per group is small, and therefore it is possible that type II error may be responsible for the lack of significance seen between groups. However the matrix design of the study allowing comparisons between several groups reduces the risk of this. Also, significant differences are detected between the groups for all *ex-vivo* renal function and metabolism parameters, WP and CP AUC_{IRR} and post-transplant renal function and survival, so any differences not detected are likely to be small.

One concern over the use of normothermic perfusion with an oxygenated and nutritious perfusate is that it may become contaminated with bacteria or fungi. This may be responsible for the late rise in IRR and deterioration in renal function seen during WP, certainly this has been reported in the past in kidneys that have been normothermically perfused. The potential for microbial contamination to occur is compounded by the prohibitive cost of sterile organ cassettes and oxygenators sold only for single use, and which cannot be sterilised. They were washed with water and detergent and rinsed with hydrogen peroxide then sterile water, but ideally new sterile cassettes would have been used each time. New cassettes were used after 5 WP experiments, or when there was evidence of gross contamination. This problem was addressed in this study by making the perfusate up in a flow hood, and by the administration of antibiotics, penicillin and streptomycin. Microbiological analysis of the perfusate at different time points of the perfusion and with cassettes of differing ages was intended during the cadaver experiments, however the public health

laboratory which would have had to process the samples was unwilling to handle porcine samples.

A problem with the autotransplant phase of the study was that the primary end-point of post-transplant function, animal survival for 2 weeks, is not as 'hard' and clear-cut as it seemed on planning the experiments initially. There are several reasons for this. Firstly, when an animal health was declining to the extent that its suffering was deemed unacceptable according to the severity limit of the home office licence, by the licence holders or animal care and welfare staff, it was terminated rather than allowed to die. This is clearly desirable from the point of view of minimising animal suffering, but immediately blurs the end-point of animal survival. Secondly, the diagnosis of death from renal failure is made on the basis of a post-mortem examination that reveals intact, patent vascular and ureteric anastomoses, with no other obvious cause of death. Death from haemorrhage was taken to be due to renal failure, where accompanied by a purpuric rash, as the bleeding was considered to be secondary to a uraemia. As several animals appeared to have recovering renal function at the time of death as evidenced by a plateau or decline of serum creatinine (see graphs 6.5, 6.8 and 6.9) this seems to be a rather harsh end-point, with the possibility that alternative causes of death had been missed, particularly sepsis. Also several animals died with lower serum creatinines and ureas than others that survived, again making the end-point rather indistinct.

Of concern, the IRR rises and *ex-vivo* renal function deteriorates by 3 hours of WP, and since IRR and *ex-vivo* function reflect warm ischaemic damage prior to WP, the late deterioration of these parameters during WP may reflect additional damage during prolonged WP, despite the oxygen consumption. Although this concern is not proven by the post-transplant results, this may represent type II error.

The late rise in IRR may be due to microbial contamination as discussed above. Alternative explanations include the depletion or absence of a vital ingredient in the perfusate, or the accumulation of a toxin or vaso-active metabolite in the recirculating perfusate. PFC particles in suspension tend to be unstable in size, with larger particles tending to increase in size, and smaller particles decreasing. Larger particles would have two potential drawbacks. Firstly they may become so large as to clog the microvasculature, in a phenomenon similar to sludging of erythrocytes. Secondly they tend to fall out of suspension due to their density, and therefore not contribute adequately to oxygenation. The latter problem could be addressed with a more local and regular supplier of emulsified PFC, so that the particle size was more reliably constant. However the only source available for the project in the quantities required was kindly provided as a favour from a lab in the University of Ulm, Germany who sent a batch as and when they were able. The problem of microbial contamination could be investigated and eliminated as described above. The other possible causes, of metabolite substrate depletion or toxin accumulation would require further investigation and intervention, most likely an iterative process gradually prolonging the length of time for which *ex-vivo* function, metabolism and IRR are stable. This would certainly be worth doing, as a separate project, and may improve the efficacy of WP as a preservation technique, as suggested by others. However this lay well outside the bounds of time available for this thesis.

The survival time of two weeks permitted no analysis of the long-term effects of the preservation methods on post-transplant function.

A final limitation in interpreting the results of this study is that the sensitivity and specificity of the parameters assessed for predicting post-transplant outcome have been determined retrospectively. In order to verify their use as prognostic

instruments, the experiments would need to be repeated, and the cut-off values used to prospectively predict post-transplant viability, with the prediction made by a researcher blind to the WIT.

8.3 Justification of methods

Choice of oxygen carrier

The choice of PFC as an oxygen carrier for this experiment was made on the basis that the alternatives, blood or cfHb solutions are reported to be nephrotoxic or otherwise deleterious in *ex-vivo* perfusion as detailed in chapter 2. The more recent studies demonstrating excellent organ preservation with white cell filtered autologous blood based perfusates have only been reported since this study was underway, whereas studies of renal preservation using PFC emulsion had been published supporting their use.

Choice of oncotic agent

The choice of oncotic agent is justified by the role albumin plays as a carrier molecule for nutrients and metabolites, in contrast to the situation in CP where HES has been shown to be superior, but the role of the oncotic agent is purely physical. The use of tissue culture medium without growth factors is justified by initial experiments in which vascular endothelial growth factor was added to the perfusate in concentrations used in tissue culture, without altering the *ex-vivo* function at all (unpublished observations).

Duration of cold perfusion

The duration of cold perfusion was determined by a previous study suggesting that the clinical benefits of CP were not enhanced by perfusion beyond 6 hours [214]. Also in an experimental model, the IRR during CP fell to a plateau within 6 hours for a variety of WIT, encompassing the WIT range examined in this study [377].

The duration of WP was determined by the observation during the cadaver studies that *ex-vivo* renal function, metabolism and vascular tone deteriorated by three hours of perfusion, suggesting the possibility of further damage to the kidneys beyond this point. Whilst the IRR was decreasing in the peak phase and then stable in the plateau phase, it was considered possible that the perfusion may be beneficial to the kidney by allowing recovery of metabolism without the presence of neutrophils to exacerbate reperfusion injury. The subsequent cold ischaemic times on ice were adjusted so that all groups had comparable total ischaemic times between retrieval and transplantation.

Autotransplant model

There was inevitably a period of cold storage between retrieving the kidneys and establishing them on WP or CP apparatus. This period of CS was kept constant so that its effects would be uniform across groups.

The autotransplant model with contralateral nephrectomy is a well established technique for examining the effects of ischaemia and different methods of renal preservation [152, 170, 400-409]. The contralateral nephrectomy can be delayed if severe ATN is anticipated [152, 400], but it was not in this study after review of the literature [152, 400]. The porcine model is most frequently reported, and was ideal for the study

because of the similarity to human size allowing the kidneys to be established on human perfusion circuits, the ready availability of animals (Foot and Mouth Disease outbreaks not withstanding), and the relative ease in obtaining a home office licence for the study (compared with primates).

The periods of functional assessment

In this study, the cadaveric model was used firstly to confirm that WIT correlated with warm *ex-vivo* function, secondly to ascertain when *ex-vivo* function may best differentiate between kidneys with different WIT, revealing that function during the first hour is the most discriminating. The same preliminary study was not necessary for AUC_{IRR} during CP, as a study by Knight and Nicholson revealed that this parameter differs most between WIT groups during the first hour of perfusion [137].

8.4 Relation to the literature

The correlation of WIT with *ex-vivo* renal function is consistent with other reports of experimental WP, as discussed in chapter 3.8. Similarly, that *ex-vivo* function correlates with post-transplant function has also been reported, as has the correlation between IRR during CP with WIT and post-transplant function (see chapter 3.7). The measurement of GFR post-transplant to validate the use of serum creatinine as an index of renal function has not been reported. Although the time to peak and time to return to baseline of serum creatinine has been reported [410], the calculation AUCcr describes more precisely the derangement in renal function, and has been correlated to GFR.

This study finds no individual *ex-vivo* perfusion parameter in either WP or CP which predicts post-transplant function better than prior knowledge of the WIT. This is because there is no overlap in the ranges for all but one parameter between WIT groups. AUC_{IRR} during WP is the exception to this, and the slight overlap actually reduces specificity of this parameter for predicting IF. No other study has demonstrated a viability test superior to the known WIT. Naturally in the uncontrolled NHBD situation the WIT is often only an estimate.

That the state of the tissues histologically did not correlate with post-transplant viability is consistent with other reports in the literature [153].

We did not find that WP was superior to hypothermic preservation techniques as has been reported for kidneys and other organs (Chapter 2.12). This may be due to deficiencies in the perfusion system used in this study as discussed above. It would be interesting to see if the excellent results using warm perfusion techniques could be reproduced by other groups using the same methodologies.

We found instead that WP was equivalent to CS and CP in terms of survival. CP was found to yield superior early graft function to CS and WP, for kidneys with minimal WIT similar to clinical HBD donors. This has been controversial in the literature (chapter 2.8). Of note there is a particular suggestion that CP is likely to be of more notable benefit for marginal kidneys including NHBD. Our study did not have enough 30 minute WIT survivors to compare early function.

There is no obvious reason to explain the differences between this study and the reports in the literature in terms of animal survival after 30 minutes warm ischaemia [152, 400]. The 30 minute WIT groups seem to represent a fairly critical model in that 20% of animals survive, and several other animals have recovering renal

function at the time of death. Therefore, relatively small differences in management, (e.g. diet) may substantially change survival.

The numbers in this study are too small to attempt to derive a composite predictive tool of post-transplant function combining several *ex-vivo* factors into a viability index. Although this has been suggested [63, 149, 309, 399], and attempted by some [63, 309], it has not been backed up by statistical analysis demonstrating superiority to any individual factor. It may well be that such an index will improve upon individual factors, and that this will be the way that an objective viability test will be developed that has adequate sensitivity and specificity to be of clinical use. However this remains to be demonstrated.

The finding that AUC_{IRR} during CP was no better than knowledge of WIT in predicting post-transplant function is broadly consistent with the report that IRR does not improve on the assessment of viability made on 'clinical grounds' [411], in which one of the clinical grounds used to judge viability was the estimated WIT. The results reported using the clinical grounds of estimated WIT, clinical details of the donor and the macroscopic appearances of the kidneys at retrieval operation have such low PNF rates and good long-term function [41], that it is difficult to improve upon them with an objective test. This remains a desirable aim in order to remove the subjective nature of viability assessment, and therefore facilitate the more widespread use of NHBD kidneys. Other centres specialising in the use of NHBD kidneys report the use of viability assessment tests (chapter 2), and some reporting an improvement in reducing PNF rates as a result [63]. The differences between centres may represent differences in the subjective assessment made of viability.

That WP was only possible for 3 hours without indirect evidence of kidney injury occurring is interestingly consistent with the reports of Brasile et al, who

consistently use 3 hours of 'EMS technology', without giving an account of the reasons for the period of WP chosen. The 3 hour period is consistent for PFC [304] and cfHb [309] based perfusates. The potential benefits of such relatively short periods of WP is supported originally by the work of Kootstra [313] with his intermittent normothermic autologous blood perfusion study prolonging the possible preservation duration, and then by Brasile et al as above. However the more recent work with kidneys [265] and other organs [264, 269] demonstrating longer effective WP preservation with autologous blood may indicate that ultimately blood-based perfusates will ultimately prove superior to the use of artificial oxygen carriers. The frustrating history of attempts to manufacture acellular artificial blood would tend to support this conjecture.

8.5 Implications of the study

The use of WP may allow other centres wishing to establish NHBD programs additional objective information to that described in chapter 3 to assist with the determination of viability. It does not suggest any parameter which may in itself be used as a reliable viability test. The same problems beset the parameters investigated in this study as for those reviewed in chapter 3, i.e., the parameters all correlate with post-transplant function to a greater or lesser extent, but none well enough to be used as a clinically diagnostic test alone. This phenomenon may be described as a 'grey area', to indicate that it is impossible to set both sensitive and specific 'cut-off' points in the ranges of values of potential viability tests, as the correlation of values to viability is not tight enough. The 'grey area' is too broad for the tests individually to be of clinical use.

The use of WP preservation as described has been confirmed as not deleterious to renal function, which is important if *ex-vivo* function is to be used in the pre-transplant assessment of kidneys.

The study suggests that for HBD kidneys CP is the optimum preservation method currently clinically available, and should be used to improve immediate graft function. This has cost implications, as CP requires expensive equipment and consumables and some training in the expertise of operation. However these costs may well be offset by the reduction in post-operative dialysis requirement, and hospital stay. For HBD kidneys, DGF probably correlates with long-term survival, so the benefits of CP may extend beyond the immediate post-operative period.

The sensitivity of the ex-vivo parameters may be better than subjective assessment in predicting which kidneys will function. The evidence for this statement is drawn from the sensitivity of most of the parameters investigated in this study of 75% for IF, and a comparison of the findings of Newcastle and Leicester. Both centres report similar NHBD graft viability rates, but Newcastle transplant 1.4 kidneys per NHBD retrieval operation, compared with the Leicester experience of transplanting 0.5 kidneys per retrieval [42, 63]. This implies that to get good graft function rates post-transplant using subjective criteria, a high price is paid in the discard of potentially viable kidneys. This statement assumes that a similar specificity could be achieved for predicting PNF as is achieved for predicting IF by changing the 'cut-off' points for the parameters used. There is no direct evidence to support this.

