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PHENTOLAMINE TREATMENT OF ISCHEMICALLY DAMAGED HYPOTHERMICALLY PERFUSED CANINE KIDNEYS

by

© GRAHAM BRUCE PIERCY, MBBS, MRCS, LRCP

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

EXPERIMENTAL SURGERY

DEPARTMENT OF SURGERY

EDMONTON, ALBERTA

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled PHENTOLAMINE TREATMENT OF ISCHEMICALLY DAMAGED HYPOTHERMICALLY PERFUSED CANINE KIDNEYS submitted by GRAHAM BRUCE PIERCY in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in EXPERIMENTAL SURGERY.

.....
Supervisor

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.....

DATED September 23 1975.

To Wendy and Joanna with all my love,

and

To my Father and late Mother, whose great generosity and support led to fulfillment of my dream.

ABSTRACT

While kidney transplantation offers the best chance of rehabilitation to patients suffering from chronic renal failure, the world-wide dearth of cadaver organs has meant that demand for such organs far outstrips supply. Many centers have therefore had occasion to use kidneys which have been harvested in less than optimum conditions, such as prolonged hypotension or other anoxic insult. While prolonged warm ischemia is without doubt harmful to subsequent renal function, several workers have suggested that the main element responsible for this impaired function is renal vasospasm: moreover they have demonstrated a marked improvement in preservation and subsequent function when the alpha adrenergic blocker phenoxybenzamine was used as a pre-treatment. Phentolamine, a rapid acting alpha adrenergic blocker has also been suggested for the reversal of agonal vasospasm during hypothermic pulsatile perfusion, which might allow transplantation of kidneys which would otherwise not be considered suitable.

A controlled trial was therefore devised involving canine nephrectomy, a 24-hour preservation period (consisting of one hour of Collins preservation , 22 hours of Belzer preservation and a final hour of Collins preservation) and subsequent autotransplantation with immediate contralateral nephrectomy. Each dog was allocated

on a randomized basis to a warm ischemia group of zero, 15, 30 and 60 minutes, achieved by clamping of the renal artery for the requisite time period. The kidneys were then either treated with 15 milligrams of intraarterial phentolamine after placement on the perfusion apparatus, or were placed in the control group, in which no treatment was given. During perfusion, flow and pressures were followed, and where single perfusions were undertaken, perfusate renin and lactic acid assays were also performed.

After implantation, renal function was followed with serial blood urea nitrogen and serum creatinine measurements. Six weeks postoperatively, survivors were sacrificed and all kidneys subjected to light microscopy: a representative number from each group were also subjected to electron microscopy.

Twenty-five dogs were excluded from the series because of technical failure or death unrelated to transplant function, leaving a total of 54 animals in the study. Survival was found to be adversely affected by increasing length of warm ischemia, but no statistically significant difference could be found between control and treatment groups. Postoperative renal function studies demonstrated no improvement when kidneys were treated with intraarterial phentolamine: nor were the perfusion parameters of flow and diastolic pressure significantly improved by treatment.

Moreover, when mean flows and diastolic pressures of each warm ischemia group were compared, no significant pattern emerged to suggest that these parameters are reliable indices of subsequent renal function. Light and electron microscopic studies did not demonstrate any behefit accruing from the use of phentolamine. Lactic acid and renin assays of the perfusate were found to be of little predictive value as to subsequent renal function, though the number of kidneys so analyzed was small.

In conclusion, this study shows that intraarterial phentolamine confers no benefit upon ischemically damaged canine kidneys in terms of survival, postoperative renal function, perfusion parameters, or microscopic analysis. Moreover it is suggested that perfusion parameters such as flow and pressure are poor guides to renal viability, and that since perfusate lactic acid and renin assays in this study were of little prognostic value, the search for an ex vivo renal viability test must continue.

The great tragedy of Science - the slaying of a beautiful hypothesis by an ugly fact.

-Thomas Henry Huxley 1825-1895

How can what an Englishman believes be heresy? It is a contradiction in terms.

- George Bernard Shaw 1856-1950

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CHAPTER I

A. INTRODUCTION

Since the first publication on hypothermic pulsatile preservation of kidneys (Belzer et al., 1967), the ability to store kidneys has come to be recognized as an essential part of human renal transplantation programs. The advantages of such preservation have been referred to by many authors and include maintenance of organ viability, ease of transportation to a different centre, time for adequate tissue typing and time for preparation of the recipient; in addition, preservation allows testing of function prior to transplantation, modification of donor organ antigenicity, and time for 'repair' of a damaged organ (Slapak, 1971). To this list of advantages may be added the convenience of performing human kidney transplantation on an elective basis. Some of these advantages are theoretical only: in practice only maintenance of organ viability, ease of transportation, time for preparation of the recipient and elective surgery have been realized as irrefutable advantages, while 'repair' of renal damage during hours of perfusion on an artificial circuit at 10 degrees centigrade would seem unlikely; however reversal of vasospasm

undoubtedly occurs (Belzer et al., 1970; Kiser et al., 1971).

While kidney preservation is unquestionably of great value for the above reasons, the number of cadaver kidneys available for transplantation has not increased in recent years, and demand almost always exceeds supply. In Britain, for example, at least 3000 kidneys are needed each year if every patient who could be treated is to be offered such a chance; and this figure is considered by many to be a conservative one (British Medical Journal, 1974). In that country moreover, differences of interpretation of the Human Tissue Act preclude the removal of organs before the heart has stopped beating, and one major centre, that of Professor R.Y. Calne at Addenbrooke's Hospital in Cambridge, had to cease renal transplantation in 1974 because of a lack of donor organs.

These problems are reflected in the long waiting lists at other transplantation centers in North America and Europe, while at the University of Alberta Hospital there is currently a waiting list of 30 patients (D.S. Silverberg - personal communication). Part of the problem without doubt is inadequate publicity as evidenced by the greater success of some areas than others in obtaining donor organs; and many schemes have been devised to increase public awareness of the transplantation program and thereby also increase the

potential availability of cadaver kidneys. These schemes, however, have met with varying degrees of success and consequently alternative avenues have to be explored.

Many kidneys which would otherwise be satisfactorily transplanted have to be discarded because of ischemic damage sustained as a result of prolonged hypotension or cardiac arrest in the donor; and this is especially the case where organs, for one reason or another, may not be removed from heart-beating cadavers. Many authors have emphasized the deleterious effect of warm ischemia on the kidney (Bogardus and Schlosser, 1956; Johnson et al., 1972; Woods et al., 1972; McCabe and Fitzpatrick, 1972; Miller and Alexander, 1972a, 1973; Sinha et al., 1973; Sterling et al., 1972; Hart and Manax, 1971). Sterling et al. (1972) found that not one of eleven machine preserved human kidneys subjected to greater than 15 minutes of warm ischemia had immediate function on transplantation. Hart and Manax (1971) found that only one out of four canine kidneys subjected to one hour of warm ischemia and preserved for 18 hours by Collins technique (Collins et al., 1969) could support life after delayed contralateral nephrectomy. Johnson et al. (1972) found that 40 minutes of warm ischemia was compatible with immediate return of function after 24 hours of hypothermic pulsatile preservation but 60 minutes of warm ischemia was compatible only with delayed return of function under the

same preservation conditions. Moreover Collins preservation for the same periods of warm ischemia produced uniformly poorer results. Scott et al. (1971) achieved poor results using Collins preservation with as little as 15 minutes of warm ischemia; and Moberg et al. (1971) achieved good preservation in kidneys subjected to clinically realistic warm ischemia with hypothermic pulsatile preservation only. Calne et al. (1972) felt that Belzer's technique was superior to Collins' technique in the preservation of ischemically damaged kidneys. Løkkegaard et al. (1973) however found that both preservation methods worked equally well with pig kidneys subjected to one hour of warm ischemia provided that pretreatment with chloropromazine was achieved. While it is not the purpose of this thesis to compare the Belzer and Collins methods of preservation, it would seem that most authors consider hypothermic pulsatile preservation to be superior in the storage of ischemically damaged kidneys; and that pre-preservation warm ischemia has a harmful effect on kidneys.

Much work has been directed towards minimizing or reversing the ischemic insult both before and during preservation; and several authors have emphasized that technique of nephrectomy appears to have a very significant effect on renal ischemia since rough handling leads to cortical vasospasm and subsequent poor cortical perfusion

(Markland, 1964; Kiser et al., 1971). Belzer et al. (1970) found occasional instances of acute tubular necrosis during their early experience with live renal donors, where hypotension had demonstrably not occurred: it was felt that this was attributable to unnecessary traction on the kidney or excessive dissection around the renal artery. Sinha and his co-workers (1973) found that a 'clumsy' nephrectomy was more harmful to ex vivo perfusion parameters, such as flow rate and oxygen consumption than 30 minutes of warm ischemia. Woods (1971) preserved canine kidneys ranging from three to seven days and attributed his success in part to allowing the kidney time to 'recover' after manipulation and before nephrectomy. While it is true that some degree of vasospasm from excessive handling is reversible in the best possible donor situation, this may not be the case in a cadaver organ with damage attributable to agonal and pre-agonal causes. Some authors (Najarian et al., 1966; Woods, 1971) have also emphasized the importance of adequate hydration and good renal function at the time of nephrectomy, but these conditions would pertain only to living related donors or donors in the category of brain death; as Belzer and Kountz (1970) have pointed out "if satisfactory donors are limited to those in whom cerebral death has occurred, but who have normal blood pressure, urinary output and renal function at the time of donor nephrectomy, then transplantation will continue to be a rare

occurrence".

Many workers (Belzer et al., 1970; Carriere et al., 1966; Kane et al., 1966; Lauson et al., 1944; Lillehei et al., 1964) have described the occurrence of renal vasoconstriction in shock, and prolonged periods of donor hypotension should also be avoided where possible (Carroll et al., 1969). Sterling et al. (1972) described an incidence of acute tubular necrosis of 58% when cadaver donors were in shock and on vasopressors prior to nephrectomy, as opposed to a 27% incidence without shock or vasopressors.

While ischemic injury has an obvious influence on cellular and subcellular metabolism, the vasomotor response of the kidney to hypotension, cardiac arrest or other anoxic insult has also been shown to be of great importance with regard to post-transplant renal function. Belzer et al. (1970), prompted by the lack of correlation between the warm ischemic interval and subsequent function in 32 human cadaver kidneys, found in a series of experiments in pig kidneys that renal vasoconstriction, occurring in the agonal period and persisting during isolated hypothermic perfusion, was the primary cause of poor perfusion parameters. Moreover these authors felt that persistent renal vasoconstriction was the most common cause of post-transplantation renal failure, since such kidneys continued to be vasoconstricted after revascularization in the recipient, thereby

introducing, as it were, a period of warm ischemia in the post-transplantation period. These workers also found that the alpha blocker phenoxybenzamine, administered intravenously prior to cardiac arrest, diminished the degree of vasoconstriction in pig kidneys as evidenced by flow and pressure measurements on pulsatile perfusion. In the same experiment, attempts were made to reverse vasospasm during the cold flush with Ringer's lactate solution immediately following nephrectomy and prior to placement on pulsatile perfusion. Various agents were tried including phenoxybenzamine, acetylcholine, propranolol, 2% procaine and 10% procaine buffered to a pH of 7.4: however, only 10% buffered procaine was effective in reversing vasospasm.

When the same group (Pryor et al., 1971) did similar experiments involving pig kidney homotransplantation but without any attempt at immunosuppression, phenoxybenzamine and 10% buffered procaine administered as described above, were again found to be effective in reversing agonal vasospasm and producing immediate renal function; while phenoxybenzamine was even found to be effective when given five minutes after cardiac arrest followed by open cardiac massage. Gump et al. (1972) confirmed that regional adrenergic blockade could be achieved with phenoxybenzamine selectively injected into the renal artery, and that this drug protected the canine kidney against vasoconstriction

from both norepinephrine and hemorrhagic shock. Smith, G.V., et al. (1971) also found phenoxybenzamine pretreatment led to protection of kidneys in hypotensive dogs. Braf et al. (1972) demonstrated that phenoxybenzamine added to Collins C3 solution led to good recovery of kidneys subjected to 30 minutes of warm ischemia and 24 hours of Collins preservation, provided contralateral nephrectomy was delayed.

When applied to human cadaver renal transplantation, Belzer and Kountz (1970) found that donor pretreatment with phenoxybenzamine reduced the incidence of postoperative dialysis from 64% to 8%. Sterling et al. (1972) obtained 69% immediate function with phenoxybenzamine pretreatment, but only 31% without its use.

Løkkegaard (1971) investigated the effect of various vasodilators and heparin on renal vascular resistance at the beginning of hypothermic perfusion and found heparin tended to increase resistance, an effect which could be counteracted with either Papaverine or Lidocaine. However when Papaverine was used with 24 hours of Collins preservation by Sharzer and Lawton (1973), it was found to be inimicable to satisfactory preservation. Løkkegaard et al. (1973) also demonstrated a protective effect of chlopromazine on pig kidneys subjected to one hour of warm ischemia followed by 24 hours of preservation. Fernando et

al. (1973) found that furosemide added to their perfusate solution improved renal blood flow as measured by 133 Xenon washout technique, though total preservation time was short. Panijayanond et al. (1973) found that furosemide, both intravenously as a pretreatment and as an additive to Collins solution, enhanced renal function significantly. These workers postulated the vasodilator effect of furosemide as being the primary efficacious factor, but also suggested other mechanisms including effects on renal blood flow, tubular fluid flow, and tubular fluid concentration, and a specific inhibition of tubular epithelial mitochondria resulting in reduced metabolic demand during storage. Sinha et al. (1973) found that furosemide decreased oxygen consumption in the isolated perfused kidney by 25% and postulated that it might afford protection by inhibition of sodium reabsorption of the renal tubules, thus decreasing the total oxygen requirements of the organ. Mannitol however had no effect on oxygen consumption. Nanninga et al. (1969) infused various compounds into in situ rat kidneys, and found that furosemide and ethacrynic acid significantly reduced renal ischemic damage, while furosemide also increased the survival rate. The effect of sodium nitroprusside on the isolated kidney was studied by Bastron and Kalcyanides (1972) and was found to have a weak renal vasodilator effect; the authors concluded that it would offer no protection against renal ischemia in association

with hypotension; however this drug has not been studied in the transplantation situation. Wolf and Wigton (1971) found that streptomycin had a vasodilator effect in the perfused canine kidney, probably due to a direct effect on vascular smooth muscle, and suggested its use in isolated organ perfusates.

While these approaches have been based on the theory of reversing agonal renal vasoconstriction, other workers have assessed the effect of ischemia on cellular and subcellular metabolism in an attempt at pharmacological reversal of such effects. Vasko et al. (1972) found that allopurinol, both as a pretreatment for 48 hours and for seven days postoperatively, protected in vivo kidneys subjected to 120 minutes of warm ischemia with immediate contralateral nephrectomy. However no preservation period was involved. Toledo-Pereyra and Najarian (1973a) subjected canine kidneys to 24 hours of hypothermic pulsatile preservation with cryoprecipitated plasma following one hour of warm ischemia at 25 degrees centigrade: they obtained 100% survival when allopurinol was added to the perfusate and given for 14 days postoperatively. The same group (Toledo-Pereyra et al., 1974) confirmed the beneficial effect of allopurinol with ischemically damaged kidneys perfused with plasma substitutes. Investigating the possible mechanism of this drug, Cunningham et al. (1974) found that allopurinol

pretreatment led to significantly higher concentrations of ATP, ADP and AMP in ischemically damaged rat kidneys compared to their controls. This higher concentration of purine energy compounds was maintained and improved upon in the post-ischemic recovery phase of the treated animals whereas controls showed a greatly reduced recovery, which was not maintained. These authors therefore postulated that protection against ischemia was afforded by the ability of allopurinol to inhibit xanthine oxidase in the catabolism of purine metabolites such as xanthine and hypoxanthine, thus making them available for resynthesis to ATP, ADP and AMP when the ischemic insult was removed. In this context it is interesting to note that Keaveny et al. (1972) found renal cortical ATP to be best maintained by Collins solution, and suggested that this might explain its effectiveness as a preservation medium.

Since the breakdown of lysosomes under slightly acidic conditions with release of lytic enzymes may be responsible for ischemic cell necrosis, Lotke and Schwartz (1970) investigated the role of these organelles in nonpulsatile kidney preservation under normobaric and hyperbaric conditions. They found that lysosomal stabilizing agents such as hydrocortisone and chloroquine protected cell function as measured by PAH and oxygen uptake in cortical slices, while hypothermia and hyperbaria appeared to delay

release of lysosomal enzymes. Starling et al, (1973) measured lysosomal enzyme release in isolated perfused kidneys and found that methylprednisolone given as a pretreatment and in the perfusate led to decreased enzyme release and decreased vascular resistance, although renal implantation was not performed in this study. Miller and Alexander (1973) found that the same drug, given two hours before a two-hour warm ischemic period with immediate contralateral nephrectomy, gave a significant protective effect; however no preservation period was included. These authors postulated lysosomal stabilization as the primary beneficial effect since lysosomal enzymes can initiate the kinin system, disrupt vascular endothelium and initiate vasoconstriction. Woods (1971) obtained successful long-term pulsatile preservation of kidneys using high doses of methylprednisolone in his perfusate. Villasante et al. (1971) suggested that thyroxine increased oxygen consumption in preserved renal cortical slices, but did not prevent edema formation or adverse histopathological effects.

While hypothermia is considered an essential prerequisite of organ preservation in its ability to reduce the oxygen consumption of perfused kidneys to 5% of normal at 10 degrees centigrade (Levy, 1959), the value of hyperbaric oxygen remains controversial. Much of the early work (Manax et al., 1964; Makin and Howard, 1965; Ladaga et

al., 1966) was done prior to the development of perfusion preservation, and the latter method appears to give superior results (Pegg, 1970). Ackermann and Barnard (1966) used the combination of perfusion and hyperbaria to preserve kidneys for 24 hours, but Basso et al. (1967) were unable to demonstrate significant benefit with this combination. More recently Snell et al. (1972) described successful preservation of canine kidneys exposed to 45 and 60 minutes of warm ischemia, using hypothermic hyperbaric trickle preservation - these workers considered their results to be at least comparable to those achieved with continuous hypothermic perfusion (Scott et al., 1971; Johnson et al., 1972).

Some, though by no means all, of the methods outlined above have led to improved preservation and utilization of kidneys which might otherwise have been discarded. While it is imperative that the best use be made of all available organs, it is also evident that a patient who receives an unsuccessful transplant suffers a major setback in the long-term care of his chronic renal failure. In Britain, almost a third of cadaver kidney transplants never function adequately (Baxby et al. 1974), and there is an obvious need for a reliable organ viability test. Belzer et al. (1967, 1970, 1972) suggest the use of perfusion characteristics such as flow, pressure and perfusate pH but some authors

(Magnusson and Kiser, 1971; Uehling and Cossman, 1972) have found that these parameters do not always correlate with the eventual functional capability of cadaver kidneys. Other viability tests are based upon renal function during isolated perfusion and include redox potential (Couch et al., 1967), succinic dehydrogenase activity (Lannon et al., 1967), PAH uptake (Lannon et al., 1971), inulin extraction (Malinin and Hollerman, 1972) and oxygen consumption (Sinha et al., 1973). Belzer et al. (1968a) and Ashby et al. (1969) have suggested that tubular function tests are unsatisfactory, stating that urine produced during perfusion is an ultra-filtrate differing only in its protein content, and being produced in amounts directly proportional to the perfusion pressure. Oxygen consumption tests were also considered to be of limited value, since at temperatures between 5 and 10 degrees centigrade the kidney uses only limited amounts of oxygen, so that changes are not great enough to predict function. Measurement of redox potential was considered to jeopardize sterility. Some authors have recommended the tetrazolium dye test (Smith, R.B., et al., 1967; Khastagir et al., 1968) but this procedure requires a biopsy: moreover Lannon et al. (1971) and Uehling and Cossman (1972) found it to be of little value since it tested the oxygen utilization enzyme system, which is not highly sensitive to ischemia. The fact that cell damage in the stored organ can result in release of cellular enzymes

or other cytoplasmic contents has led some workers to assay the perfusate in an attempt to discover a satisfactory viability test. Increased lactate dehydrogenase levels are considered useful by some (Belzer et al., 1968a; Ashby et al., 1969; Magnusson and Kiser, 1971; Kohn and Ross, 1971; Kiser et al., 1971; Grundmann and Pichlmaier, 1973), but Johnson (1972) was unable to correlate levels of this enzyme with success or failure of the transplant. Sterling et al., (1972) assessed lysosomal enzymes cathepsin-D and betaglucuronidase, while Calman and Bell (1973) and Cunningham et al. (1974) suggested tissue levels of ATP, ADP and AMP as indicators of organ viability. Angiographic evaluation of the isolated perfused kidney has been used by Alfidi and Magnusson (1972), but contrast medium used led to extensive renal damage which could be prevented only by removal from the circuit of effluent containing the contrast medium. Renal blood flow at 60 minutes post-implantation was considered by Ariyan et al. (1971) to be a reliable indicator of renal survival, but this method obviously suffers from not being a true ex vivo test. Johnson et al. (1972) have found that a perfusate pH fall in the first hour is a clear indication that the kidney is irreversibly injured and Johnson et al. (1973) and Baxby et al. (1974), continuing this work, have suggested that perfusate levels of lactic acid are very reliable in the detection of ischemically damaged kidneys. Moore et al. (1974) have

mentioned perfusate renin concentrations as being substantially elevated in long-term perfusion experiments but no further details were supplied.

B. FORMULATION OF THE PROBLEM AND OBJECTIVES

Since 1955, when the first successful cadaver renal transplant was reported (Hume et al., 1955) this procedure has become a well established and well accepted method of treatment in the armamentarium against chronic renal failure; and while the immunological aspects of transplantation still pose a considerable problem, there is no doubt that many transplant recipients have achieved a more complete rehabilitation than that offered by other treatment modalities such as chronic hemodialysis.

The key factor limiting the more widespread use of cadaver kidney transplantation is the availability of donor organs, and this fact is borne out by the long waiting lists at most transplant centers in North America and Europe. This shortage of kidneys, together with the unacceptably high incidence of failure of cadaver kidneys due to ischemic damage sustained prior to donor nephrectomy has prompted investigation into methods of protecting kidneys in hypotensive donors. The recognized association between persistent renal vasoconstriction and the development of

tubular necrosis has led to the use of the alpha blocker phenoxybenzamine to prevent the development of vasospasm during the agonal period prior to donor nephrectomy (Belzer et al., 1970). Phenoxybenzamine does prevent renal vasoconstriction but requires 30 to 60 minutes to become effective (Smith, G.V., et al., 1971; Miller and Alexander, 1972b). Its use therefore would seem to be limited to donors in the category of brain death with gradually deteriorating vital signs. Since many potential kidney donors are admitted in shock or subsequently become hypotensive, a drug requiring up to one hour to become effective in preventing renal vasospasm may be of little benefit. Miller et al. (1974) considered that an alpha adrenergic blocking agent with a more rapid onset of action even in poor perfusion states would better facilitate the harvesting of suitable kidneys, especially from non-heartbeating cadavers. Since phentolamine is a well known alpha adrenergic blocking agent that has these properties, these workers evaluated this drug both as a pre-treatment intravenously and intraarterially on the Belzer LI 400 hypothermic pulsatile perfusion apparatus in an attempt to reverse established vasospasm. Vasospasm was achieved by asphyxiation of pigs, leading to anoxia and cardiac arrest.