This clearly indicates the need for a model of PNF to be used rather than of DGF/IF, in order to determine the value of WP preservation. Should WP prove to be of benefit clinically as the result of future research (see below) then there would be considerable costs involved. In addition to the perfusion machine used in CP, a water

bath and circulation pump are required. In addition to the disposable perfusion cassettes, a membrane oxygenator is required. For clinical purposes these would be single use items, an each oxygenator used in the study cost £180. Additional costs would be incurred in manufacturing the warm perfusate under pharmacological conditions of quality control, the individual reagents cost circa £80 per perfusion, with incurring any charges for the emulsification of the PFC or making up the solutions. The biochemical and blood gas analysis of the perfusate would have to be paid for also. However more than any of these, a major increase in training requirements and work load would be generated, as WP is technically more demanding and labour intensive than either hypothermic preservation technique.

8.6 Areas for future research

The discussion above throws up many avenues for further research. The use of the cadaver model with minimally ischaemic kidneys to refine the perfusate along the lines discussed above to improve the *ex-vivo* function, metabolism and vascular dynamics, and stabilise them for longer period would be a sensible starting point. It may be necessary to infuse some metabolic substrates throughout the perfusion, as they are consumed. Once a clear improvement is demonstrated *ex-vivo*, further auto-transplant work would be justified to verify the improved condition of the kidney. In this manner it may be possible to progress from a situation in which limited WP does no harm, to one in which limited or prolonged WP improves post-transplant renal function by 'resuscitating' it from ischaemic injury in the absence of many of the factors responsible for reperfusion injury, as is suggested in the literature.

The auto-transplant model may need to be altered according to the information that is required from it. In order to detect subtle changes in post-transplant function an immediate or early post-transplant nephrectomy is required in order to detect transient changes in renal function by GFR or AUC_{IRR} before the function is fully recovered. This would be appropriate for HBD transplant research into preservation efficacy, using kidneys with minimal WIT. However in order to determine viability in NHBD research a considerably delayed contralateral nephrectomy is required to determine PNF rates. ATN may recover up to 6 weeks after onset, and so to distinguish between DGF and PNF clinically, the contralateral nephrectomy should be delayed this long. A surrogate end-point may partially avoid this problem, in that the histology may indicate ATN rather than cortical necrosis, suggesting that the kidney may recover life-supporting function.

Ultimately, an optimised WP preservation system would require clinical trials. In addition to validating the results across species, these would be needed to assess whether the predictive value of *ex-vivo* function would be maintained or degraded by the other variables known to influence transplant outcome, both immunological and non-immunological.

Appendix A

Reagent, equipment, animal and service suppliers

Reagent/ Equiptment/ Service/ Animal	Supplier
Abbot Aroset Biochemical Analyser	Abbott systems. Bedford, UK
ABL Radiometer 625 blood gas analyser	ABL. Copenhagen, Denmark
Actrapid insulin	Novonordisk, UK
Animal care and welfare	Biomedical Services, Leicester University
Animal diet	Various sources, through Biomedical Services
Atropine	Smith Kline Beecham, UK
Augmentin	Smith Kline Beecham, UK
Azaperone	Farma, UK
Belzer's II Machine perfusion solution	Transmed, Illinois, USA
Bovine Albumin. Fraction V	Sigma. Gillingham, UK
Buprenorphine	Colman Pharmaceuticals, USA
Bupivicaine	Braun, UK
Capiox SX 100 hollow fibre oxygenator	Terumo. Liverpool, UK
Cellstor 60ml pots – 10% formal saline, 4% formaldehyde	Cell Path. Newton Powys, Wales, UK.
Creatinine, anhydrous	Sigma. Gillingham, UK
Diathermy machine	Conmed. Boston, MA, USA
Dexamethasone	Allergan, UK
Emulsification of perflourodecalin with RPMI 1640	Department of Biomaterials, University of Ulm, Germany

Fetal bovine serum	Sigma. Gillingham, UK
Gas mixture 95%O_2/5%CO_2	BOC Gases. Guilford, UK
Haemaccel	Behring, UK
Halothane	ICI, UK
Hyper-osmolar citrate solution	Baxter. Kent, UK
Isoflourane	ICI, UK
Perflourodecalin	F2 chemicals. Sellafield, UK
Phenobarbitone (Na^+)	Univet. Bicester, Oxon
Portex tubing	Portex, Gillingham, UK
Prolene sutures	Ethicon, UK
Propofol	Transmedics
RM3 pulsatile perfusion machine and MOX 100 pulsatile perfusion cassettes	Waters medical systems. Rochester, MN, USA
RPMI-1640 medium	Sigma. Gillingham, UK
Saline solution 0.9%	Baxter. Kent, UK
Silk sutures	Ethicon, UK
Surgical instruments	Landmark Surgical Equipment, Merseyside, UK
Swabs and Packs	Baxter, UK
Ureteric stents 12 French 8 cm	Bard, UK
UW cold storage solution	DuPont, UK
Water heater/pump	Grant instruments. Cambridge, UK
Vascaths 40 cm double lumen	Bard, UK
Vascular slings	Ethicon, UK
Vicryl sutures	Ethicon, UK

Appendix B

Set up of perfusion systems

WP apparatus

1. On the RM3, check the AC power cord is connected and that the installed back-up battery is charged.
2. Ensure the pump-head inlet is connected to the cassette (Figure A).
3. Place the cassette on the RM3 and screw into place.
4. Connect the temperature probe to the cassette and the RM3.
5. Attach flow probes, ensuring that the right probe in connected from the right RM3 connection to the right arterial tubing and complete the same for the left.
6. Connect the pump-head outlet to the hollow-fibre oxygenator blood inlet port (Figure B).
7. Connect the hollow-fibre oxygenator blood outlet port to the bubble trap.
8. Set the thermostatic bath to 32°C and connect it to the hollow-fibre oxygenator heat exchanger via the water ports.
9. Connect the 95%O_2/5%CO_2 supply, at a flow of 1L/min, to the hollow-fibre oxygenator (figure B) gas inlet port.
10. Add 700ml of the perfusion fluid to the cassette, and switch on power.
11. Press SYSTEM on the display menu then turn ON the pulse pump, zero the pressure and enter the experiment identification code. Turn OFF the circulation pump.
12. Attach pressure tubing to the pressure transducer.
13. De-bubble the pump-head by gently squeezing it until all the bubbles have passed.
14. Using a fine needle syringe, add or remove air to adjust the fluid level in the bubble trap to the centre line.
15. Attach the cannulated renal arteries to the arterial tubing.
16. Readjust fluid level in the bubble trap to the centre line (maintain this throughout perfusion).
17. Adjust systolic pressure to 100mmHg.
18. Check and readjust systolic pressure throughout perfusion to maintain it constant

Cold Perfusion Apparatus

1. On the RM3, check the AC power cord is connected and that the installed back-up battery is charged.
2. Ensure the pump-head inlet is connected to the cassette (Figure A).
3. Place the cassette on the RM3 and screw into place.
4. Connect the temperature probe to the cassette and the RM3.
5. Attach flow probes, ensuring that the right probe in connected from the right RM3 connection to the right arterial tubing and complete the same for the left.
6. Connect waterbath pump tubing and drain tubing to the inlet and outlet of the countercurrent cooler on the cassette.
7. Add 1.5 litres of cold water to the waterbath, and fill the reservoir with ice.
8. Add 600ml of the perfusion fluid to the cassette.
9. Switch on power.
10. Press SYSTEM on the display menu then turn ON the pulse pump and circulation pump, zero the pressure and enter the experiment identification code.
11. Attach pressure tubing to the pressure transducer.
12. De-bubble the pump-head by gently squeezing it until all the bubbles have passed.
13. Using a fine needle syringe, add or remove air to adjust the fluid level in the bubble trap to the centre line.
14. Attach the cannulated renal artery(ies) to the arterial tubing.
15. Readjust fluid level in the bubble trap to the centre line (maintain this throughout perfusion).
16. Adjust systolic pressure to 60mmHg. Check and readjust systolic pressure throughout perfusion to maintain it constant.
17. Add ice to waterbath at intervals to ensure cooling effective