When given as a pre-treatment phentolamine produced rapid alpha adrenergic blockade as shown by high initial

flow rates and low diastolic pressures on the perfusion apparatus. Perhaps more significantly, five to 15 milligrams of phentolamine given intraarterially within ten minutes of being placed on perfusion, dissipated vasospasm as evidenced by the perfusion characteristics of flow and pressure, and the gross appearance of the kidney. In the human situation, phentolamine pre-treatment to cadaver donors resulted in improved initial perfusion characteristics and markedly superior initial renal function, reflected both in the serum creatinine and creatinine clearance, and in the decreased need for post-operative hemodialysis. When phentolamine was given to isolated perfused human kidneys, improvement in flow rates was obtained, but this improvement was found to be related to the warm ischemic interval - if this was greater than 25 minutes, no benefit could be obtained. This observation led to the suggestion that the infusion of phentolamine during isolated perfusion might separate out poor hemodynamic parameters caused by vasospasm from those caused by irreversible ischemic injury. While the benefits of donor pretreatment have been well documented (Belzer et al., 1970; Pryor et al., 1971; Sterling et al., 1972; Løkkegaard, 1973) little has been published to indicate that vasospastic kidneys can be successfully treated during the preservation period. The work of Miller and associates (1974) if confirmed, would seem to present a useful method of reversing vasospasm in cadaver kidneys when no

opportunity for donor pretreatment had occurred, thus making available organs which might not otherwise be considered for transplantation. In addition, an ex vivo viability test in which vasospastic kidneys might be differentiated from ischemically damaged kidneys would indeed be useful. Since the animal experimental model of these authors precluded the possibility of autotransplantation, a controlled trial of the use of phentolamine in isolated canine kidney perfusions has been set up with the following major objectives:

1. To evaluate the effect of intraarterial phentolamine, given during hypothermic pulsatile preservation, on perfusion parameters of canine kidneys subjected to varying periods of warm ischemia.

2. To determine if intraarterial phentolamine given during pulsatile preservation may reverse vasospasm and thereby improve post-transplant renal function as determined by (a) serial blood urea nitrogen and creatinine studies, and (b) survival.

3. To determine if the kidney can undergo extensive tissue necrosis and be non-responsive to this drug, yet still maintain reasonable flow and pressure characteristics on preservation.

4. To determine, where circumstances allow, if serial lactic acid and renin studies during preservation will help

to differentiate the viable from the non-viable kidney.

5. To determine if there is a demonstrable light or electron microscopic difference between the treated and the untreated groups.

CHAPTER II

METHODOLOGY

Each kidney transplant, whether double or single, involved a two day period. Early on the first day, 1000 cc of cryoprecipitated pooled canine plasma, which had previously been collected in acid citrate dextrose, was rapidly thawed in a hot water bath: and drugs added as illustrated in Table 1. (Belzer et al., 1967). Previous work at this Institute has indicated that the addition of distilled water is not necessary since the ACD in which blood is collected has an equivalent diluent effect (Milner, J. - personal communication). The head for the Belzer LI 400, previously sterilized, was now prepared, and the perfusate, after millipore filtration to a pore size of 0.22 microns, was placed in the apparatus (Belzer et al., 1968b). Throughout this research, the Belzer LI 400 machine was kept in the Clinical Sciences Building, approximately half a mile from the Surgical Medical Research Institute, where the animal transplants were done: this was to ensure availability of the machine for the human transplant program.

In the early stages of this research only one dog was

Table 1

Belzer Solution

Cryoprecipitated plasma	1000 ml
$NaHCO_3$	18 ml
Decadron (4 mg/ml)	2 ml
Insulin (80 U/ml)	1.5 ml
Penicillin G (500,000 U/ml)	1 ml
$MgSO_4$ (1 gm/2 ml)	2 ml
Phenolsulfonphthalein (P.S.P.)	2 vials

operated on at a time in order to gain information on perfusate lactic acid and renin levels: later two dogs were operated on on the same day. Adult mongrel dogs weighing between 15 and 30 kilograms and fasted for 12 hours prior to operation were sedated with acepromazine maleate following which anesthesia was induced with halothane. The same agent was used to maintain anesthesia through an endotracheal tube. No pretreatment was given other than intravenous normal saline and furosemide 40 mg, the latter being administered approximately 15 minutes prior to occlusion of the renal artery. In particular, heparinization was not employed since this would not have simulated the human cadaver situation.

A midline incision was used to open the abdomen, and the spleen retracted to expose the left kidney (Fig. 1). The renal vein was carefully cleared of surrounding tissue with a combination of blunt and sharp dissection until an adequate length had been exposed; the posterior peritoneum was then cleared from the margin of the kidney. The kidney was freed from perinephric tissue so that the renal artery could be approached and isolated posteriorly: it was found that this minimized the incidence of double arteries, since many apparently double arteries became single at the origin of the renal artery from the aorta. If true double arteries were found, the right kidney

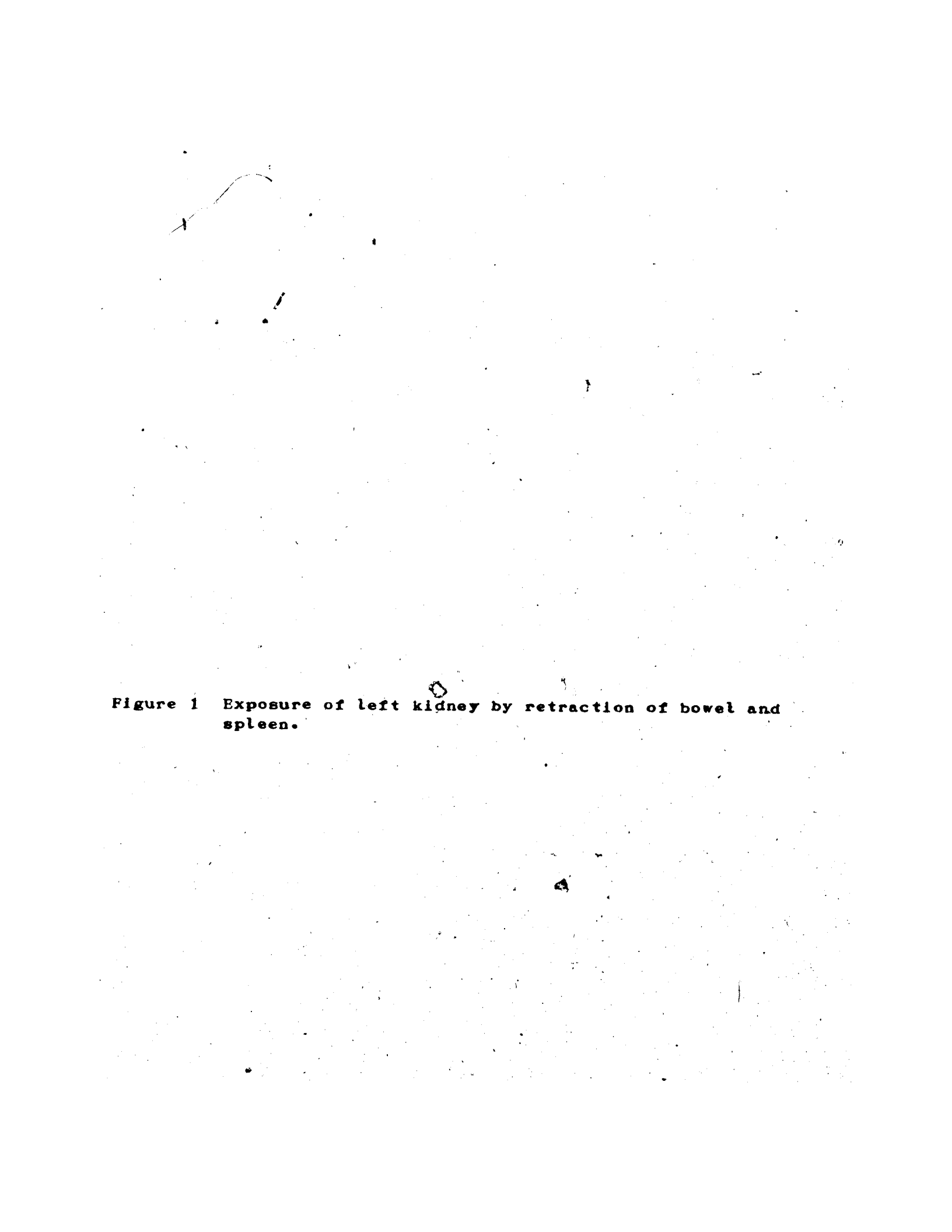


Figure 1 Exposure of left kidney by retraction of bowel and spleen.

was explored and only two procedures had to be abandoned because of bilateral complete arterial duplication.

Adequate ureteral length was obtained by blunt dissection down to the level of the pelvic brim (Fig. 2): the ureter was then transected and ligated with 00 cotton. When the kidney had been satisfactorily isolated from its bed, the renal artery was ligated in continuity and the abdominal viscera returned intraperitoneally so that the kidney could be subjected to the required period of warm ischemia. At the end of this period, the kidney was once again exposed, the renal vein ligated with 00 cotton at the point of entrance of the gonadal vein, and both vessels divided. The kidney was placed in a bowl of iced saline slush, the artery cannulated and immediately perfused with 200-300 cc of Collins C3 solution (Hartley et al., 1971), cooled to 4 degrees centigrade (Table 2). When this had been accomplished, the kidney was put in iced saline slush in a sterile transport jar, which in turn was placed within a styrofoam cooler packed with ice. Exactly 40 minutes after the kidney from the first dog was removed, the second dog was nephrectomized in similar manner, allowing 20 minutes for transport of both kidneys to the perfusion apparatus.

In view of this period of transportation to and from the perfusion apparatus in another building, the preservation protocol included an hour of Collins

Figure 2 Isolation of left kidney, renal vessels and divided ureter.

Table 2

Collins Solution (C_3)

KH_2PO_4	2.05 g/litre
$K_2HPO_4 \cdot 3H_2O$	9.70 g/litre
KCl	1.12 g/litre
$NaHCO_3$	0.84 g/litre
Heparin	5000 U/litre
Glucose (50% w/v)	50 ml/litre
Procaine HCl (10% w/v)	20 ml/litre
$MgSO_4 \cdot 7H_2O$ (50% w/v)	14.4 ml/litre

preservation before, and an hour at the end of, 22 hours of Belzer preservation. During the transportation of kidneys to the perfusion apparatus, hemostasis was secured and the incision closed by the author's surgical technician, Mr. Malcolm Wharton. A three layer closure was employed, continuous 0 chromic to the linea alba, continuous 00 chromic to the subcutaneous tissue and 0 chromic to the skin, the latter being closed in subcuticular fashion. The animals were then returned to the vivarium where they were allowed water ad libitum until the next morning.

Prior to placing the kidneys on the Belzer machine, perfusate samples were taken for pH; as were samples for lactate and renin assays if one kidney only was to be preserved. When each kidney was introduced into the apparatus, care had to be taken to ensure that the patency of the renal artery was not compromised by torsion. The systolic pressure was then set at 60 mm Hg and the pulse at 60 to 70 beats per minute. Oxygen flow rate was kept at one litre per minute and this ensured a pO_2 of approximately 200 mm Hg. The CO_2 input was maintained at 140 to 150 cc per minute, which ensured a pH in the 7.3 to 7.5 range. Flow rates, pressure and pH were recorded immediately after placement, at one hour and at 22 hours immediately prior to removal from the circuit.