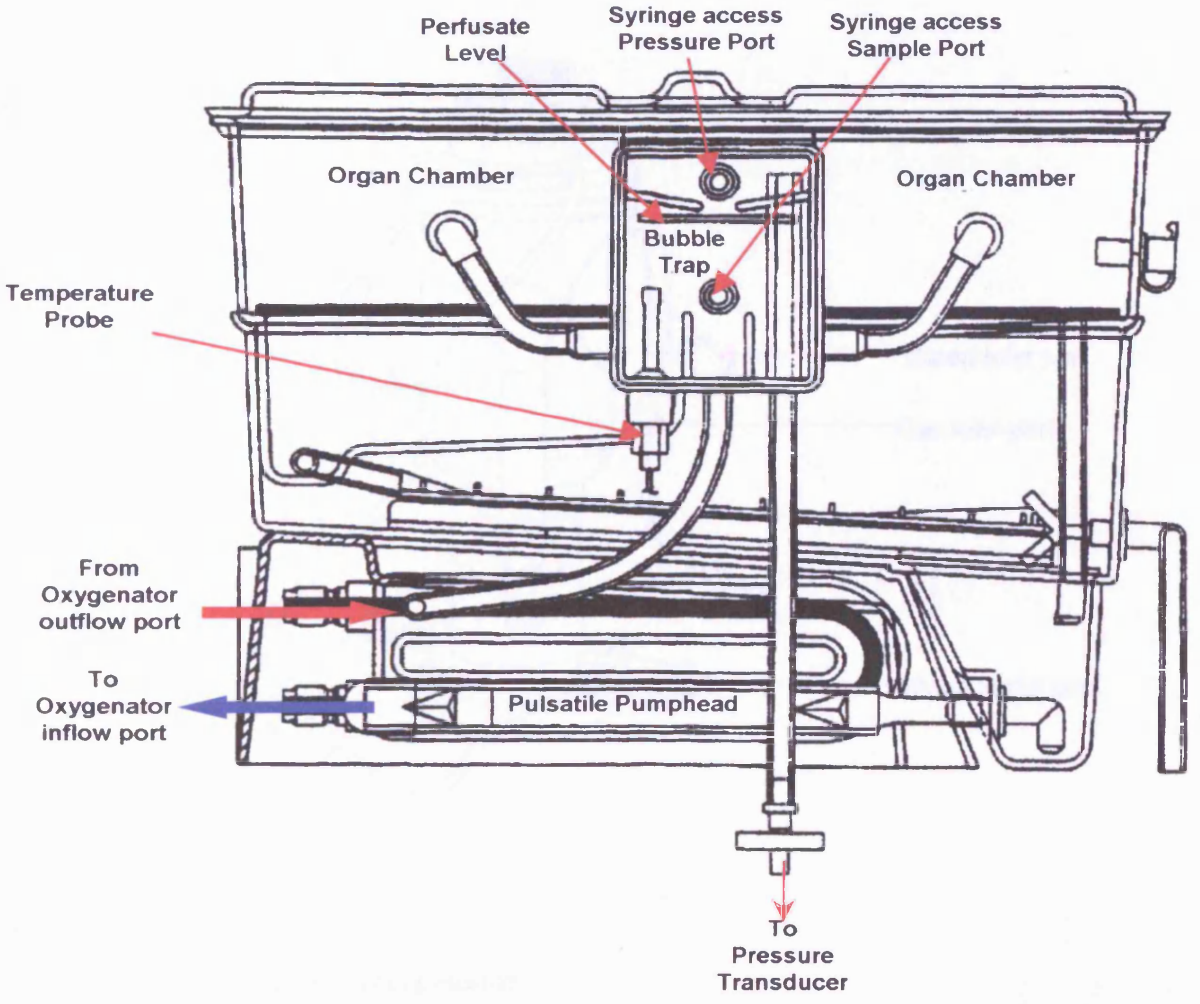


Figure A

Waters M3 pulsatile perfusion machine cassette

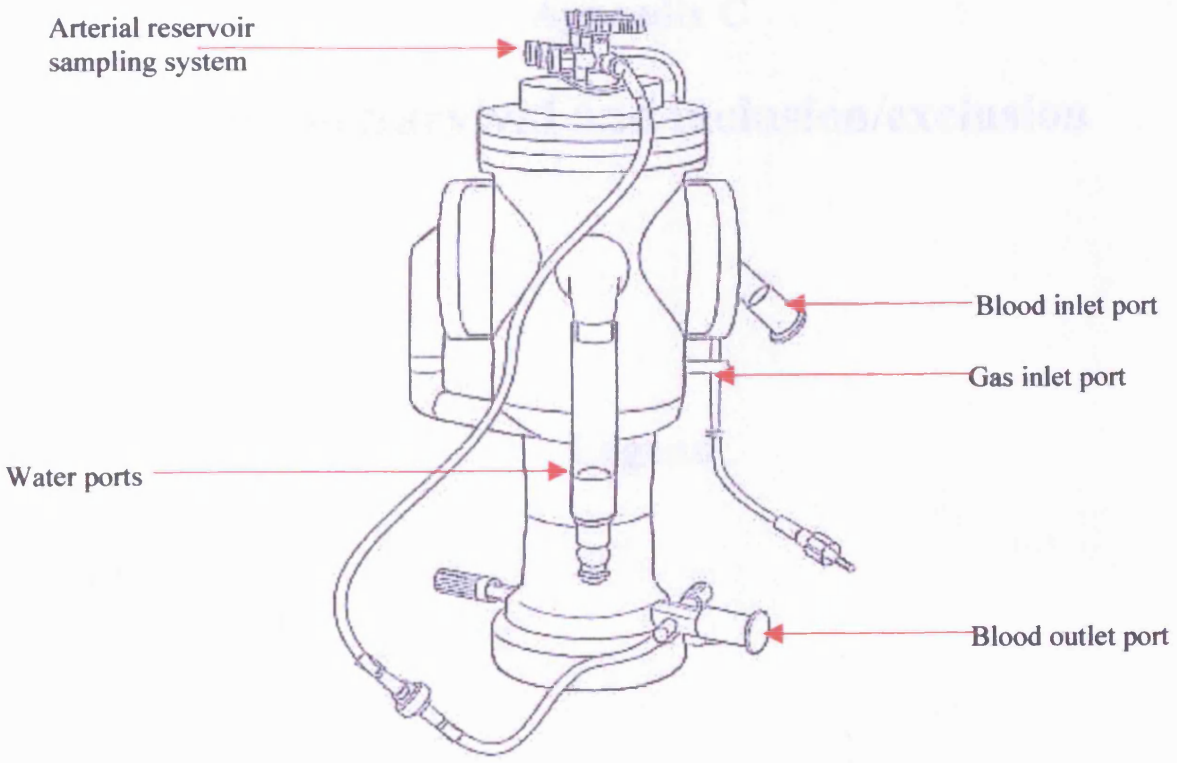


Figure B

Capiox SX 10 Hollow Fibre Oxygenator

Appendix C

Animal Survival and inclusion/exclusion

Legend

:included
:excluded

Annotation	Key
PIN	Pig identification number
WIT 1	Warm ischaemic time (minutes)
CIT	Cold ischaemic time (hours)
WIT 2	Anastomosis time (minutes)
Pres	Preservation Method
Surv	Days survived
Post mortem findings/Cause of death	

PIN No.	WIT 1	CIT	WIT 2	Pres	Surv	PM
1	3	24.1	31	CS	2	kidney well perfused, anastomoses intact and widely patent. Enormous blood loss from aorta during retrieval.
2	3	n/a	n/a	CS	0	Died on induction for transplant operation, large left flank haematoma from nephrectomy site
3	5	25.27	25	CS	14	Normal kidney, anastomoses intact
4	4	25.17	25	CS	14	Normal kidney, anastomoses intact
5	n/a	n/a	n/a	n/a	-1	Died on table during nephrectomy of malignant hyperpyrexia
6	2	20.11	25	CS	14	Normal kidney, anastomoses intact
7	4	21.24	32	CS	7	Well perfused kidney, anastomoses intact
8	31	21.17	28	CS	6	Found dead, PM huge haematoma, pupuric rash over body, kidney and bladder normal, anastomoses patent
9	31	22.43	32	CS	14	Well perfused, all anastomoses patent and intact
10	30	23.58	33	CS	2	Single kidney. Infarcted. Renal artery thrombosis, pedicle kinked.
11	n/a	n/a	n/a	n/a	-1	Vascular clamp slipped off renal pedicle during 30 minute WIT, in situ reperfusion injury, terminated
12	3	22.36	27	CS	14	Normal kidney, anastomoses intact
13	31	21.07	30	CS	2	although kidney appeared well perfused, only pinhole patency of renal artery
14	30	23.11	40	CS	0	arterial thrombosis on table - therefore terminated
15	30	22.32	27	CS	2	kidney and all anastomoses normal. Reason for termination = lame left leg (refractory)
16	30	23.14	32	CS	3	Kidney well perfused, and the anastomoses widely patent.
17	31	22.07	34	CS	6	kidney well perfused except for approx 10-20% supplied by lower polar artery. Anastomoses patent.
18	30	22.56	37	CP	7	kidney, anastomoses, ureter all fine. Pupuric rash over body, and enormous haematoma in transplant bed.
19	32	21.48	30	CP	2	Initially passing urine, then injured during restraint -> urine leaking through wound. Ureteric anastomosis disrupted.
20	31	22.4	37	CP	5	kidney well perfused, anastomoses widely patent. Bladder full of offensive urine and pus.
21	30	23.11	27	CP	5	kidney well perfused, making urine (clear). Anastomoses intact and widely patent.
22	32	21.23	28	CP	6	kidney well perfused, making urine (clear). Anastomoses widely patent. SB full of blood (pig vomiting pre-op)
23	31	22.51	31	CP	14	Normal kidney, anastomoses intact
24	30	22.55	31	CP	4	necrotic kidney, renal vein thrombosis (questioned at the time of transplantation)
25	30	24.18	36	CP	5	2/3 kidney well perfused, 1/3 (lower pole) frank cortical necrosis. Anastomoses intact.
26	30	23.44	31	WP*	0	Porcine serum rather than fetal bovine serum used
27	31	22.14	34	WP*	6	Porcine serum rather than fetal bovine serum used

28	32	21.51	27	WP	7	Anastomoses fine, ureter fine, kidney well perfused.
29	30	22.39	30	WP	14	Despite the excellent clinical course, there was a collection of pus around the kidney.
30	31	22.16	32	WP	5	Anastomoses fine, ureter fine, kidney well perfused, some urine in the bladder, clear and inoffensive
31	30	24.06	40	WP	5	50% of the kidney perfused,anastomoses intact and patent.
32	30	24.32	30	CP	5	Collection of pus inside kidney and bladder. Anastomoses intact and patent.
33	30	23.55	34	CS	8	Kidney well perfused,urine in bladder. Large amount of blood/fluid around transplanted kidney.
34	30	24.4	30	WP	4	Kidney alittle mottled,anastomosis intact and patent. Small amount of haemturia in bladder.
35	30	20.5	30	CP	4	Kidney well perfused anastomosis intact. No urine in the bladder. Renal failure.
36	2	24.42	35	CP	5	Kidney grossly enlarged, patchey areas of cortical necrosis. Very little urine in the bladder, stent in place but ureter obstructed
37	2	24.4	27	CP	14	Kidney well perfused anastomosis intact. Normal kidney.
38	2	21.22	27	WP	14	Normal kidney, anastomoses intact
39	2	24.49	29	WP	14	Normal kidney, anastomoses intact
40	2	22.32	30	WP	7	Kidney mostly necrotic tissue, anastomosis all intact and patent
41	2	24.11	32	WP	4	Kidney well perfused, anastomoses intact and patent. Large haematoma around transplanted kidney.
42	2	23.19	34	WP	4	Found dead, stomach full of blood. Kidney well perfused, anastomoses intact and patent, urine in bladder.
43	1	21.5	29	WP	3	Kidney necrotic in places, anastomosis intact and patent, no urine in bladder.
44	1	24.43	27	WP blood	14	Normal kidney,anastomosis intact. Large haematoma around transplanted kidney.
45	2	23.01	27	WP	14	Normal kidney, anastomoses intact
46	2	22.55	28	CP	14	Normal kidney, anastomoses intact and patent.
47	2	24.08	24	CP	4	Kidney grossly enlarged, anastomosis intact and patent.
48	2	25.11	32	CP	14	Normal kidney, anastomoses intact and patent.
49	2	23.47	24	CP	14	Normal kidney, anastomoses intact and patent.

Appendix D

Raw data

Biochemistry

Perfusion

Post-transplant

GFR

O_2 Consumption

IRR

Animal weight

Kidney weight

Biochemistry

Autotransplant WP: perfusion biochemistry at one hour

Minimal WIT

Creatinine concentration ratios

PIN	perfusate	urine	Ratio
38	324	2630	8.11728 4
39	208	2338	11.2403 8
40	169	3346	19.7988 2
43	143	3414	23.8741 3
45	274	2300	8.39416 1

K+ ratios

38	8	61	7.625
39	12	60	5
40	7	91	13
43	5	78	15.6
45	9	71	7.88888 9

Na+

38	160	97	0.60625
39	163	58	0.35582 8
40	165	23	0.13939 4
43	154	63	0.40909 1
45	170	59	0.34705 9

Glucose

38	16.4	14.1	0.85975 6

30 minutes WIT

PIN	perfusate	urine	ratio
28	303	476	1.57095 7
29	351	594	1.69230 8
30	284	498	1.75352 1
31	322	500	1.55279 5
34	315	513	1.62857 1

28	9	41	4.55555 6
29	8	23	2.875
30	8	34	4.25
31	12	37	3.08333 3
34	11	26	2.36363 6

28	153	109	0.71241 8
29	163	111	0.68098 2
30	157	99	0.63057 3
31	170	118	0.69411 8
34	157	121	0.77070 1

28	13.1	15.3	1.16793 9

			0.52343				1.23478
39	12.8	6.7	8	29	11.5	14.2	3
							1.22429
40	14.2	2.2	0.15493	30	10.7	13.1	9
			0.12711				1.24489
43	11.8	1.5	9	31	9.8	12.2	8
							1.36641
45	11.6			34	13.1	17.9	2

Protein

			0.83076				
38	65	54	9	28	64	80	1.25
							1.19047
39	68	34	0.5	29	63	75	6
			0.52238				1.28571
40	67	35	8	30	63	81	4
							1.29508
43	50	21	0.42	31	61	79	2
							1.27868
45	70			34	61	78	9

Cadaver experiments: perfusion biochemistry at one hour

WIT <10 mins

Animal No	Na	K	protein	glucose	creatinine
1					
Perfusate	148	8	69d	contaminate	389
left urine	136	28	4d	contaminate	1249
Right urine	99	65	1.9d	contaminate	261
2					
Perfusate	141	6	63	7.6	377
left urine	95	43	6	1.3	3804
Right urine	115	35	8	2	3211
3					
Perfusate	126	19	68d	contaminate	506
left urine	15	74	3d	contaminate	4545
Right urine	31	52	5d	contaminate	1299
4					
Perfusate	159	8	62	6.1	375
left urine	133	21	16	6	1058
Right urine	130	35	8	4.7	1143
5					
Perfusate	155	10	67	7.4	1035
left urine	127	41	3	2.4	1583
Right urine	74	64	2	0.5	2268

WIT 30 mins

Animal No	Na	K	protein	glucose	creatinine
1					
Perfusate	147	8	61	8.3	428
left urine	144	30	35	7	1767
Right urine	147	20	44	9.1	889
2					
Perfusate	141	11	63	4.4	252
left urine	110	28	14	2	1443
Right urine	131	38	13	3.6	1347
3					
Perfusate	158	9	61	6	369
left urine	135	40	21	3.5	2567
Right urine	137	38	23	4	2407

WIT 60 mins

Animal No	Na	K	protein	glucose	creatinine
1					
Perfusate	165	7	66	5.6	529
left urine	127	37	7	1.5	1003
Right urine	130	39	9	1.7	582
2					
Perfusate	141	12	51	5	448
left urine	147	22	45	5.5	807

Right urine 3	145	19	48	5.1	777
Perfusate	145	10	62	5.8	414
left urine	145	21	56	7.3	1255
Right urine	156	13	65	8.4	596

Urea and creatinine values before and after autotransplantation

Daily serum creatinines (umol/ml)															
Day	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Min WIT	**CS**														
PIN 3	105	148	625	921	1083	1117	1066	1098	1063	956	728	467	350	306	251
PIN 4	106	199	543	770	1029	1247	1278	951	1062	328	203	175	163	155	161
PIN 6	176	236	639	1011	1174	1266	1071	1086	1047	973	768	575	664	661	556
PIN12	102	212	755	1125	1401	1655	1731								
PIN7	146	208	583	833	1229	1459	1876	2238							
30 WIT	**CS**														
PIN 8	98	181	648	977	1152	1119	1046								
PIN9	125	201	671	1039	1352	1537	1502	1419	1244	696	426	320	255	249	231
Pin 16	141	206	702	1097	1312										
PIN 17	132	240	657	894	1208	1393	1455	1593							
PIN 33	120	239	652		1558	1908	1916	1940	1945						
30 WIT	**CP**														
PIN 18	111	175	639	872	1138	1429	1748	1677	1574						
PIN 21	151	202	620	830	1095	1314	1496								
PIN 23	120	167	576	904	1148	1493	1648	1697	1513	1107	768	521	375	301	324
PIN 25	109	189	740	989	1287	1465	1732	1957							
PIN 35	129	199	657	1032	1394	1650									

30 WIT	**WP**															
PIN 28	97	140	530	754	909	1168	1387	1443	1590							
PIN 29	133	172	706	941	1096	1359	1504	1624								
PIN 30	128	165	683	914	1050	1253	1476									
PIN 31	119	195	719	998	1354	1698	1943									
PIN 34	150	269			1220	1617										
Min WIT	**CP**															
PIN 37	139	209	594	965	1259	1635	1585	1000	655	401	309	293	258	254	256	
PIN 46	109	143	272	229	231	252	254	196	150	136	132	141		136	142	
PIN 47	71	212	564	789	1070	1270										
PIN 48	130	139	669	754	950	1037	1048	963	799	595	476	391	352	313	283	
PIN 49	114	188	582	657	515	376	356	297	225	199	193		167			
PIN 50	129	193	657	523	363	362	442	406	317	251	221	206	212	218	217	
Min WIT	**WP**															
PIN 38	143	213	676	1159	1657	1775	854	642				331	316	314		
PIN 39	115	184	690	1049	1409	1753	2131	2192	1862	1385	963	714	583	466	372	
PIN 43	109	186	690	1061	1234											
PIN 45	133	198	568	782	1052	1341	1434	1289	1090	973	618	407	334	316	268	