The next morning, the animals were anesthetized in a

similar manner to day one, except that no preoperative tranquilization was used. After prepping and draping, the midline incisions were opened and extended to approximately one inch from the pubis. The lower abdomen and pelvis were exposed and after creation of a right retroperitoneal pouch adjacent to the common iliac artery and vein, the latter vessels were carefully dissected free of investing tissue for a distance of several centimeters from the aortic and inferior vena caval bifurcations (Fig. 3). Contralateral nephrectomy was then performed, both vessels and ureter being ligated with 00 cotton.

At this point the author adjourned to the perfusion apparatus where the kidneys were removed within 40 minutes of one another and perfused with freshly prepared Collins solution. They were then returned to the Surgical-Medical Research Institute where implantation was commenced. Total body heparinization was achieved with 300 units per kilogram of heparin; the common iliac vessels were then ligated and divided distally after being clamped proximally. After clearing the vascular stumps of blood by saline lavage, the kidney was placed with its anterior surface lying posteriorly in the right iliac fossa, and an end-to-end common iliac to renal vein anastomosis performed with continuous 6-0 silk (Fig. 4). The common iliac artery was anastomosed to the renal artery in like manner, after which

Figure 3 Isolation of common iliac artery (lying anteriorly) and common iliac vein.

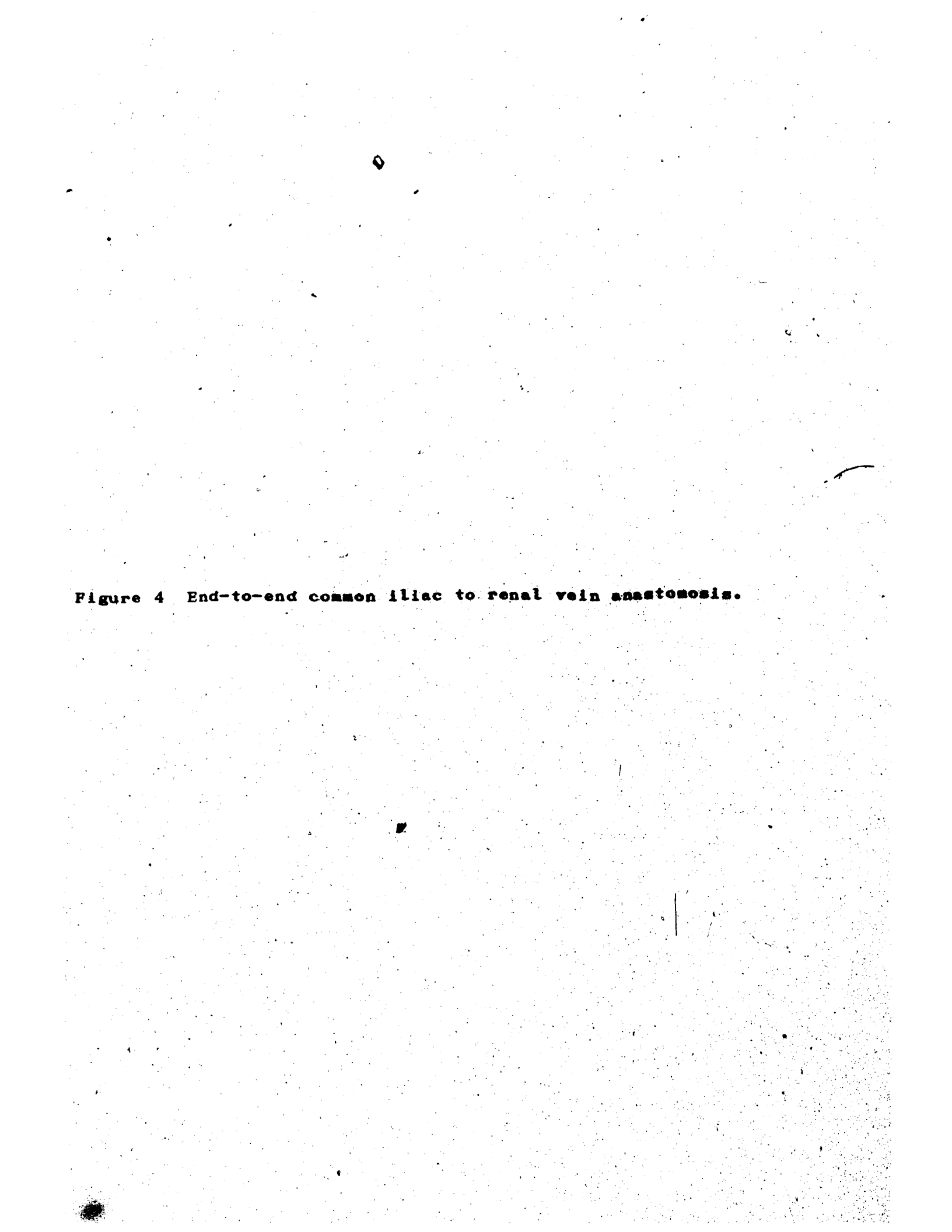


Figure 4 End-to-end common iliac to renal vein anastomosis.

the clamps were immediately released (Fig. 5). Intravenous normal saline, with 40 mg of furosemide was given rapidly together with the appropriate amount of protamine sulfate to reverse the heparin effect. The whole vascular anastomosis took between 18 and 28 minutes, allowing sufficient time for hemostasis as necessary, prior to commencing the implantation on the second dog. This was accomplished in the same manner as in the first dog, after which each ureter was implanted in turn into the bladder. An intravesical approach through an anterior bladder incision was used to fashion a submucosal tunnel, terminating near the trigone. The ureter was then pulled through this tunnel, spatulated, and anchored with multiple interrupted 4-0 chromic catgut sutures. Patency of the orifice was verified by passing a probe, after which the bladder was closed using continuous 4-0 chromic catgut through the mucosa, and interrupted 2-0 chromic catgut through the muscular layers. The kidney was placed in the retroperitoneal pouch, the edges of which were approximated with interrupted sutures. Closure of the abdominal incision was in three continuous layers: 00 chromic catgut to the subcutaneous tissues, and 000 subcuticular polyethylene to the skin.

In the postoperative period, the animals were given a full diet, and serial blood urea nitrogen (Fawcett and Scott, 1960) and serum creatinine (Owen et al., 1964)

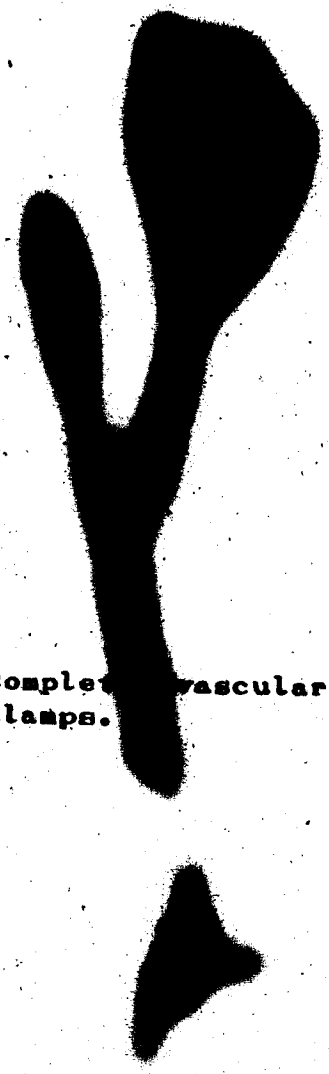


Figure 5 Complete vascular anastomoses after release of clamps.

measurements were performed. Six weeks postoperatively the surviving animals were sacrificed by exsanguination and all kidneys were sectioned for light microscopy. In addition, representative numbers for each group were selected for electron microscopy.

Perfusate renin activity was measured using the NEN Angiotensin I (^{125}I) radioimmunoassay kit (Haber et al., 1969), and perfusate lactic acid assayed by the Sigma kit method #826. Statistical analysis was made using standard techniques and a short computer programme on the University of Alberta IBM 360/67 computer. Data was plotted directly from the calculated results using the Calcomp Plotter. Student's t-test was used for comparison of the means of the creatinines and blood urea nitrogens, and a 2x2 contingency table for the survival data.

CHAPTER III

RESULTS

The two main groups in this experiment consisted of a control and a treatment group. These were in turn divided into four sub groups based upon the length of warm ischemia to which the kidneys were subjected, i.e. zero, 15, 30, and 60 minutes. Each animal was allocated on a random basis to one of these eight sub groups; and when the surgical part of this study had been completed, there were between five and nine animals in each sub group (Table 3). A total of 54 animals were included in the study, while 25 were excluded for the following reasons.

(1) Intussusception

The most common reason for exclusion was intussusception which was found in ten dogs at autopsy. No explanation for the frequency of this complication can be given, but it appears to be disproportionately more common in dogs undergoing the autotransplantation model as described earlier than in dogs subjected to other major intra-abdominal surgery such as gastrectomy (D.C. Secord - personal communication). Few other experimental protocols,

Table 3: Experimental Groups

Number of Dogs in Each Subgroup

	Control Dogs	Treated Dogs
Minutes of Warm Ischemia	No Intraarterial Therapy	Phentolamine (Rogitine) 15 mg
0	5	6
15	7	7
30	9	8
60	6	6

however, demand two operations within a 24-hour period and it may be that this factor combined with postoperative vomiting and uremia contribute to the pathogenesis of intussusception in dogs. It is probable that some cases of intussusception are agonal events, but fears that exclusion might introduce a bias into this study appear unfounded, in that distribution is equally divided between the treated and untreated groups. In addition, there appears to be no correlation with duration of warm ischemia: the number in each group, according to increasing warm ischemic intervals, was 3, 0, 3, and 4 respectively.

(2) Technical difficulties with the perfusion apparatus

Five dogs were excluded under this category. The main problem was found to be related to control of gas inflow which is notoriously sensitive in the Belzer LI 400. Two dogs were excluded because the carbon dioxide inflow fell to zero overnight during perfusion of their kidneys, thus raising perfusate pH to unphysiologic heights. Similarly, two other animals were excluded because on one occasion the oxygen tank valve was insufficiently opened, resulting in an hypoxic perfusion.

The remaining dog was excluded because of poor positioning of the cannula in a renal artery with a low bifurcation - this led to inadequate and uneven perfusion

because the tip of the cannula was distorting the bifurcation.

(3) Perfusate problems

(a) Collins solution: In the early stages of this study, Collins solution was found to precipitate with excessive ease, resulting in the exclusion of two dogs from the series. C3 solution was used in this study, and Chapman (1971) found that reduction of the magnesium content by one sixth led to elimination of problems with precipitation. In practice, it was found that immediate use of Collins solution after it was made up seemed to eliminate turbidity and for this reason the author was accompanied to the perfusion apparatus by Mr. Ken Proskow, biochemistry technician, who mixed the individual components of the solution just prior to the removal of each kidney. Occasionally, crystalline deposits were noticed in the arterial cannula before the kidney was placed on the Belzer apparatus: but provided these were irrigated out with plasma, no untoward effects were noted.

(b) Cryoprecipitated plasma: Pooled canine plasma was used and this was obtained from dogs (operated upon earlier in the series) which were sacrificed after the requisite six week postoperative period. Variable degrees of hemolysis were noted in the plasma thus collected, and excessive

hemolysis was an indication for not using such plasma.