Serum urea levels (mmol/l)															
Day	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Min WIT	**CS**														
PIN 3	4.1	7.9	22	36.9	39.4	42.3	45.8	52.7	54.3	49.5	36.1	25.3	19.2	15.3	10.9
PIN 4	4.6	18.7	29.4	34.2	38.7	43.6	42.9	41.3	53.6	12.3	6.4	4.1	4.6	4.9	6.2
PIN 6	3.8	4.1	20.1	34.2	43.7	47.4	49.5	55.7	53.4	51.1	43.3	36.4	35.6	38.8	35.9
PIN12	5.7	21.6	30.3	43.3	38.5	42.6	50.5	43.3							
PIN7	3.4	6.1	17	24.5	34.8	39.7	41.9	48.4							
30 WIT	**CS**														
PIN 8	3.6	6.5	19.5	30.4	41.6	45.1	53								
PIN9	4	7.1	21.7	34.1	41.5	51.1	54.4	60.6	62.2	39	18.2	10.4	7.6	6.6	6.2
Pin 16	3.6	5.7		41.4	48.1										
PIN 17	3.7	6	23.2	37.5	44.7	50.3	53.6	60.4							
PIN 33															
30 WIT	**CP**														
PIN 18	5.1	9.5	24.4	39.2	46.7	55.9	60	64.3	68.4						
PIN 21	4.8	19.3	27.4	39.8	38.5	42.6									
PIN 23	4	12	26.3	33.8	40.4	46.2	52.9	55	41.7	28.6	20.1	13.2	8	5.5	4.8
PIN 25	5.3	15	28.5	37	40.6	43.2	49.2	53.4							
PIN 35	4.5	7.8	21.9	36.9	43.7	45.9									

30 WIT	**WP**															
PIN 28	3.7	7.2	19.6	31.2	39.9	52.7	56.3	61.3	64.9							
PIN 29	3.9	14.2	25.3	32.3	38.4	40.5	44.7	47.8	49	30.7	23.4	18.6	15.2	14.7	17	
PIN 30	4.8	5.7	21.6	30.3	43.3	38.5	42.6									
PIN 31																
PIN 34	6	9.3				56.2										
min WIT	**CP**															
PIN 37	4.6	6.4	13.9	25.1	35.3	43.9	40.9	31.8	19.5	12.6	11.7	10.2	9.4	9	9.2	
PIN 46	4.4	4.8		7.5	6.7	8	8.9	7.8	4.4	4.9	4.3		5	5.1	5.4	
PIN 47	1.6	5.4	11.5	15.9	19.3	27.1										
PIN 48	3.6	6.6	12.3	16.2	18.3	20.7	24.3	26.5	25.7	19.2	15.5	13.9	13.2	11.4	10.1	
PIN 49	4.1	6.3	16.3	17.6	12.8	10.1	11.2	9.3	5.8	4.5	4.2		4.8			
PIN 50	3.7	9.2	17.8	13.4	8.6	9.5	11.1	10.6	9	7.1	5.7	5	4.5	4.8	5.7	
min WIT	**WP**															
PIN 38	5	6.4	18.8	26.6	33	33.4	18.6	15.5				9.4	7.5	6.9		
PIN 39	4.2	7	19	29.3	38	45.6	55	66.6	62.9	47.6	30		14.7	8.6	5.9	
PIN 43	3.4	5.8	32.4	37.6												
PIN 45	4.5	7.5	13	16.8	21.5	25.4	28.2	27.5	28.5	29.7	23.6	16.3	11.8	10.1	8.3	

Autotransplant GFR

PIN	Sample	time (min)	[I] mg/ml	Point clearance	Clearance
6	1	150	0.492	8	16
	2	195	0.471	10	
	3	240	0.455	10	
	4	300	0.418	13	
9	1	150	0.523	28	34
	2	203	0.451	32	
	3	242	0.42	32	
	4	300	0.378	32	
23	1	150	0.36	101	92
	2	195	0.313	92	
	3	245	0.257	89	
	4	300	0.194	90	
33	1	145	0.429	18	18
	2	195	0.419	16	
	3	245	0.39	17	
	4	300	0.393	14	
37	1	150	0.276	58	51
	2	195	0.238	57	
	3	240	0.19	62	
	4	300	0.195	49	
39	1	152	0.499	11	20
	2	228	0.458	13	
	3	272	0.439	13	
	4	330	0.382	18	
44	1	148	0.417	57	11
	2	192	0.431	42	
	3	250	0.413	36	
	4	305	0.397	32	
38	1	150	0.19	68	61
	2	195	0.171	62	
	3	242	0.144	62	
	4	300	0.118	61	
45	1	150	0.393	27	22
	2	195	0.365	27	
	3	240	0.345	26	
	4	300	0.326	24	
48	1	151	0.305	29	36
	2	186	0.289	29	
	3	241	0.255	30	
	4	301	0.207	35	

49	1	150	0.169	79	78
	2	195	0.127	85	
	3	240	0.11	79	
	4	305	0.091	73	
22	1	150	0.23	53	54
	2	195	0.2	53	
	3	242	0.177	51	
	4	307	0.132	56	
46	1	155	0.175	113	103
	2	195	0.171	94	
	3	245	0.12	100	
	4	300	0.092	97	

Oxygen consumption

Cadaver experiments: oxygen consumption at one hour

<10 min Animal No	WIT O2 (ml/min)	30 mins Animal No	WIT O2 (ml/min)	60 mins Animal No	WIT O2 (ml/min)
1	16.87881 (a) 17.45009 (v)	1	14.14859 (a) 15.20854 (v)	1	9.95731 (a) 11.47242 (v)
3	16.68883 (a) 21.27119 (v)	2	17.58207 (a) 19.70688 (v)	2	6.468798 (a) 7.485323 (v)
4	19.36775 (a) 21.08933 (v)	3	10.40489 (a) 11.36 (v)	3	10.21882 (a) 12.88742 (v)

Autotransplant experiments: oxygen consumption at 1 hour

Minimal WIT	ARTERIAL p02 (kPa)	VENOUS p02 (kPa)	30 min WIT	ARTERIAL p02 (kPa)	VENOUS p02 (kPa)
PIN 38	73.89	45.42	PIN 28	78.47	42.31
PIN 39	68.2	40.4	PIN 29	75.52	39.63
PIN 40	83.74	46.5	PIN 30	84.6	40.37
PIN 43	73.31	28.85	PIN 31	74	30.43
PIN 45	87.67	38.61	PIN 34	71.79	27.96

IRR

Intrarenal vascular resistance: cadaveric experiments

Minimal WIT

Animal	1		2		3		4		5	
kidney	left	Right	left	right	left	Right	left	right	left	right
	0.985075	0.375	0.382199	0.370558	2.475	0.916667	3.16129	0.695035	0.343612	0.3436
	0.393548	0.297561	0.247863	0.242678	1.695652	0.821053	0.604317	1.473684	0.324561	0.3915
	0.273408	0.240924	0.240175	0.22541	1.432836	0.827586	0.586207	1.133333	0.328283	0.3915
	0.296552	0.262997	0.275556	0.238462	0.962963	0.981132	0.534591	0.714286	0.31405	0.4871
	0.335793	0.268437	0.314159	0.252669	1.141304	1.153846	0.538961	0.568493	0.313008	0.5833
	0.280702	0.195122	0.345455	0.263889	1.534247	1.454545	0.540881	0.5	0.305439	0.5983
	0.562162	0.380952	0.328889	0.25784	2.5	1.590909	0.575342	0.459016	0.305785	0.5781
	0.38674	0.263158	0.302326	0.240741	1.787234	1.448276	0.601449	0.430052	0.297071	0.5378
	0.412088	0.277778	0.297561	0.235521	2.525	1.836364	0.625899	0.430693	0.283333	(
	0.376963	0.257143	0.276699	0.222656	2	1.606557	0.697183	0.482927	0.26971	0.4676
	0.359606	0.253472	0.262443	0.221374	1.90566	1.246914	0.692308	0.466321	0.266667	0.4444
	0.319444	0.230769	0.241667	0.208633	1.426471	0.989796	0.677966	0.484848	0.253731	0.4146
	0.307692	0.226667	0.228571	0.201439	1.219178	0.936842	0.548148	0.391534	0.240741	0.3892
	0.282158	0.214511	0.216867	0.197802	1.228571	0.905263	0.389222	0.320197	0.235507	0.3757
	0.26506	0.205607	0.208835	0.191882	1.295775	0.929293	0.335196	0.272727	0.226027	0.3567
	0.249042	0.19697	0.199203	0.185874	1.089888	0.906542	0.313725	0.238806	0.217687	0.3316
	0.231343	0.186186	0.192913	0.182836	1.045455	0.910891	0.279817	0.208191	0.211604	0.3147
	0.21978	0.180723	0.194656	0.186813	0.989474	0.959184	0.283105	0.202614	0.203226	0.2957
	0.214545	0.178248	0.185714	0.178694	0.828829	0.958333	0.266376	0.188854	0.197492	0.2825
	0.203448	0.172515	0.180212	0.176471	0.772358	1.021505	0.252252	0.176101	0.189189	0.2658
	0.193333	0.16763	0.176471	0.180851	0.666667	1.126437	0.239837	0.16573	0.181818	0.
	0.187097	0.164306	0.177305	0.171821	0.637584	0.703704	0.22619	0.157459	0.184848	0.244
	0.178683	0.158774	0.177632	0.174757	0.638889	0.707692	0.220472	0.155556	0.175953	0.2272
	0.18038	0.157025	0.174051	0.171875	0.635714	0.706349	0.212598	0.153846	0.175595	0.2201
	0.173913	0.153846	0.169811	0.168224	0.646617	0.699187	0.209924	0.158501	0.169697	0.2081
	0.171779	0.153005	0.170886	0.168224	0.559748	0.695313	0.203008	0.151261	0.165079	0.2023
	0.166667	0.150685	0.169279	0.168224	0.508571	0.674242	0.198502	0.149718	0.165109	0.202
	0.160714	0.147139	0.169279	0.169279	0.480447	0.637037	0.190476	0.143251	0.167183	0.2007
	0.159763	0.147139	0.170347	0.169279	0.5	0.619403	0.18705	0.141689	0.166667	0.1963
	0.170279	0.148248	0.169811	0.169279	0.482353	0.594203	0.187726	0.142077	0.164306	0.1889
	0.163142	0.145553	0.171429	0.170347	0.4375	0.571429	0.189781	0.139037	0.159091	0.1794
	0.167683	0.147453	0.169811	0.170347	0.433862	0.546667	0.18638	0.142857	0.160458	0.1777
	0.165644	0.145553	0.170347	0.170347	0.421875	0.536424	0.183453	0.134565	0.160458	0.1755
	0.166667	0.145161	0.170347	0.170886	0.39	0.513158	0.180851	0.134921	0.159091	0.172
	0.17134	0.147453	0.170347	0.171429	0.371981	0.509934	0.180851	0.133858	0.158192	0.1707
	0.171875	0.147059	0.170347	0.171429	0.356808	0.5	0.1777	0.132124	0.156425	0.1676
	0.17134	0.146667	0.170886	0.171429	0.340909	0.483871	0.178947	0.133159	0.157303	0.165
	0.17284	0.148936	0.170886	0.172524	0.328889	0.465409	0.178694	0.136126	0.157746	0.165
	0.170347	0.147541	0.166667	0.16879	0.311688	0.441718	0.177083	0.134211	0.15864	0.165
	0.172308	0.149333	0.170347	0.172524	0.301724	0.419162	0.182143	0.132468	0.157746	0.1651
	0.176471	0.152406	0.169811	0.173077	0.294606	0.405714	0.182143	0.134211	0.157303	0.1651
	0.180685	0.155496	0.169279	0.172524	0.285714	0.380435	0.189474	0.144	0.153409	0.1592
	0.183801	0.158177	0.170347	0.172524	0.2749	0.353846	0.182759	0.143631	0.156069	0.1597
	0.186916	0.16	0.169811	0.173633	0.269076	0.338384	0.183746	0.136842	0.156342	0.1582
	0.188854	0.162234	0.170347	0.173633	0.244094	0.300971	0.211864	0.131926	0.158683	0.157
	0.191358	0.164021	0.16875	0.173633	0.266055	0.320442	0.176056	0.138504	0.160606	0.1591

0.193252	0.167109	0.168224	0.173633	0.256604	0.302222	0.173913	0.137203	0.161677	0.1597
0.19788	0.170213	0.16875	0.173077	0.257576	0.294372	0.174497	0.136126	0.161194	0.1592
0.221402	0.20202	0.167183	0.171975	0.255725	0.288793	0.173913	0.136483	0.160237	0.1588
0.208333	0.191693	0.169231	0.174051	0.250951	0.28821	0.180272	0.137662	0.159763	0.1583
0.212014	0.188679	0.167173	0.174051	0.25	0.285714	0.177852	0.136247	0.160237	0.1569
0.214035	0.191824	0.167173	0.174051	0.249042	0.276596	0.177258	0.135204	0.164179	0.159
0.221831	0.2	0.167683	0.174051	0.244186	0.265823	0.183051	0.139535	0.164671	0.160
0.219512	0.201278	0.167173	0.174603	0.24031	0.258333	0.181518	0.143979	0.164671	0.1608
0.214286	0.197368	0.167173	0.175159	0.237354	0.25	0.17608	0.137306	0.167665	0.1637
0.214533	0.199357	0.164634	0.171975	0.235294	0.244898	0.182432	0.137405	0.166667	0.1632
0.209622	0.196141	0.167683	0.175159	0.246094	0.250996	0.181818	0.137755	0.167164	0.1637
0.20438	0.190476	0.164134	0.171975	0.238095	0.235507	0.1843	0.138462	0.169811	0.1621
0.205479	0.192926	0.164634	0.171975	0.228464	0.222628	0.185567	0.139896	0.175159	0.1708
0.202749	0.189103	0.165138	0.172524	0.227642	0.215385	0.181208	0.142105	0.175385	0.1696
0.202749	0.189711	0.164134	0.172524	0.203704	0.187713	0.180505	0.139665	0.175926	0.1706
0.206897	0.194175	0.163142	0.172524	0.2	0.183673	0.183051	0.143617	0.174847	0.1727
0.205479	0.194805	0.164634	0.173077	0.203636	0.186047	0.18	0.142857	0.176471	0.1716
0.206897	0.19544	0.165138	0.173077	0.205776	0.184466	0.187075	0.142857	0.177019	0.1727
0.204082	0.196078	0.164634	0.173077	0.200704	0.1875	0.186667	0.146982	0.180685	0.1757
0.204082	0.196721	0.161585	0.170418	0.203448	0.189103	0.181518	0.146277	0.177019	0.1732
0.207483	0.200658	0.166154	0.174194	0.204152	0.187898	0.186667	0.150943	0.179567	0.1752
0.208191	0.200658	0.16358	0.170968	0.204861	0.187898	0.189831	0.147757	0.179567	0.1762
0.209622	0.200658	0.162577	0.171521	0.204861	0.187302	0.187075	0.144357	0.177215	0.1739
0.210345	0.199346	0.163077	0.171521	0.207612	0.189274	0.188889	0.144068	0.182692	0.1792
0.211073	0.199346	0.162577	0.170968	0.207612	0.188679	0.199219	0.14956	0.182109	0.1786
0.209622	0.200658	0.164596	0.170968	0.210345	0.191223	0.203125	0.152047	0.18038	0.1764
0.206186	0.19802	0.164596	0.171521	0.209622	0.191824	0.20155	0.152047	0.182965	0.1790
0.208191	0.200658	0.164596	0.171521	0.213793	0.196203	0.204633	0.156342	0.183544	0.1801
0.201954	0.194357	0.165109	0.172078	0.213793	0.195584	0.207692	0.161194	0.184713	0.1790
0.214286	0.202454	0.164087	0.171521	0.217993	0.198738	0.210728	0.166163	0.185304	0.1795
0.223729	0.195266	0.163077	0.171521	0.21875	0.198738	0.211321	0.172308	0.185304	0.1795
0.212121	0.186944	0.165625	0.172078	0.222222	0.201258	0.217899	0.195122	0.185304	0.1806
0.199357	0.192547	0.165109	0.171521	0.22028	0.2	0.219512	0.171429	0.181529	0.1786
0.211921	0.194529	0.164596	0.171521	0.224265	0.203333	0.226891	0.175896	0.185304	0.181
0.194268	0.191824	0.164596	0.171521	0.220149	0.198653	0.23431	0.18241	0.184713	0.1818
0.195513	0.190031	0.165109	0.170968	0.230189	0.208191	0.238494	0.184466	0.184127	0.1818
0.194888	0.190625	0.166667	0.175325	0.235741	0.213058	0.247899	0.192182	0.183544	0.182
0.242754	0.195335	0.165644	0.174194	0.237548	0.213058	0.257384	0.199346	0.184127	0.182
0.198083	0.196825	0.166154	0.174194	0.242308	0.216495	0.261603	0.203947	0.185304	0.182
0.199357	0.194969	0.163077	0.170968	0.242308	0.215753	0.266376	0.211073	0.187702	0.181
0.199357	0.194969	0.164087	0.171521	0.247104	0.219178	0.281553	0.214815	0.187702	0.181
0.2	0.196203	0.166667	0.174757	0.249027	0.219178	0.296875	0.22619	0.189542	0.181
0.199357	0.196203	0.167183	0.175325	0.25	0.219931	0.310881	0.239044	0.190164	0.181
0.203226	0.201923	0.164596	0.172078	0.254902	0.224138	0.324742	0.258197	0.190789	0.181
0.201278	0.201923	0.168224	0.175325	0.256917	0.225694	0.335052	0.282609	0.190789	0.1818
0.203822	0.205788	0.168224	0.175325	0.261905	0.229965	0.342391	0.288991	0.194079	0.1849
0.209677	0.209003	0.169753	0.176849	0.266932	0.234266	0.371429	0.302326	0.194079	0.1843
0.203077	0.206897	0.161491	0.168285	0.264706	0.232472	0.384615	0.3125	0.194079	0.1855
0.215385	0.213415	0.174194	0.181208	0.273128	0.24031	0.408537	0.328431	0.194719	0.1855
0.206154	0.211356	0.170279	0.177419	0.277533	0.244186	0.432099	0.341463	0.194719	0.186
0.213415	0.217391	0.167183	0.174194	0.283186	0.248062	0.447205	0.352941	0.195364	0.186
0.217262	0.222561	0.170807	0.177419	0.293333	0.255814	0.440994	0.348039	0.195364	0.186
0.207101	0.213415	0.170279	0.176849	0.290179	0.252918	0.484848	0.376471	0.195364	0.186
0.222222	0.228188	0.174455	0.180064	0.297674	0.260163	0.515625	0.390533	0.197279	0.1877

0.219745	0.226974	0.174455	0.180645	0.302326	0.265306	0.543307	0.410714	0.199324	0.19218
0.218069	0.234899	0.17284	0.180645	0.31068	0.273504	0.555556	0.416667	0.197232	0.18269
0.215434	0.226351	0.182099	0.190323	0.314286	0.278481	0.581395	0.449102	0.197917	0.18269
0.217949	0.227425	0.173913	0.180064	0.319048	0.2827	0.578947	0.484277	0.199301	0.1821(
0.219048	0.228477	0.174455	0.180064	0.327014	0.292373	0.6	0.436364	0.204225	0.18412
0.22619	0.2375	0.176101	0.180064	0.331754	0.297872	0.646552	0.465839	0.204947	0.18412
0.218954	0.231034	0.174603	0.180328	0.33	0.298643	0.66087	0.466258	0.205674	0.18354
0.222222	0.239726	0.181818	0.184211	0.345178	0.311927	0.666667	0.469136	0.207143	0.1829(
0.234899	0.251799	0.180064	0.183607	0.348485	0.316514	0.704762	0.477419	0.210714	0.1867(
0.243827	0.252396	0.183871	0.1875	0.350785	0.317536	0.75	0.524476	0.21147	0.1867(
0.238806	0.253968	0.183871	0.186885	0.367021	0.334951	0.745098	0.546763	0.215827	0.18987
0.226866	0.247557	0.185065	0.1875	0.37234	0.343137	0.737374	0.51049	0.215827	0.19108
0.225519	0.25	0.187097	0.190164	0.37766	0.351485	0.77551	0.531469	0.213235	0.1883'
0.229607	0.251656	0.187097	0.190789	0.384615	0.357143	0.77551	0.524138	0.215613	0.1901(
0.225519	0.250825	0.187097	0.190164	0.392265	0.367876	0.785714	0.527397	0.219331	0.192(
0.22619	0.251656	0.190939	0.194079	0.39779	0.371134	0.793814	0.52027	0.219331	0.192(
0.224299	0.245734	0.190939	0.194079	0.40884	0.38342	0.793814	0.52027	0.219331	0.192(
0.233438	0.254296	0.190939	0.194079	0.40884	0.38342	0.793814	0.52027	0.223048	0.1973(
0.228916	0.24918	0.194175	0.197368	0.423529	0.39779	0.8125	0.496183	0.225926	0.201
0.231928	0.256667	0.196774	0.200658	0.434524	0.414773	0.810526	0.52381	0.22963	0.2052
0.234043	0.255814	0.196141	0.200658	0.437126	0.412429	0.802083	0.52381	0.242424	0.2084
0.23494	0.258278	0.2	0.203947	0.453416	0.434524	0.8125	0.537931	0.233463	0.203
0.236527	0.260726	0.203226	0.207237	0.465409	0.43787	0.833333	0.531915	0.25	0.2202
0.238806	0.266667	0.204473	0.210526	0.471698	0.443787	0.862069	0.547445	0.245968	0.2155
0.246201	0.271812	0.205128	0.211221	0.471698	0.443787	0.863636	0.558824	0.242915	0.2120
0.25	0.277027	0.210356	0.214521	0.471698	0.443787	0.862069	0.551471	0.245902	0.2142
0.25	0.283276	0.209003	0.215232	0.465839	0.441176	0.842697	0.551471	0.25	0.2170
0.251479	0.289116	0.212219	0.218543	0.465839	0.438596	0.921053	0.57377	0.25	0.2194
0.252941	0.292517	0.214744	0.222591	0.465839	0.436047	0.77451	0.537415	0.254098	0.2230
0.259587	0.302405	0.215434	0.223333	0.465839	0.433526	0.8	0.539007	0.256198	0.2238
		0.21865	0.226667	0.47205	0.44186	0.8	0.546763	0.2625	0.2282
		0.219048	0.23	0.47205	0.44186	0.8	0.546763	0.261411	0.2290
		0.223642	0.233333	0.46875	0.436047	0.78125	0.547445	0.261803	0.2284
		0.227564	0.237458	0.477987	0.44186	0.802083	0.557971	0.267241	0.2348
		0.233227	0.244147	0.481013	0.449704	0.802083	0.557971	0.273913	0.2395
		0.236422	0.248322	0.487013	0.451807	0.791667	0.550725	0.276316	0.2423
		0.242038	0.255892	0.489796	0.455696	0.77551	0.562963	0.28	0.2451
		0.246006	0.260135	0.496454	0.463576	0.734513	0.560811	0.285714	0.250
		0.250794	0.266892	0.496454	0.463576	0.72973	0.554795	0.290179	0.2549
		0.25641	0.271186	0.496454	0.463576	0.736364	0.558621	0.29148	0.2559
		0.262821	0.277966	0.496503	0.467105	0.78	0.577778	0.294643	0.2598
		0.267516	0.285714	0.496552	0.467532	0.736364	0.554795	0.299107	0.263
		0.273312	0.289116	0.496552	0.467532	0.721739	0.553333	0.299539	0.2685
		0.281553	0.296928	0.489655	0.461039	0.706897	0.550336	0.303318	0.2807
		0.282958	0.30137	0.496552	0.464516	0.698276	0.554795	0.315789	0.2920
		0.288026	0.305842	0.493151	0.464516	0.692308	0.554795	0.317308	0.2920
		0.292605	0.312715	0.493151	0.464516	0.680672	0.558621	0.323671	0.2951
		0.296774	0.317241	0.493151	0.467532	0.691667	0.568493	0.326733	0.2972
		0.300971	0.321799	0.493151	0.467532	0.672	0.56	0.338384	0.3059
		0.309446	0.33101	0.489933	0.467949	0.666667	0.556291	0.343434	0.3119
		0.31068	0.334495	0.493243	0.467949	0.672	0.56	0.348485	0.3165
		0.315961	0.339161	0.493243	0.467949	0.672	0.556291	0.350515	0.3177
		0.322476	0.347368	0.493243	0.467949	0.651163	0.556291	0.357513	0.3239
		0.32459	0.348592	0.493243	0.467949	0.636364	0.56	0.368984	0.3349