(4) Distemper

Two dogs were excluded because of distemper, diagnosed independently by a veterinarian. Extension of the quarantine period (previously two weeks) to three weeks led to elimination of further cases of distemper since the final week of quarantine was spent at the Surgical-Medical Research Institute where symptoms of the disease were more readily detected.

(5) Surgical error

Five dogs could not be included in the series because of morbidity and mortality related to surgical error. Two dogs were lost because of renal artery thrombosis and one because of an unsuccessful double artery anastomosis. One dog died within hours because of massive hemorrhage from the arterial anastomosis, and one dog was found to have died of obstructive uropathy from peripelvic hematoma.

(6) Anesthetic death

There was one intraoperative anesthetic death.

Survival

Table 4 shows the number and percentage of survivors in

Table 4: Survival - Treated versus Controls ($p>0.4$ in all groups)

	Control Dogs No Intraarterial Therapy			Treated Dogs Phentolamine (Rogitine) 15 mg		
Minutes of Warm Ischemia	Survived	Died	% Age Survival	Survived	Died	% Age Survival
0	5	0	100	6	0	100
15	7	0	100	7	0	100
30	7	2	78	6	2	75
60	1	5	17	2	4	33

each group: 100% survival was achieved in both treated and untreated groups with zero and 15 minutes of warm ischemia; but survival in the 30 minutes warm ischemia group was only 78% and 75% for untreated and treated groups respectively. With one hour of warm ischemia there were only two survivors in the treated group and one in the untreated group. In terms of survival, there is self-evidently no difference between treated and untreated groups at zero and 15 minutes of warm ischemia while at 30 and 60 minutes the difference in survival was not statistically significant ($p>0.4$). From these figures one may conclude that intraarterial phentolamine during perfusion of ischemically damaged kidneys has no effect on the survival of dogs autotransplanted with these kidneys.

Serum Creatinine Data

Serum creatinines were measured frequently in the postoperative period, and although every effort was made to ensure that samples were taken on the same days postoperatively, some individual figures have been extrapolated from the nearest adjacent results for the purpose of calculating the mean values. Figures 6, 7 and 8 represent mean serum creatinines with standard errors plotted against days post-transplant for the survivors of the zero, 15 and 30 minute warm ischemia groups

Figure 6 Zero Minutes Warm Ischemia Group: Mean serum creatinine (milligrams percent) with standard error plotted against days post-transplant. No significant differences found except on the first post-transplant day when the control value is lower than that of the treatment group. ($p<0.05$).

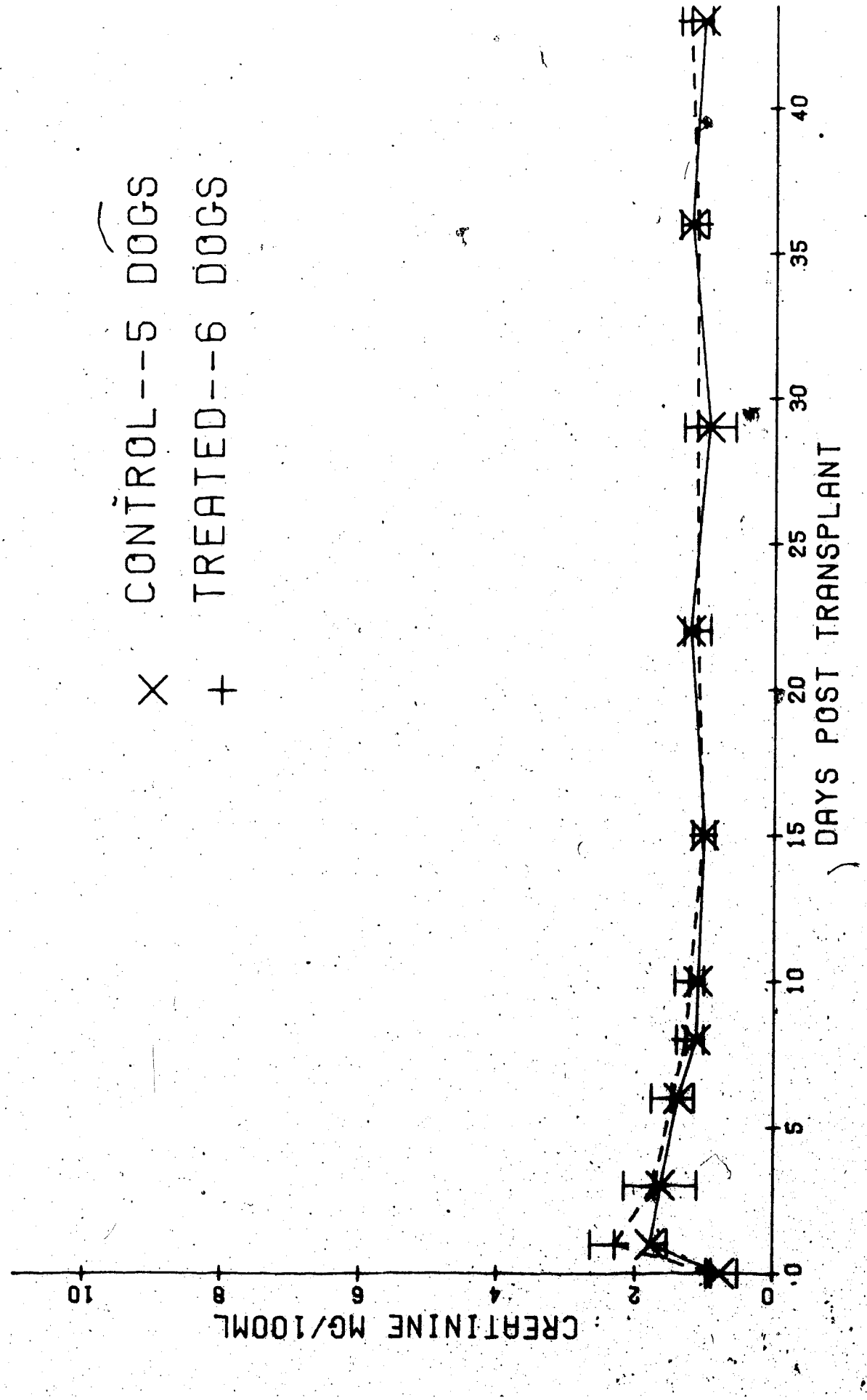
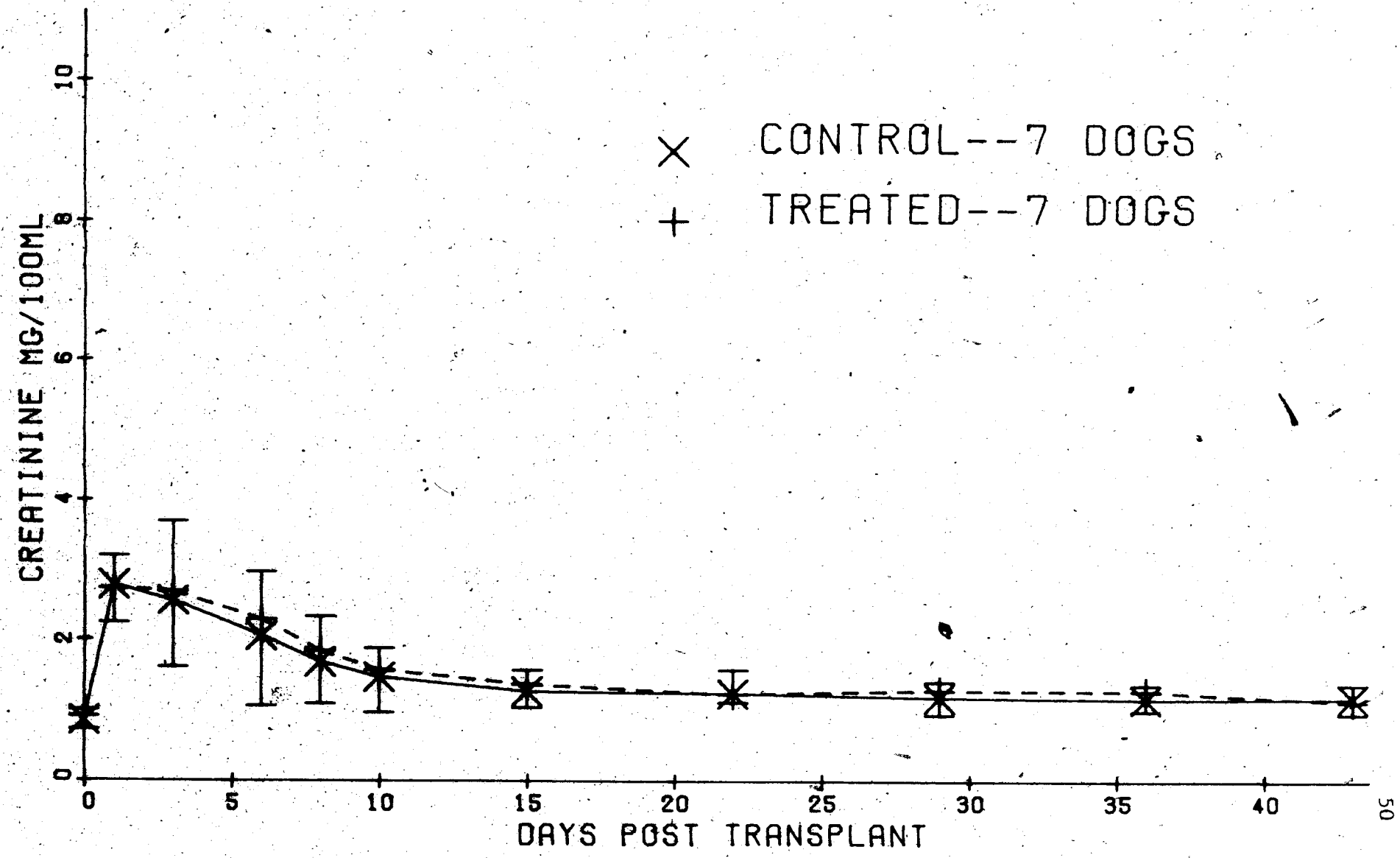

X CONTROL--5 DOGS
+ TREATED--6 DOGS
CREATININE MG/100ML
0
2
4
6
8
10
0
5
10
15
20
25
30
35
40
DAYS POST TRANSPLANT

Figure 7 <u>15 Minutes Warm Ischemia Group</u>: Mean serum creatinine (milligrams percent) with standard error plotted against days post-transplant. No significant differences between control and treatment groups.