		0.330065	0.355634	0.496599	0.470968	0.642857	0.5625	0.374332	0.3414
		0.331148	0.355634	0.496599	0.470968	0.712963	0.620968	0.380435	0.3465
		0.336634	0.360424	0.493243	0.470968	0.706422	0.601563	0.387978	0.3
		0.343333	0.366548	0.496599	0.470968	0.712963	0.596899	0.392265	0.3585
		0.322835	0.353448	0.5	0.467949	0.706422	0.596899	0.398876	0.3659
		0.413978	0.48125	0.5	0.470968	0.712963	0.596899	0.40678	0.3730
		0.430108	0.5	0.496599	0.467949	0.719626	0.592308	0.410112	0.3782
		0.44	0.513333	0.5	0.470968	0.747573	0.596899	0.418079	0.383
		0.45977	0.540541	0.503448	0.470968	0.762376	0.592308	0.421348	0.3886
		0.462428	0.540541	0.5	0.470968	0.728155	0.581395	0.426966	0.3937
		0.47619	0.551724	0.459677	0.428571	0.809524	0.612613	0.435028	0.3989
		0.487952	0.566434	0.465649	0.438849	0.754902	0.601563	0.440678	0.406
		0.485207	0.594203	0.5	0.480519	0.762376	0.596899	0.424419	0.3924
		0.497041	0.608696	0.5	0.480519	0.76699	0.617188	0.442857	0.4052
				0.506757	0.487013	0.759615	0.617188	0.480263	0.4424
				0.506757	0.483871	0.769231	0.625	0.486842	0.4457
				0.517007	0.490323	0.771429	0.632813	0.496689	0.4518
				0.517007	0.493506	0.764151	0.632813	0.503311	0.4578
				0.517007	0.493506	0.780952	0.645669	0.509934	0.4638
				0.517007	0.496732	0.79798	0.642276	0.52	0.469
				0.517007	0.496732	0.822917	0.652893	0.52	0.469
				0.52027	0.503268	0.772727	0.60177	0.47482	0.4258
				0.52381	0.506579	0.861111	0.632653	0.546154	0.4863
				0.510204	0.496689	0.840426	0.658333	0.54	0.4939
				0.504065	0.484375	0.84375	0.658537	0.523179	0.4876

30 minutes WIT

Animal No.	1		2		3	
Kidney	left	right	left	right	Left	right
	0.745098	0.449704	2.131579	0.852632	0.019608	0.333333
	0.613793	0.549383	0.881188	0.613793	0.55303	0.682243
	0.567073	0.588608	0.89	1.059524	0.39881	0.350785
	0.520958	0.537037	0.852459	1.083333	0.353659	0.297436
	0.488636	0.49711	1.297872	1.605263	0.368421	0.302885
	0.505747	0.494382	1.728395	2.153846	0.310044	0.303419
	0.54386	0.505435	4.307692	2.604651	0.327586	0.35023
	0.5375	0.488636	1.79661	2.12	0.359447	0.380488
	0.541935	0.461538	6.571429	3.538462	0.382353	0.408377
	0.512821	0.42328	5.944444	2.815789	0.427027	0.42246
	0.5	0.412698	5.789474	1.746032	0.493976	0.445652
	0.459302	0.379808	27	1.588235	0.548611	0.461988
	0.424581	0.36019	2.518519	1.283019	0.564286	0.473054
	0.406593	0.350711	7	1.26506	0.519231	0.445055
	0.387435	0.342593	5.941176	1.278481	0.51634	0.424731
	0.374384	0.330435	3.607143	1.216867	0.531034	0.409574
	0.350467	0.316456	2.8	1.139535	0.613636	0.430851
	0.349057	0.313559	2.475	1.076087	0.692308	0.433155
	0.33945	0.307054	2.285714	1.066667	0.640625	0.468571
	0.367442	0.331933	1.811321	1.090909	0.633588	0.471591
	0.344186	0.317597	1.59322	1.032967	0.630769	0.473988
	0.331776	0.300847	1.342857	0.921569	0.615385	0.467836
	0.319444	0.292373	1.210526	0.859813	0.577778	0.445714
	0.302632	0.278226	1.168831	0.79646	0.565217	0.440678
	0.2827	0.255725	1.109756	0.80531	0.506757	0.407609
	0.275424	0.246212	1.022472	0.777778	0.480519	0.38342
	0.266393	0.238971	0.989011	0.762712	0.440994	0.353234
	0.256809	0.234043	0.938776	0.754098	0.423529	0.341232
	0.243542	0.219269	0.948454	0.747967	0.38172	0.307359
	0.244444	0.212219	0.910891	0.747967	0.367021	0.293617
	0.237918	0.203822	0.893204	0.747967	0.340206	0.276151
	0.22963	0.196203	0.883495	0.739837	0.313433	0.255061
	0.219512	0.192073	0.857143	0.743802	0.303738	0.25
	0.211806	0.18209	0.824074	0.735537	0.296804	0.243446
	0.215054	0.179104	0.810811	0.775862	0.278481	0.230769
	0.201413	0.169139	0.787611	1.072289	0.264463	0.21843
	0.195286	0.173134	0.5	0.741667	0.258333	0.211604
	0.195946	0.174174	0.747899	0.684615	0.252101	0.205479
	0.186885	0.168142	0.721311	0.666667	0.242678	0.199313
	0.187919	0.164223	0.685039	0.654135	0.231405	0.193103
	0.184564	0.158501	0.661654	0.619718	0.224	0.190476
	0.188356	0.157143	0.637037	0.597222	0.21374	0.186047
	0.185567	0.154286	0.607143	0.551948	0.217544	0.191358
	0.186851	0.152113	0.573427	0.522293	0.202055	0.181538
	0.183391	0.148045	0.51773	0.474026	0.200692	0.181818
	0.187266	0.151057	0.468085	0.425806	0.202166	0.18123
	0.184028	0.148876	0.496933	0.447514	0.197183	0.175
	0.184669	0.149296	0.433862	0.394231	0.189003	0.171875
	0.18662	0.149718	0.417989	0.393035	0.188153	0.169279

0.184028	0.149296	0.396825	0.365854	0.187943	0.165109
0.18662	0.148876	0.383784	0.342995	0.1875	0.166154
0.189964	0.149718	0.373626	0.328502	0.182143	0.161392
0.190813	0.152542	0.351351	0.31401	0.183099	0.161994
0.192857	0.152113	0.333333	0.300429	0.179856	0.160256
0.194245	0.152975	0.306306	0.275304	0.179856	0.160772
0.192308	0.158501	0.284404	0.255144	0.175627	0.157556
0.191638	0.15896	0.261905	0.233051	0.176895	0.160656
0.197183	0.163743	0.236364	0.203922	0.174216	0.159236
0.202899	0.16	0.212	0.189964	0.171617	0.1571
0.202899	0.161383	0.192727	0.167722	0.170068	0.15674
0.207885	0.166667	0.182456	0.165605	0.177083	0.156923
0.212996	0.17052	0.180212	0.160377	0.167235	0.151703
0.21223	0.171014	0.176678	0.157729	0.167808	0.153605
0.215054	0.175439	0.173145	0.154088	0.174216	0.155763
0.212014	0.173913	0.176678	0.158228	0.16443	0.146269
0.20073	0.167683	0.170732	0.157556	0.162712	0.145015
0.220588	0.185759	0.175	0.157051	0.162252	0.14497
0.21831	0.182891	0.176259	0.157051	0.171329	0.151235
0.219081	0.183976	0.177305	0.161812	0.169014	0.147692
0.217544	0.185075	0.174377	0.159091	0.170819	0.148607
0.225352	0.191617	0.179211	0.159744	0.175439	0.152905
0.221805	0.187302	0.178182	0.15655	0.164948	0.143713
0.222222	0.186047	0.177536	0.157051	0.168421	0.146789
0.210526	0.176991	0.175627	0.157051	0.171429	0.148148
0.215139	0.172524	0.175627	0.155556	0.176471	0.15
0.216216	0.176101	0.175	0.155063	0.174545	0.147239
0.228873	0.193452	0.183099	0.165605	0.175373	0.147335
0.2397	0.192771	0.16955	0.156051	0.167883	0.142415
0.242537	0.197568	0.171329	0.155556	0.164286	0.136905
0.237548	0.194357	0.173759	0.154088	0.164948	0.136364
0.254386	0.205674	0.172535	0.155063	0.164948	0.134831
0.264228	0.210356	0.17037	0.158076	0.17301	0.136986
0.266393	0.211726	0.170139	0.158065	0.182432	0.138462
0.274262	0.221843	0.174216	0.161812	0.179054	0.137306
0.279661	0.229167	0.176056	0.160256	0.183391	0.136598
0.279167	0.234266	0.177936	0.159744	0.189091	0.137203
0.283262	0.235714	0.178571	0.159236	0.193309	0.137203
0.292035	0.249057	0.177936	0.160772	0.192308	0.137363
0.297778	0.25283	0.177936	0.162338	0.192308	0.13624
0.302222	0.257576	0.179211	0.162866	0.192913	0.134986
0.310811	0.264368	0.180505	0.16129	0.200803	0.139665
0.3125	0.272374	0.184116	0.162939	0.202429	0.138889
0.31982	0.276265	0.184116	0.16242	0.205645	0.142857
0.325792	0.28125	0.184783	0.16242	0.207317	0.144886
0.325893	0.285156	0.18705	0.165605	0.203125	0.140541
0.327434	0.287938	0.18705	0.165605	0.205224	0.144737
0.330396	0.294118	0.191336	0.168254	0.203774	0.144385
0.330435	0.300395	0.192029	0.167722	0.205993	0.143979
0.331797	0.303797	0.19708	0.169811	0.201493	0.143236
0.339713	0.308696	0.198529	0.167702	0.200772	0.143646
0.35	0.316742	0.199262	0.166154	0.206226	0.148045
0.353234	0.324201	0.202952	0.168712	0.20155	0.144444
0.356436	0.331797	0.211679	0.178462	0.205993	0.148649

0.363184	0.336406	0.202899	0.175	0.20438	0.146597
0.365979	0.334906	0.206522	0.179245	0.210332	0.151194
0.378947	0.344498	0.202899	0.176101	0.205993	0.147849
0.382199	0.350962	0.226923	0.189711	0.206897	0.14876
0.387435	0.357488	0.215328	0.181538	0.208333	0.150273
0.39267	0.362319	0.214545	0.183801	0.209125	0.152355
0.406593	0.373737	0.215328	0.184953	0.210526	0.153425
0.416667	0.380711	0.218978	0.1875	0.2	0.146199
0.424581	0.385787	0.21978	0.186916	0.216216	0.159544
0.426829	0.384615	0.223938	0.187097	0.214286	0.158333
0.431034	0.394737	0.237903	0.197324	0.209924	0.15625
0.45614	0.40625	0.236948	0.196667	0.216216	0.16
0.458824	0.408377	0.239837	0.197987	0.214559	0.160458
0.468085	0.415094	0.241803	0.20068	0.209924	0.158501
0.487342	0.4375	0.244813	0.202749	0.209125	0.15942
0.493671	0.443182	0.251029	0.211806	0.209924	0.15942
0.460526	0.419162	0.251012	0.216028	0.214022	0.173653
0.503448	0.45625	0.26971	0.222603	0.204301	0.163324
0.490446	0.435028	0.266667	0.226148	0.229927	0.1875
0.43949	0.38764	0.268085	0.230769	0.127273	0.103245
0.427673	0.379888	0.266094	0.224638	0.174545	0.142012
0.409357	0.366492	0.26383	0.227106	0.214545	0.174556
0.39548	0.35533	0.266949	0.231618	0.231047	0.188791
0.377143	0.338462	0.27234	0.236162	0.231047	0.187683
0.415205	0.371728	0.27897	0.241636	0.232975	0.191176
0.4	0.35468	0.281385	0.243446	0.231884	0.191045
0.403315	0.363184	0.290749	0.25	0.232143	0.192308
0.403315	0.363184	0.297778	0.256705	0.234875	0.19469
0.40884	0.368159	0.300448	0.2607	0.235915	0.19764
0.406593	0.368159	0.305164	0.265306	0.237762	0.2
0.40884	0.37	0.316832	0.278261	0.239583	0.202346
0.405556	0.368687	0.325123	0.289474	0.243902	0.205882
0.415663	0.377049	0.323383	0.290179	0.247387	0.208211
0.421687	0.382514	0.338624	0.303318	0.238806	0.2
0.424242	0.380435	0.342105	0.309524	0.269076	0.22408
0.426829	0.380435	0.34555	0.314286	0.265873	0.221854
0.431138	0.382979	0.352632	0.320574	0.270161	0.224832
0.417647	0.37766	0.363158	0.330144	0.269388	0.225256
0.423529	0.380952	0.37234	0.334928	0.25523	0.217082
0.431953	0.386243	0.379679	0.341346	0.28	0.237736
0.440476	0.393617	0.392473	0.350962	0.278481	0.23913
0.446429	0.398936	0.403226	0.358852	0.2827	0.246324
0.45509	0.406417	0.408602	0.363636	0.288136	0.253731
0.469512	0.416216	0.411765	0.370192	0.291304	0.256705
0.469512	0.418478	0.414894	0.375	0.3	0.265385
0.47205	0.422222	0.427807	0.386473	0.305677	0.269231
0.481013	0.429379	0.41875	0.378531	0.308036	0.269531
0.487342	0.435028	0.448485	0.41573	0.316514	0.277108
0.484277	0.435028	0.462963	0.418994	0.324074	0.282258
0.487179	0.44186	0.471338	0.432749	0.325581	0.284553
0.5	0.448485	0.477707	0.441176	0.331731	0.289916
0.506757	0.454545	0.484076	0.449704	0.341463	0.300429
0.510067	0.463415	0.486842	0.453988	0.35122	0.310345
0.510067	0.463415	0.506757	0.474684	0.351759	0.311111