CONTROL--7 DOGS
TREATED--7 DOGS
CREATININE MG/100ML
0
2
4
6
8
10
0
5
10
15
20
25
30
35
40
DAYS POST TRANSPLANT

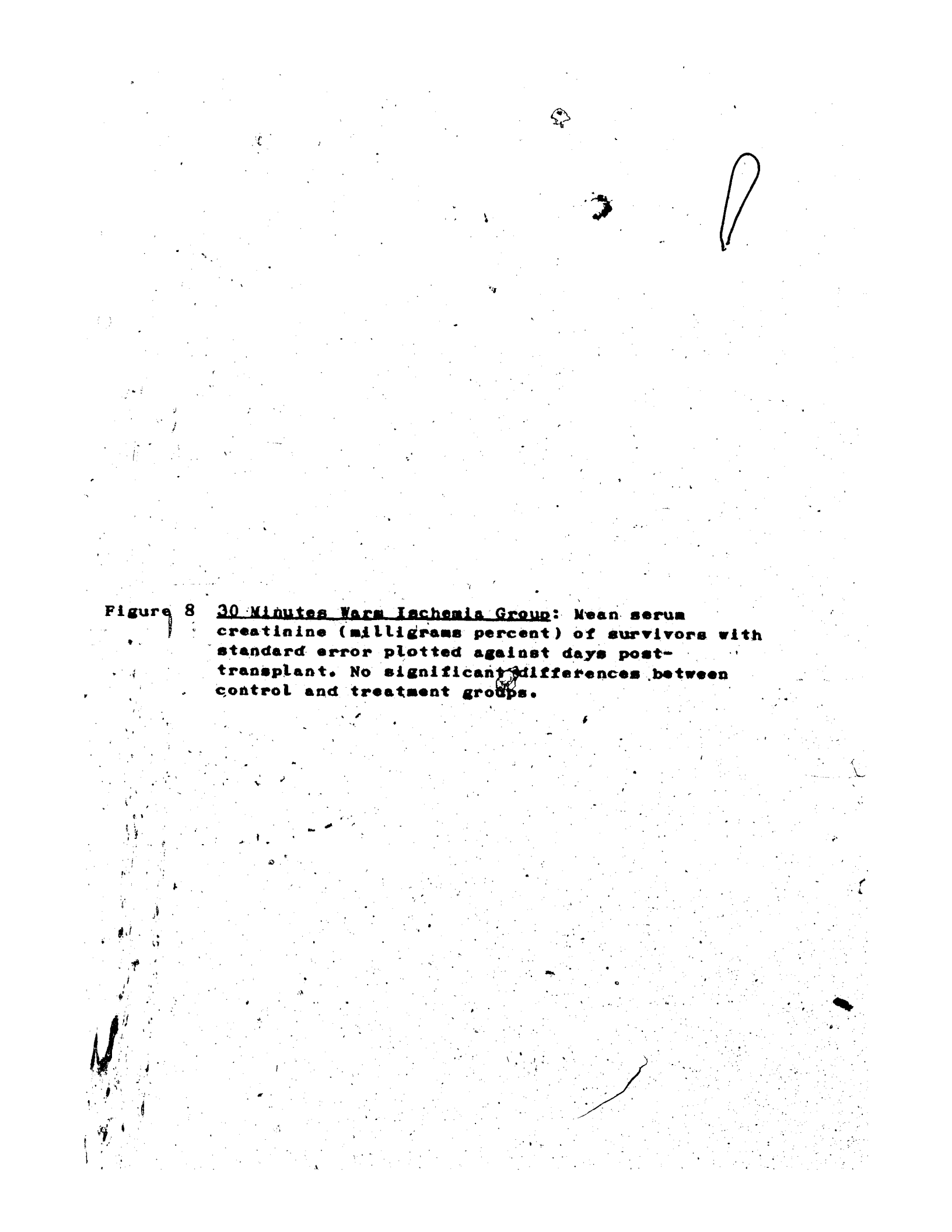
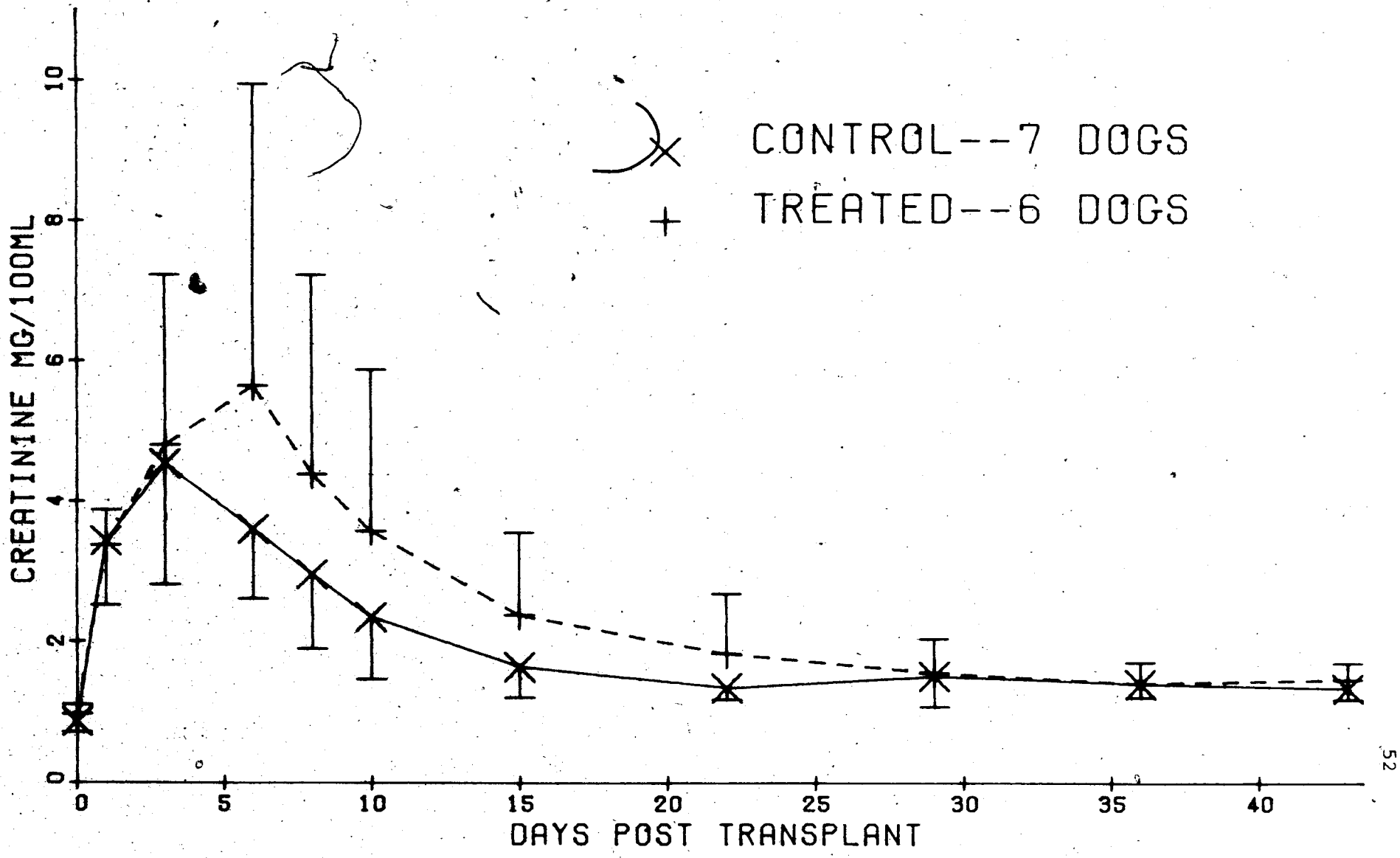


Figure 8 <u>30 Minutes Warm Ischemia Group</u>: Mean serum creatinine (milligrams percent) of survivors with standard error plotted against days post-transplant. No significant differences between control and treatment groups.


CONTROL--7 DOGS
TREATED--6 DOGS
CREATININE MG/100ML
0
2
4
6
8
10
0
5
10
15
20
25
30
35
40
DAYS POST TRANSPLANT

respectively. Maximum average creatinine elevation occurred on the first day post-transplant except in the case of the 30 minutes warm ischemia group where the maximum rise occurred on day three in the control group and on day six in the treated group. While the rise in serum creatinine is seen to be proportional to the duration of warm ischemia, no statistical difference is demonstrated between control and treatment groups; except on the first day post-transplant of the zero minutes warm ischemia group where the control value is significantly lower than the value for the treated group ($p<0.05$). This suggests that intraarterial phentolamine actually led to inferior preservation in this group, and while the significance of this finding is debatable, it is worth noting that early average creatinine values in the other two groups are consistently, though <u>not significantly</u>, lower in the control than in the treatment group.

The values for the 60 minute warm ischemia group have not been plotted because of the small number of survivors. The sole survivor in the control group reached a maximum serum creatinine of 5.4 on the second day post-transplant, before returning towards normal; while the two survivors in the treatment group had maximum elevations of 8.9 and 9.9 on days 4 and 7 respectively. Values for the animals that did not survive tended to follow a similar pattern in both groups, in that there was an uninterrupted progression to an

elevation of 19 to 21 milligrams per cent with death occurring between days 7 and 10.

Blood Urea Nitrogen Data

As in the case of the creatinine data, some blood urea nitrogen values have been calculated from the nearest adjacent results in order to calculate mean values - Figures 9, 10 and 11 show the mean blood urea nitrogen values with standard deviations plotted against days post-transplant for the zero, 15 and 30 minutes warm ischemia groups respectively. No statistically significant difference can be demonstrated between control and treatment groups, and the curves have an analogous relationship to those plotted with the serum creatinine data. In the 60 minute warm ischemia group, the only survivor in the control group had a maximum BUN of 101 on the second day post-transplant, after which values returned towards normal. The two survivors in the treatment group had maximum elevations of 181 and 212 on days 4 and 6 respectively.

It may be concluded from both these results and the creatinine data that intraarterial phentolamine during hypothermic perfusion preservation has no beneficial effect on post-transplant function of kidneys subjected to such preservation.

Figure 9 Zero Minutes Warm Ischemia Group: Mean blood urea nitrogen (milligrams percent) with standard error plotted against days post-transplant. No significant differences between control and treatment groups.

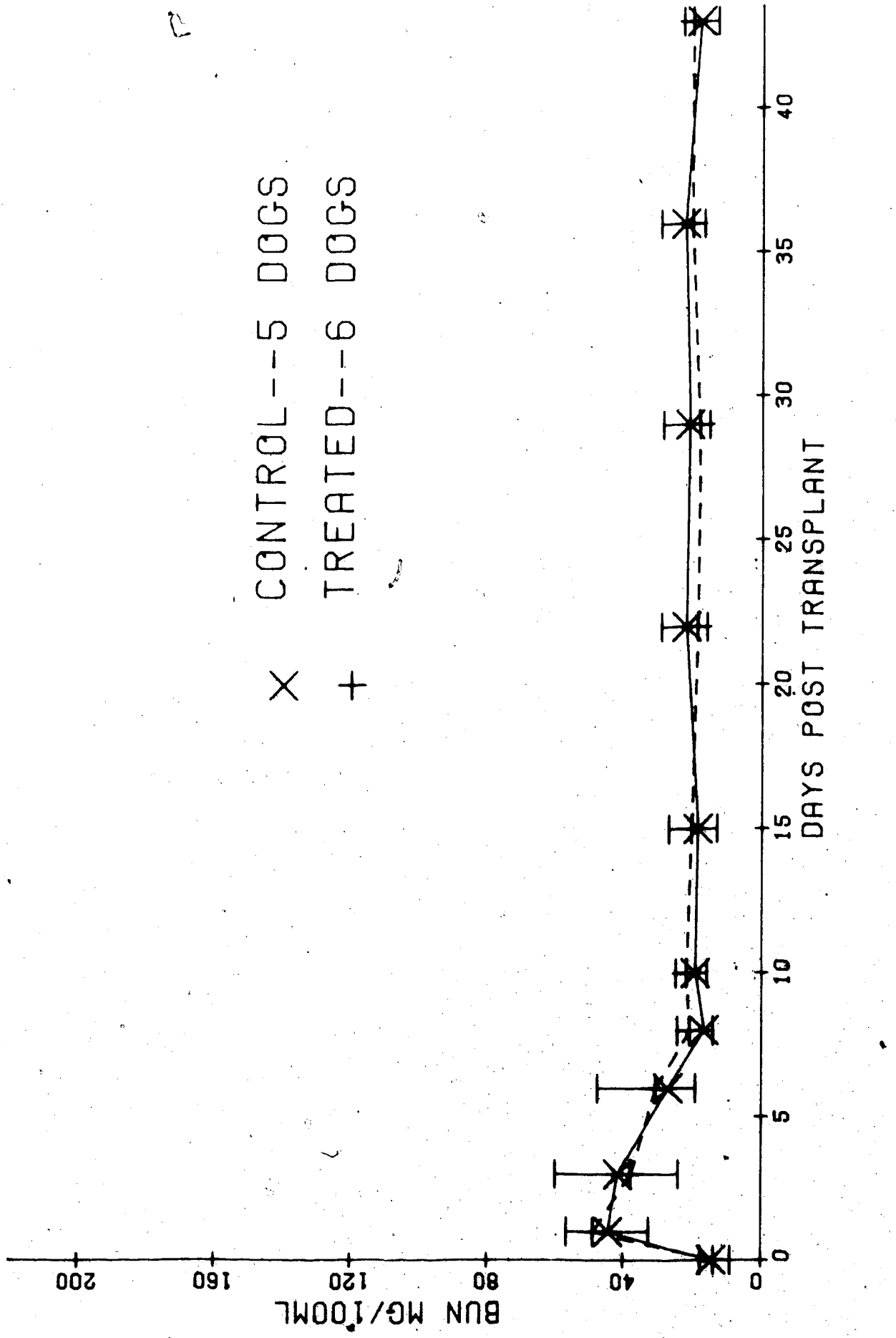

X CONTROL--5 DOGS
+ TREATED--6 DOGS
BUN MG/100ML
0
40
80
120
160
200
0
5
10
15
20
25
30
35
40
DAYS POST TRANSPLANT

Figure 10 15 Minutes Warm Ischemia Group: Mean blood urea nitrogen (milligrams percent) with standard error plotted against days post-transplant. No significant differences between control and treatment groups.

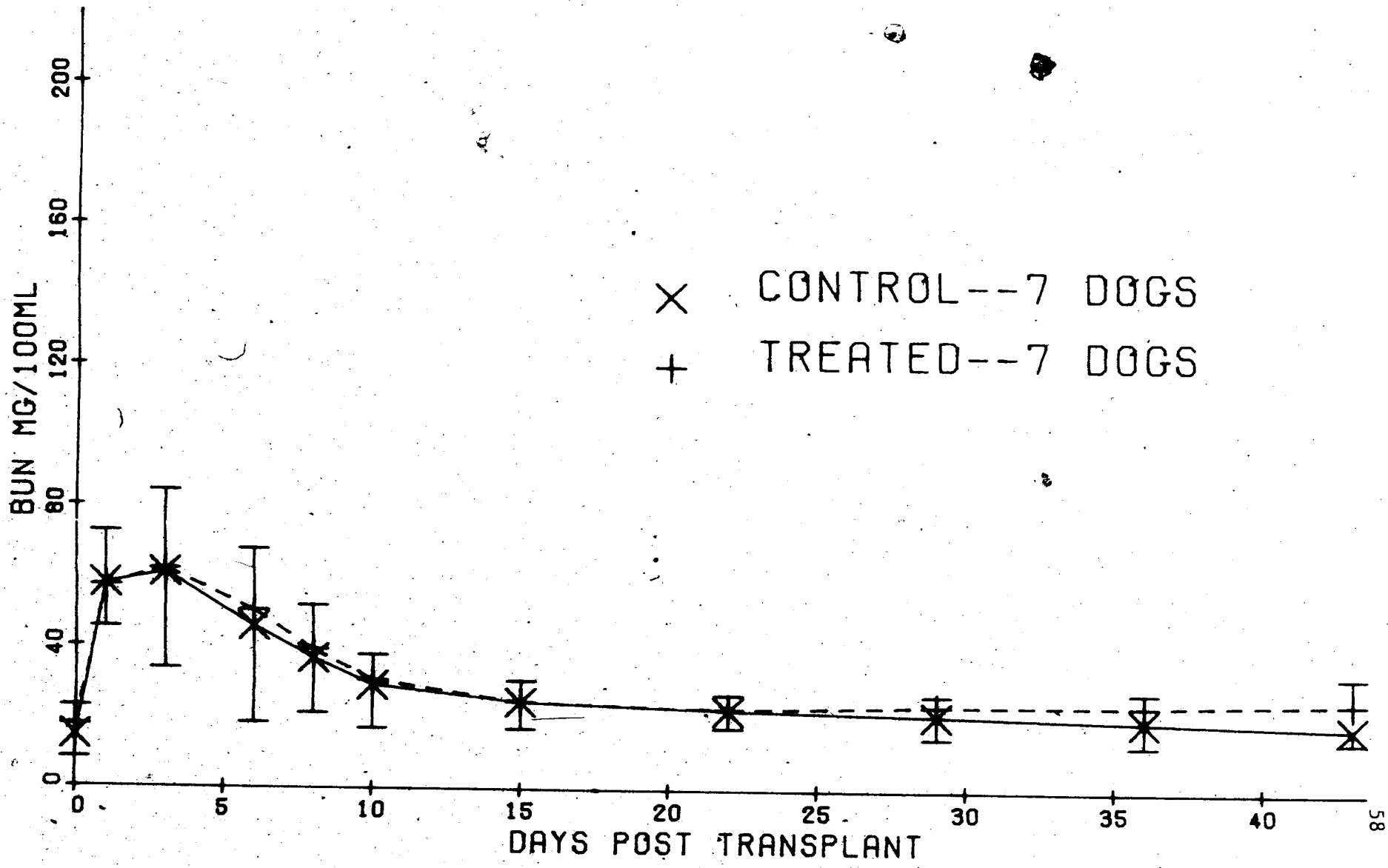
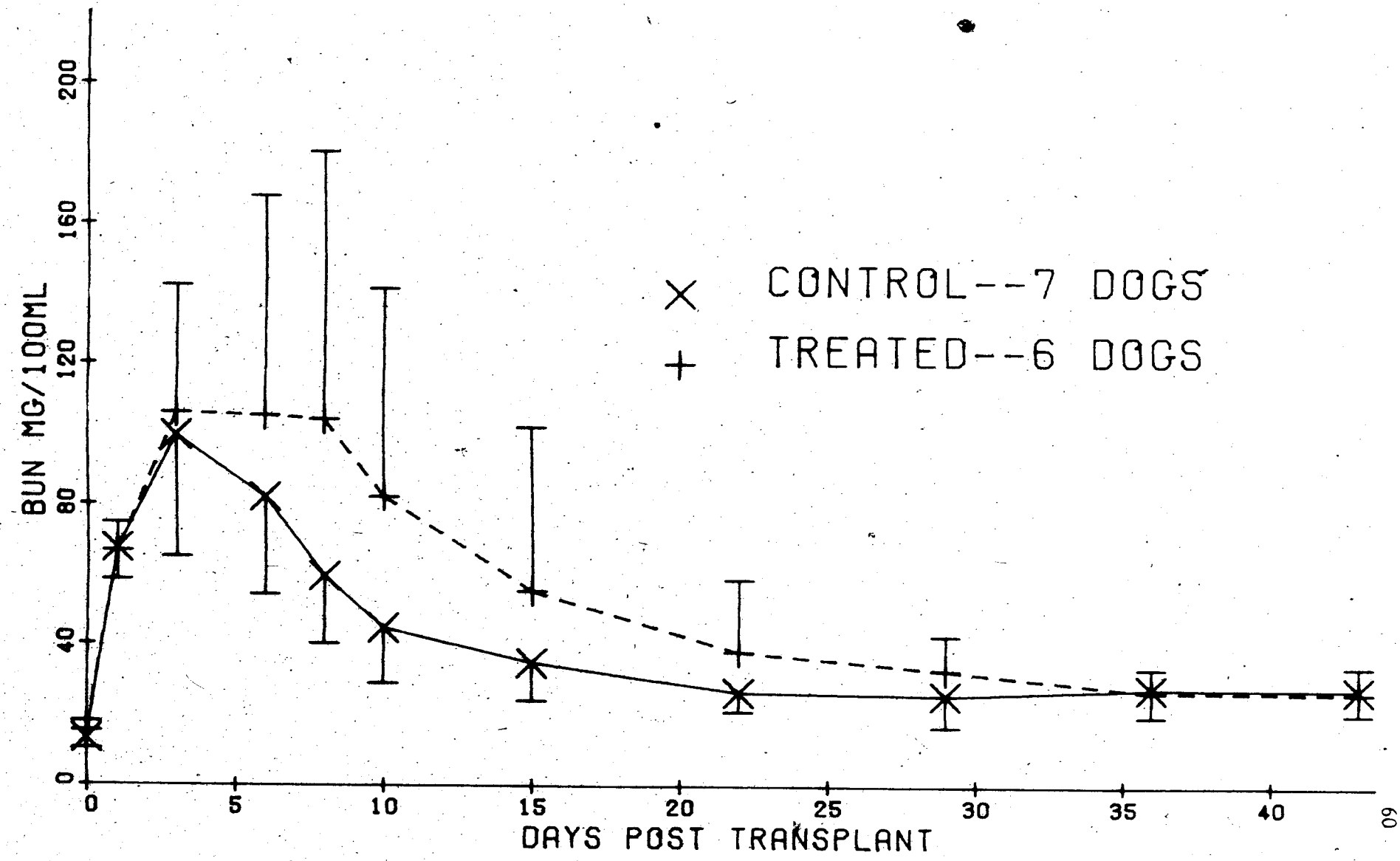

BUN MG/100ML
0
40
80
120
160
200
0
5
10
15
20
25
30
35
40
DAYS POST TRANSPLANT
CONTROL--7 DOGS
TREATED--7 DOGS

Figure 11 30 Minutes Warm Ischemia Group: Mean blood urea nitrogen (milligrams percent) of survivors with standard error plotted against days post-transplant. No significant differences between control and treatment groups.


BUN MG/100ML
0
40
80
120
160
200
0
5
10
15
20
25
30
35
40
DAYS POST TRANSPLANT
CONTROL--7 DOGS
TREATED--6 DOGS

Perfusion Pressure Studies

Kidney perfusion pressures on the Belzer LI 400 were evaluated immediately after the kidney had been placed in the apparatus, at one and at 22 hours, just before removal from the apparatus. Since the pulse rate was kept at 60 to 70 beats per minute and the systolic pressure at 60 millimeters of mercury, diastolic pressure variations provided the only variables (other than flow rates), and were recorded via a pressure transducer connected to a graph monitor. Figures 12, 13, 14 and 15 represent the mean diastolic pressure and standard error plotted against hours of perfusion for each group according to increasing duration of warm ischemia. In those kidneys subjected to no warm ischemia, the treatment group had significantly lower diastolic pressures immediately after placement ($p<0.05$) and at one hour ($p<0.002$) while at 22 hours the value was lower, though not significantly so. An evaluation of this finding is difficult, since the first reading is taken <u>prior</u> to infusion of phentolamine when there should, in theory, be no significant difference between groups - since neither group at that stage has received treatment. At all events, the remaining warm ischemia groups show no significant difference between control and treated animals, indicating that phentolamine has no effect on perfusion pressures of the isolated perfused ischemically damaged canine kidney.

Figure 12 Zero Minutes Warm Ischemia Group: Mean diastolic pressure (millimeters of mercury) with standard error plotted against hours of perfusion. A significant difference is found immediately after placement ($p<0.05$) and at one hour ($p<0.002$), control values being higher than those of the treatment group.