0.510067	0.463415	0.513514	0.484076	0.362245	0.322727
0.516779	0.469512	0.52027	0.490446	0.367347	0.328767
0.52027	0.472393	0.52027	0.49359	0.369231	0.333333
0.527027	0.478528	0.527027	0.5	0.371134	0.336449
0.527027	0.478528	0.520548	0.496732	0.374359	0.339535
0.537415	0.484663	0.554745	0.527778	0.382199	0.349282
0.548387	0.485714	0.562044	0.538462	0.388298	0.354369
0.541096	0.487654	0.562044	0.538462	0.38587	0.356784
0.544218	0.487805	0.569343	0.545455	0.385965	0.352941
0.536913	0.490798	0.537037	0.495726	0.39779	0.367347
0.536913	0.493827	0.573529	0.549296	0.413408	0.389474
0.543624	0.5	0.595588	0.566434	0.418994	0.396825
0.543624	0.5	0.591241	0.574468	0.424581	0.402116
0.543624	0.5	0.59854	0.585714	0.430168	0.409574
0.546763	0.496732	0.605839	0.592857	0.432584	0.407407
0.576923	0.524476	0.613139	0.595745	0.440678	0.412698
0.589147	0.531469	0.613636	0.591241	0.440678	0.412698
0.589147	0.531469	0.637097	0.612403	0.431034	0.403226
0.589147	0.527778	0.669421	0.613636	0.465839	0.438596
0.588785	0.512195	0.576923	0.530973	0.463855	0.44
0.612903	0.546763	0.703704	0.655172	0.46988	0.448276
0.596899	0.534722	0.624	0.604651	0.467066	0.453488
0.601563	0.538462	0.648	0.627907	0.46988	0.453488

60 minutes WIT

Animal No	1		2		3	
Kidney	left	right	left	right	Left	right
		0.335366				8.75
	1.731707	0.78022	1.328571	1.690909		
	1.566667	0.79661	1.424658	1.464789	4.947368	
	1.372881	0.736364	2.1	1.693548	0.404858	0.934579
	1.352113	0.768	3.52381	2.176471	0.979167	0.895238
	1.966102	0.913386	2.794872	2.18	1.066667	0.941176
	2.189655	1.040984	3.5	2.142857	1.172414	1
	2.192308	1.14	6.933333	2.311111	1.4	1.101124
	2.512821	1.306667	7.571429	2.52381	1.693548	1.22093
	2.878788	1.376812	12.11111	3.30303	1.912281	1.197802
	2.5	1.219512	10.6	2.717949	2.805556	1.346667
	2.292683	1.119048	8.583333	2.102041	2.395349	1.537313
	2.023256	1.035714	12	2.204082	2.212766	2
	1.777778	0.952381	1.445946	2.14	3.740741	2.348837
	1.541667	0.870588	4.333333	1.6	3.517241	2
	1.4	0.833333	4.08	1.728814	3.366667	1.655738
	1.197183	0.732759	3.322581	2.102041	2.55	1.569231
	1.025316	0.632813	2.828571	2.020408	1.98	1.455882
	0.987805	0.623077	2.615385	1.888889	1.796296	1.310811
	0.901099	0.577465	2.272727	1.724138	1.916667	1.210526
	0.828283	0.539474	2.222222	1.754386	1.72	1.088608
	0.776699	0.506329	2.454545	2.037736	1.844444	1.024691
	0.752381	0.49375	2.842105	2.571429	1.213333	0.978495
	0.733333	0.48125	3.724138	3.375	1.157895	0.77193
	0.714286	0.465839	3.925926	3.785714	1.098765	0.700787
	0.704762	0.459627	5.777778	4.333333	0.927835	0.6
	0.679245	0.444444	5.368421	4.25	0.877551	0.565789
	0.669811	0.438272	4.652174	3.689655	0.857143	0.541935
	0.660377	0.432099	4.458333	2.891892	0.790476	0.506098
	0.650943	0.428571	4.375	2.837838	0.769231	0.5
	0.646552	0.428571	4.12	2.783784	0.672131	0.476744
	0.61157	0.406593	4	2.7	0.634921	0.457143
	0.601563	0.398964	3.724138	2.511628	0.601504	0.446927
	0.578947	0.383085	3.821429	2.488372	0.560284	0.431694
	0.56391	0.373134	3.655172	2.409091	0.552448	0.429348
	0.544776	0.363184	3.785714	2.355556	0.544828	0.424731
	0.537313	0.358209	3.888889	2.333333	0.512987	0.427027
	0.518519	0.348259	3.888889	2.282609	0.49375	0.427027
	0.5	0.338308	3.75	2.282609	0.484472	0.414894
	0.489051	0.335	3.178571	1.934783	0.462963	0.396825
	0.481752	0.331658	3.653846	2.375	0.503226	0.423913
	0.464052	0.316964	2.970588	1.980392	0.487654	0.391089
	0.445161	0.306667	2.428571	2.04	0.484663	0.391089
	0.44375	0.307359	1.2	2.372093	0.481481	0.386139
	0.423313	0.294872	3.290323	1.961538	0.477707	0.378788
	0.411043	0.286325	3.290323	1.924528	0.475904	0.379808
	0.4	0.280851	2.5	1.5	0.465116	0.37037
	0.389222	0.275424	3.291667	1.975	0.464286	0.367925
	0.381503	0.269388	2.628571	1.672727	0.464286	0.373206

0.370787	0.26087	2.823529	1.714286	0.458333	0.368421
0.357542	0.253968	2.939394	1.763636	0.449704	0.345455
0.355191	0.252918	3.0625	1.75	0.443787	0.334821
0.342105	0.246212	2.939394	1.763636	0.440476	0.330357
0.331606	0.240602	3.030303	1.818182	0.440476	0.328889
0.317949	0.234848	2.361702	1.632353	0.426901	0.324444
0.309645	0.231061	2.306122	1.661765	0.426901	0.325893
0.301508	0.229008	2.138889	1.480769	0.430233	0.330357
0.3	0.229885	2.125	1.511111	0.418182	0.32093
0.296482	0.227799	1.959184	1.5	0.412088	0.321888
0.293839	0.226277	1.90566	1.485294	0.407609	0.320513
0.280543	0.216783	1.672414	1.328767	0.4	0.316239
0.275556	0.215278	1.371429	1.185185	0.402174	0.318966
0.269565	0.210884	1.24	1.177215	0.387097	0.310345
0.265217	0.20678	1.233766	1.17284	0.394595	0.304167
0.264069	0.207483	1.156627	1.185185	0.380208	0.29918
0.258621	0.204082	1.144578	1.1875	0.373057	0.298755
0.257511	0.20339	1.107143	1.177215	0.355	0.292181
0.252137	0.2	1.070588	1.166667	0.344828	0.285714
0.251064	0.2	1.047059	1.141026	0.338235	0.280488
0.246862	0.197987	0.988764	1.128205	0.325243	0.273469
0.246862	0.196013	0.924731	1.102564	0.318182	0.269231
0.246862	0.194719	0.923077	1.063291	0.301724	0.25641
0.243902	0.204082	0.901961	1.045455	0.297414	0.252747
0.244813	0.194719	0.853211	0.989362	0.291845	0.248175
0.243802	0.194719	0.857143	0.967742	0.286307	0.244681
0.243902	0.194805	0.839623	0.956989	0.271255	0.235915
0.243902	0.194805	0.814815	0.93617	0.262948	0.230769
0.241525	0.19322	0.783784	0.896907	0.255639	0.226667
0.24898	0.199346	0.769231	0.9	0.246377	0.220779
0.239837	0.191558	0.733333	0.838095	0.241007	0.218241
0.244898	0.193548	0.725	0.828571	0.236559	0.215686
0.246914	0.194175	0.691057	0.825243	0.234875	0.219269
0.253112	0.195513	0.714286	0.841584	0.231579	0.217822
0.257511	0.196078	0.661538	0.788991	0.232394	0.22
0.263598	0.200637	0.661654	0.715447	0.229167	0.22
0.2625	0.2	0.641791	0.688	0.227273	0.214521
0.266949	0.200637	0.610294	0.664	0.224561	0.214047
0.27234	0.204473	0.567376	0.64	0.22449	0.215686
0.27234	0.204473	0.557047	0.633588	0.225256	0.214286
0.277056	0.206452	0.538961	0.610294	0.222997	0.210526
0.264069	0.196774	0.51875	0.592857	0.216028	0.206667
0.277533	0.205882	0.506173	0.573427	0.216495	0.207237
0.272727	0.201923	0.481928	0.547945	0.21	0.200637
0.272727	0.201923	0.464286	0.52349	0.208469	0.198758
0.275109	0.201923	0.467949	0.52518	0.21	0.196262
0.276316	0.203226	0.449438	0.5	0.208754	0.190769
0.276018	0.203333	0.448864	0.490683	0.208754	0.19195
0.285714	0.213058	0.443182	0.481481	0.202658	0.188272
0.288991	0.217241	0.424731	0.461988	0.198052	0.188272
0.285068	0.217241	0.419355	0.453488	0.19544	0.185759
0.29148	0.224138	0.416216	0.447674	0.193548	0.185759
0.288889	0.224913	0.408377	0.438202	0.197368	0.184049
0.284404	0.221429	0.403141	0.430168	0.196078	0.183486

0.300971	0.238462	0.4	0.422222	0.194079	0.178248
0.298578	0.235955	0.4	0.422222	0.193443	0.177177
0.296651	0.234848	0.39267	0.407609	0.190939	0.177177
0.305419	0.243137	0.394737	0.40107	0.190939	0.175074
0.313433	0.248031	0.397906	0.395833	0.19281	0.174556
0.32	0.251969	0.396907	0.388889	0.185304	0.172107
0.321608	0.253968	0.394872	0.388889	0.183544	0.173134
0.328283	0.256917	0.394872	0.386935	0.183544	0.172619
0.329949	0.255906	0.396907	0.383085	0.18612	0.174556
0.335025	0.258824	0.402062	0.39	0.184375	0.175074
0.336735	0.257813	0.397959	0.386139	0.182663	0.175595
0.336735	0.256809	0.404145	0.39	0.182099	0.175595
0.340102	0.25969	0.408377	0.39	0.180124	0.173134
0.345178	0.262548	0.408377	0.39	0.185535	0.176647
0.346939	0.262548	0.406417	0.387755	0.183801	0.174556
0.352041	0.267442	0.423077	0.398964	0.183801	0.174041
0.360825	0.27027	0.428571	0.408377	0.185759	0.176991
0.362694	0.27027	0.438202	0.39196	0.182099	0.175074
0.369792	0.275194	0.435754	0.393939	0.187692	0.181009
0.369792	0.276265	0.438202	0.39196	0.181538	0.175595
0.376963	0.280156	0.440678	0.39196	0.188498	0.182663
0.375	0.282353	0.440678	0.39196	0.178462	0.172619
0.380208	0.286275	0.44382	0.39899	0.17737	0.172107
0.378238	0.287402	0.440678	0.393939	0.178788	0.175074
0.379487	0.291339	0.446328	0.39899	0.177711	0.175074
0.377551	0.29249	0.446328	0.401015	0.182371	0.178042
0.382653	0.296443	0.454545	0.406091	0.180124	0.174174
0.382653	0.297619	0.457143	0.406091	0.187919	0.180645
0.380711	0.298805	0.457143	0.40404	0.190635	0.18328
0.383838	0.302789	0.450867	0.397959	0.192691	0.187097
0.387755	0.304	0.48125	0.420765	0.191419	0.188925
0.383838	0.304	0.46988	0.412698	0.194719	0.193443
0.388889	0.308	0.46988	0.410526	0.195364	0.193443
0.385	0.310484	0.467949	0.405556	0.2	0.196078
0.39196	0.315789	0.466667	0.403141	0.203448	0.2
0.395	0.319838	0.481707	0.415789	0.210714	0.20922
0.4	0.323887	0.478788	0.413613	0.212766	0.214286
0.39801	0.325203	0.478788	0.411458	0.218638	0.223443
0.402985	0.331967	0.478788	0.413613	0.222222	0.230483
0.405941	0.336066	0.487805	0.418848	0.225926	0.234615
0.40796	0.337449	0.487805	0.416667	0.231343	0.241245
0.415	0.341564	0.496894	0.42328	0.236641	0.247012
0.42	0.347107	0.50641	0.427027	0.243243	0.254032
0.427136	0.35124	0.509677	0.429348	0.249012	0.2625
0.429293	0.352697	0.512821	0.434783	0.257937	0.273109
0.432161	0.358333	0.516129	0.432432	0.265306	0.282609
0.434343	0.358333	0.522581	0.440217	0.269388	0.28821
0.437186	0.3625	0.525974	0.440217	0.276596	0.300926
0.444444	0.366667	0.535948	0.445652	0.284483	0.311321
0.444444	0.366667	0.54	0.45	0.295154	0.323671
0.449495	0.370833	0.554054	0.458101	0.297778	0.330049
0.449495	0.372385	0.554054	0.458101	0.310185	0.345361
0.454545	0.373444	0.561644	0.458101	0.32093	0.35567
0.459596	0.379167	0.582734	0.47093	0.327014	0.361257

0.464646	0.383333	0.591241	0.476471	0.333333	0.367232
0.462312	0.384937	0.59854	0.482353	0.348039	0.383784
0.467337	0.3875	0.605839	0.488235	0.362245	0.40113
0.472362	0.391667	0.605839	0.488235	0.373057	0.409091
0.467662	0.391667	0.610294	0.488235	0.396739	0.429412
0.472637	0.395833	0.618321	0.496933	0.411111	0.435294
0.472637	0.395833	0.634783	0.503448	0.413408	0.435294
0.472906	0.4	0.632813	0.512658	0.409091	0.434483
0.477612	0.4	0.630769	0.515723	0.459119	0.480263
0.480198	0.40249	0.638462	0.522013	0.465839	0.490196
0.482587	0.40249	0.638462	0.522013	0.48125	0.5
0.482587	0.400826	0.646154	0.525	0.496815	0.503226
0.485	0.40249	0.653846	0.534591	0.509554	0.516129
0.487562	0.404959	0.653846	0.534591	0.522293	0.529032
0.495	0.410788	0.661538	0.544304	0.528662	0.535484
0.492537	0.410788	0.661417	0.54902	0.537975	0.548387
0.5	0.414938	0.674603	0.559211	0.554054	0.561644
0.5	0.416667	0.674603	0.562914	0.583942	0.592593
0.505	0.419087	0.68	0.57047	0.609023	0.609023
0.502488	0.420833	0.702479	0.586207	0.627907	0.618321
0.51	0.423237	0.730337	0.585586	0.653226	0.627907
0.517588	0.429167	0.695652	0.583942	0.686441	0.642857
0.517588	0.430962	0.699187	0.601399	0.689076	0.650794

Intrarenal vascular resistance: autotransplant experiments

30 minutes WIT, CP

PIN 18	PIN 21	PIN 23	PIN 25	PIN 35
0.921569	0.706667	0.712121	0.97561	0.155894
1.057143	0.753623	0.703125	1.054054	0.416964
1.020408	0.846154	0.730159	1.026316	0.51
1	0.773585	0.774194	1.025641	0.477679
0.960784	0.830508	0.777778	1.055556	0.496396
0.942308	0.872727	0.809524	1	0.504505
0.924528	0.888889	0.810345	1.025641	0.514545
0.924528	0.907407	0.839286	1.025641	0.542857
0.888889	0.907407	0.854545	1	0.585714
0.888889	0.925926	0.888889	1	0.6
0.849057	0.925926	0.888889	1	0.610638
0.857143	0.962264	0.907407	0.974359	0.62043
0.857143	0.961538	0.925926	0.974359	0.630435
0.857143	0.979592	0.925926	0.974359	0.651685
0.875	1	0.944444	0.974359	0.652809
0.857143	1	0.944444	0.974359	0.657303
0.857143	1	0.959184	0.95	0.653333
0.84	1.021277	0.978723	0.974359	0.657303
0.84	1	0.978723	0.974359	0.820313
0.84	1.021277	1.021739	0.95	0.73871
0.84	1.021277	1.021739	0.975	0.744262
0.84	1.021277	1.021739	0.974359	0.744262
0.84	1.021277	1.021739	0.974359	0.730645
0.82	1.021277	1.021739	0.974359	0.732258
0.836735	1.021277	1.021739	0.974359	0.719048
0.82	1.043478	1.021739	0.974359	0.740984
0.836735	1.065217	1.021739	0.974359	0.730645
0.816327	1.065217	1.043478	0.974359	0.720635
0.816327	1.06383	1.043478	0.95	0.769492
0.816327	1.086957	1.043478	0.974359	0.673913
0.816327	1.06383	1.043478	0.95	18.80366
0.822222	1.086957	1.043478	0.974359	37.60732
0.826087	1.086957	1.065217	0.974359	
0.808511	1.108696	1.065217	0.974359	
0.8125	1.108696	1.065217	0.974359	
0.804348	1.085106	1.065217	0.974359	
0.804348	1.106383	1.065217	0.974359	
0.826087	1.130435	1.065217	0.974359	
0.804348	1.130435	1.065217	0.974359	
0.826087	1.130435	1.065217	0.974359	
0.826087	1.130435	1.088889	1	
0.826087	1.130435	1.065217	1	
0.829787	1.12766	1.065217	0.974359	
0.829787	1.12766	1.065217	0.975	
0.791667	1.152174	1.065217	0.975	
0.8125	1.152174	1.065217	0.975	
0.8125	1.12766	1.065217	1	
0.8125	1.152174	1.065217	0.975	
0.791667	1.152174	1.065217	0.975	

0.808511	1.148936	1.065217	0.975
0.808511	1.148936	1.065217	1
0.808511	1.148936	1.065217	0.975
0.808511	1.148936	1.065217	0.975
0.808511	1.173913	1.065217	1
0.808511	1.173913	1.065217	1
0.808511	1.173913	1.065217	1
0.808511	1.136364	1.065217	1
0.808511	1.136364	1.065217	1
0.808511	1.136364	1.088889	1
0.808511	1.136364	1.065217	0.975

Minimal WIT, CP

PIN 37	PIN 47	PIN 48	PIN 49	PIN 50
0.462738	0.55	0.77	0.97	0.62
0.458916	0.53	0.73	0.87	0.75
0.432289	0.51	0.68	0.8	0.66
0.4225	0.49	0.65	0.78	0.63
0.416	0.48	0.62	0.73	0.63
0.421325	0.49	0.62	0.71	0.62
0.423293	0.48	0.61	0.68	0.61
0.416951	0.48	0.61	0.68	0.61
0.418537	0.47	0.6	0.68	0.6
0.410361	0.47	0.59	0.65	0.59
0.298361	0.48	0.6	0.64	0.59
0.275061	0.47	0.59	0.63	0.59
0.331481	0.48	0.59	0.61	0.59
0.357407	0.47	0.58	0.59	0.57
0.350617	0.47	0.58	0.59	0.57
0.343827	0.46	0.58	0.59	0.56
0.33913	0.46	0.57	0.58	0.56
0.337736	0.47	0.57	0.58	0.56
0.330625	0.47	0.56	0.57	0.56
0.323602	0.46	0.57	0.56	0.55
0.317901	0.46	0.56	0.57	0.55
0.317391	0.47	0.56	0.57	0.55
0.311728	0.47	0.55	0.55	0.55
0.306748	0.46	0.55	0.55	0.55
0.304294	0.46	0.54	0.55	0.55
0.303704	0.46	0.55	0.55	0.55
0.296951	0.47	0.54	0.54	0.55
0.292169	0.47	0.54	0.55	0.55
0.293373	0.46	0.54	0.55	0.54
0.284337	0.46	0.53	0.54	0.54
	0.46	0.54	0.53	0.54
	0.47	0.53	0.54	0.54
	0.46	0.53	0.52	0.54
	0.46	0.53	0.53	0.54
	0.47	0.53	0.52	0.54
	0.46	0.53	0.52	0.53
	0.46	0.53	0.51	0.53
	0.46	0.53	0.51	0.54

	0.46	0.52	0.51	0.53
	0.46	0.52	0.51	0.53
	0.46	0.53	0.51	0.53
	0.46	0.53	0.51	0.53
	0.46	0.54	0.51	0.53
	0.46	0.52	0.51	0.53
	0.46	0.52	0.51	0.52
	0.45	0.53	0.5	0.53
	0.45	0.52	0.5	0.52
	0.45	0.52	0.5	0.52
	0.46	0.52	0.5	0.52
	0.45	0.52	0.5	0.52
	0.45	0.51	0.5	0.52
	0.45	0.51	0.48	0.53
	0.45	0.51	0.49	0.53
	0.45	0.51	0.49	0.53
	0.45	0.51	0.49	0.52
	0.45	0.5	0.49	0.51
	0.44	0.51	0.49	0.51
	0.45	0.51	0.49	0.51
	0.45	0.5	0.48	0.51
	0.45	0.51	0.49	0.51

30 minutes WIT, WP

PIN 28	PIN 29	PIN 30	PIN 31	PIN 34
0.42	0.69	0.46	0.33	1.214493
0.41	0.39	0.4	0.29	0.292574
0.41	0.36	0.39	0.34	0.309596
0.4	0.3	0.4	0.36	0.357868
0.38	0.3	0.4	0.33	0.369697
0.38	0.3	0.4	0.31	0.430964
0.36	0.31	0.4	0.28	0.369347
0.35	0.3	0.38	0.27	0.224121
0.33	0.3	0.38	0.27	0.2925
0.32	0.31	0.37	0.26	0.285279
0.31	0.31	0.35	0.26	0.501546
0.3	0.31	0.32	0.27	0.225654
0.3	0.31	0.3	0.27	0.289071
0.29	0.31	0.29	0.28	0.459116
0.29	0.31	0.28	0.28	0.330601
0.28	0.32	0.26	0.28	0.39011
0.28	0.32	0.26	0.29	0.240223
0.28	0.34	0.25	0.29	0.208374
0.28	0.33	0.25	0.3	0.203365
0.28	0.34	0.25	0.31	0.204854
0.28	0.34	0.25	0.3	0.206897
0.28	0.34	0.25	0.32	0.205392
0.29	0.34	0.25	0.36	0.19717
0.28	0.34	0.25	0.36	0.164567
0.28	0.35	0.25	0.41	0.16
0.28	0.35	0.25	0.39	0.14629
0.29	0.35	0.25	0.39	0.151034
0.29	0.35	0.25	0.38	0.150859

0.29	0.36	0.25	0.38	0.136426
0.29	0.37	0.25	0.39	0.140972
0.28	0.37	0.25	0.38	0.130968
0.29	0.37	0.25	0.38	0.125566
0.29	0.39	0.25	0.38	0.150987
0.3	0.43	0.24	0.39	0.14202
0.29	0.44	0.25	0.39	0.477955
0.31	0.45	0.25	0.39	0.187302
0.31	0.46	0.25	0.39	0.245426
0.3	0.54	0.25	0.4	0.291401
0.3	0.54	0.25	0.4	0.522188
0.3	0.56	0.25	0.4	0.132609
0.3	0.59	0.25	0.4	0.155108
0.3	0.62	0.25	0.42	0.170846
0.3	0.66	0.26	0.41	0.164198
0.3	0.69	0.26	0.41	0.212654
0.3	0.71	0.26	0.41	0.208696
0.3	0.72	0.26	0.41	0.227864
0.3	0.75	0.26	0.41	0.16192
0.3	0.77	0.26	0.41	0.153988
0.31	0.79	0.26	0.41	0.162997
0.31	0.82	0.26	0.41	0.166358
0.32	0.83	0.25	0.42	0.162385
0.33	0.84	0.26	0.42	0.162385
0.33	0.8	0.26	0.43	0.158485
0.35	0.89	0.26	0.43	0.156798
0.36	0.82	0.26	0.44	0.156839
0.36	0.87	0.26	0.44	0.156098
0.36	0.89	0.26	0.44	0.156
0.38	0.84	0.26	0.44	0.164087
0.39	0.87	0.26	0.44	0.257018
0.4	0.89	0.26	0.45	0.164087

Minimal WIT, WP – values recorded every 2 minutes

PIN 38	PIN 39	PIN 43	PIN 45
0.231126	0.177273	0.42809	0.391534
0.271069	0.176364	0.404795	0.487179
0.210577	0.168935	0.514286	0.598361
0.211962	0.166766	0.469912	0.537879
0.215459	0.163772	0.224113	0.467626
0.220673	0.163526	0.219444	0.414634
0.210177	0.160423	0.189172	0.375723
0.21991	0.156845	0.155309	0.331606
0.202846	0.156587	0.153041	0.295775
0.204833	0.157704	0.159542	0.265823
0.18678	0.157229	0.159596	0.24498
0.191724	0.156287	0.161421	0.220149
0.19614	0.153824	0.161869	0.202335
0.198239	0.153353	0.164631	0.200743
0.203191	0.154839	0.164646	0.188925
0.222535	0.154971	0.166582	0.177778
0.22766	0.155848	0.169821	0.17284

0.229329	0.156598	0.170051	0.167665
0.229577	0.158112	0.170127	0.16568
0.233452	0.160119	0.171827	0.165192
0.239273	0.160059	0.171465	0.159292
0.243382	0.160831	0.172727	0.158209
0.241091	0.162575	0.174873	0.159159
0.240217	0.162874	0.177692	0.159292
0.240217	0.163554	0.179487	0.158358
0.236918	0.163855	0.181074	0.15942
0.241606	0.164048	0.18312	0.160819
0.239273	0.163939	0.185385	0.163265
0.239927	0.161078	0.185934	0.162162
0.243284	0.154467	0.187436	0.169643

Animal weight data (autotransplants)

Minimal WIT		30 minutes WIT
Preservation	Weight (Kg)	Weight (Kg)
CS	48	32
	53	37
	51	32
	45	45
	53	53
CP	55	35
	45	28
	70	28
	72	31
	70	55
	68	
WP	48	34
	54	41
	32	35
	52	68
		55

Kidney weights – pre and post perfusion – autotransplants

	minimal WIT		30 minutes WIT	
Weight (g)	**start**	**stop**	**start**	**stop**
CP	190	210	82	118
	125.8	187.2	53	94
	180.3	253.2	74	116
	117.6	187.9	76	130
	130.9	200	105	187
	124.9	199.2		
WP	98	146	105	119
	110	152	125	141
	99.2	139	98	110
	67	98	97	141
	102	130	106	154
CS	101	n/a	112	n/a
	108	n/a	105	n/a
	92	n/a	109	n/a
	103	n/a	86	n/a
	97	n/a	103	n/a

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