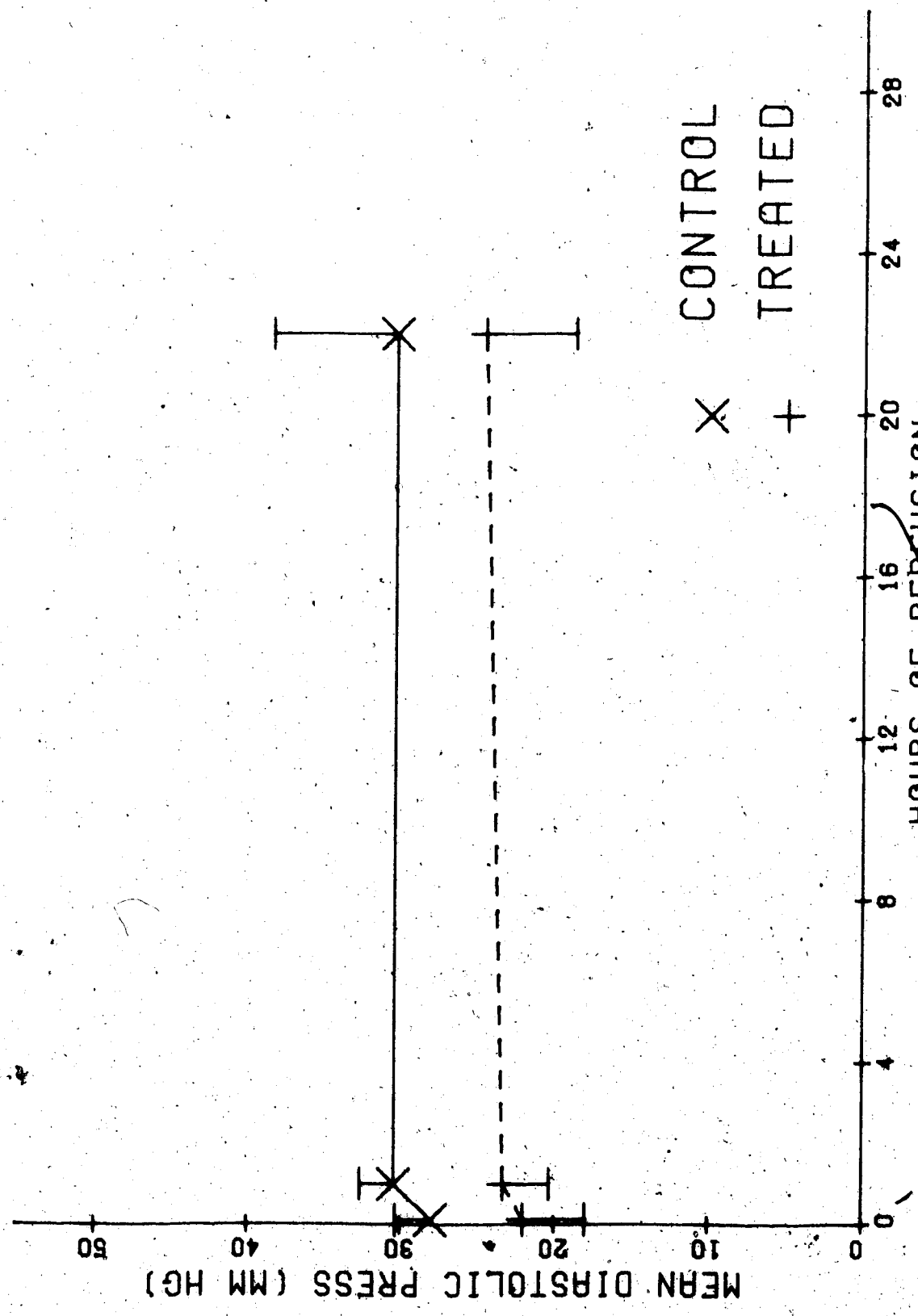

MEAN DIASTOLIC PRESS (MM HG)
0
10
20
30
40
50
HOURS OF PERFUSION
0
4
8
12
16
20
24
28
CONTROL
TREATED

Figure 13 <u>15 Minute Warm Ischemia Group</u>: Mean diastolic pressure (millimeters of mercury) with standard error plotted against hours of perfusion. No significant difference between control and treatment groups.

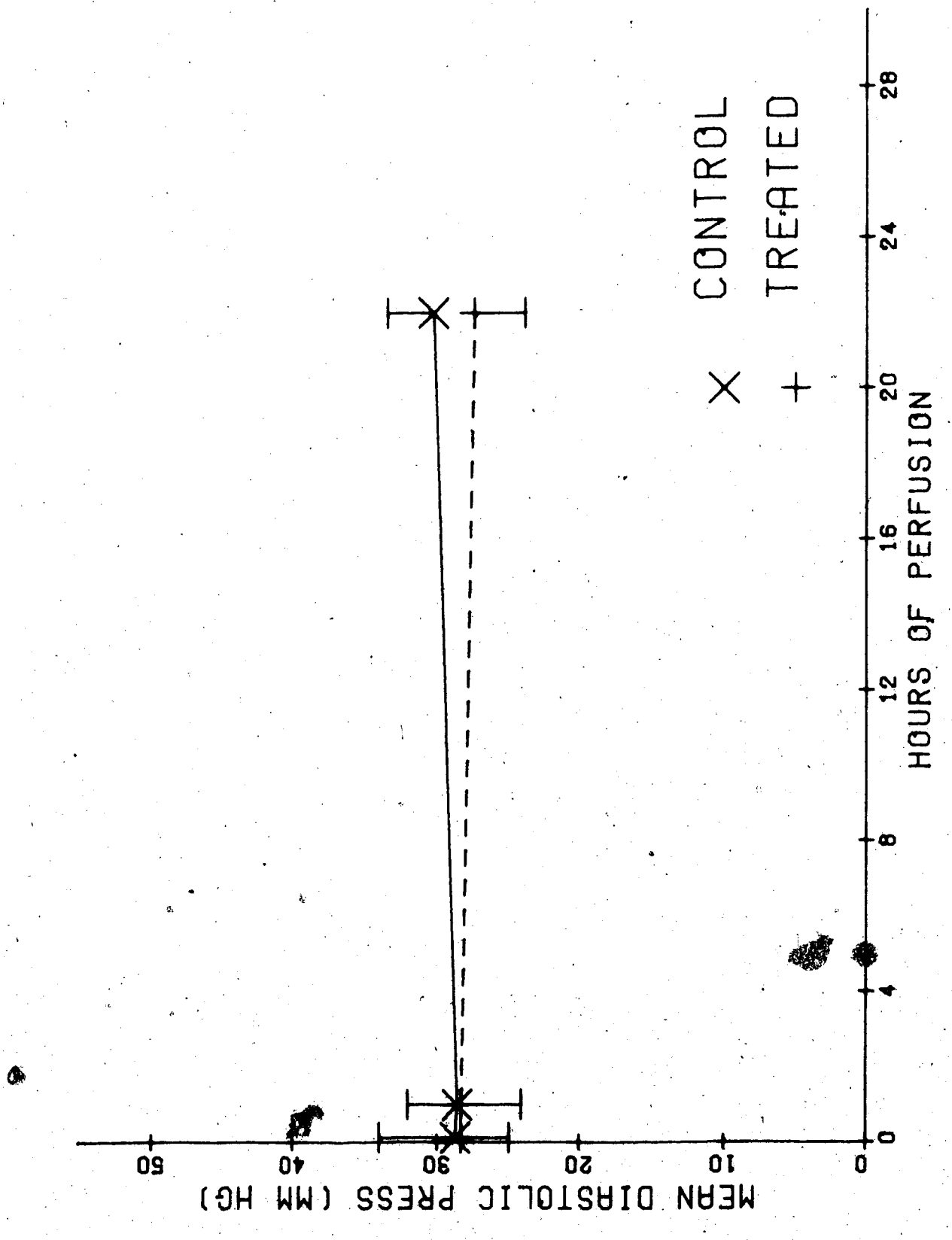

MEAN DIASTOLIC PRESS (MM HG)
0
10
20
30
40
50
HOURS OF PERFUSION
0
4
8
12
16
20
24
28
CONTROL
TREATED

Figure 14 30 Minute Warm Ischemia Group: Mean diastolic pressure (millimeters of mercury) with standard error plotted against hours of perfusion. No significant difference between control and treatment groups.

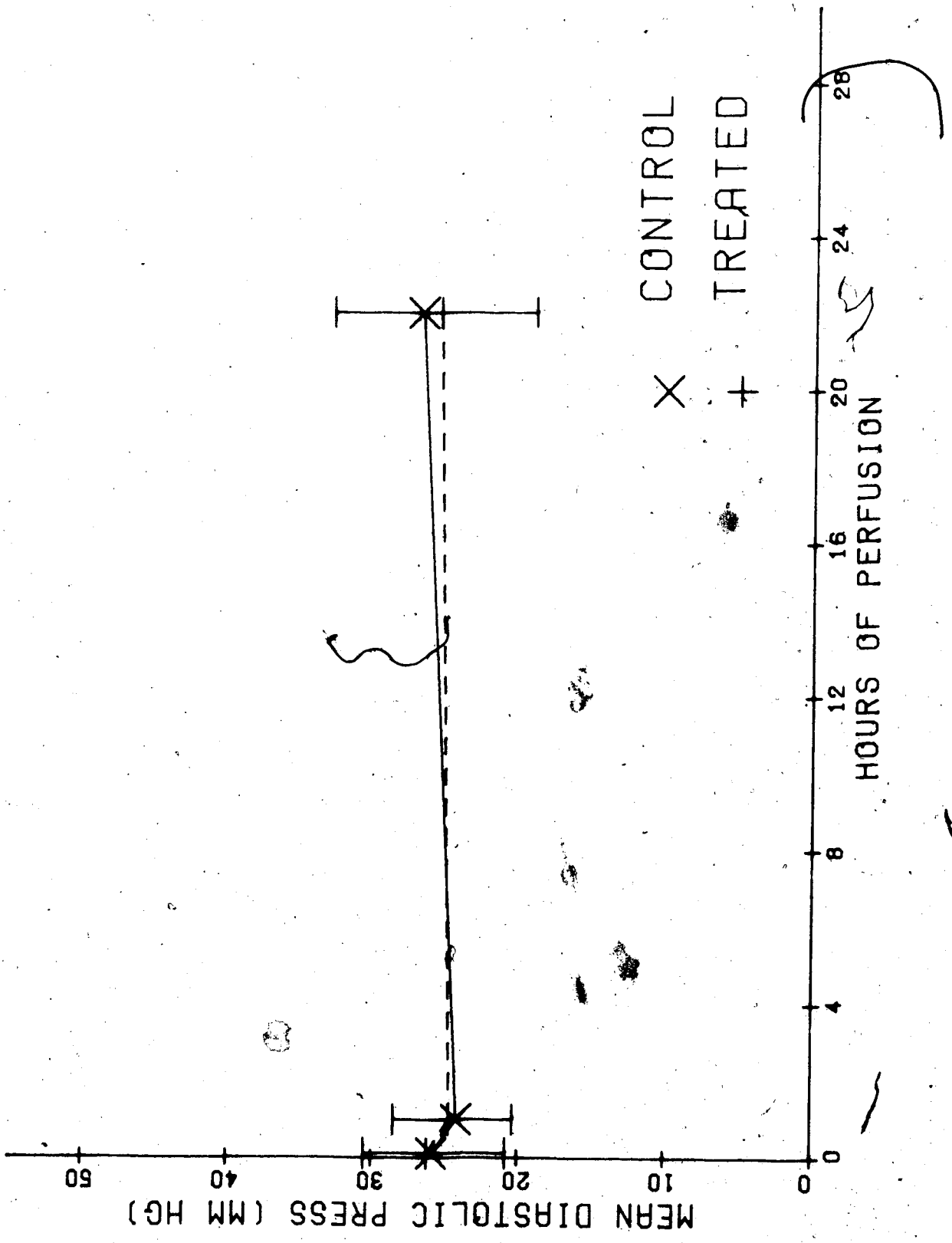
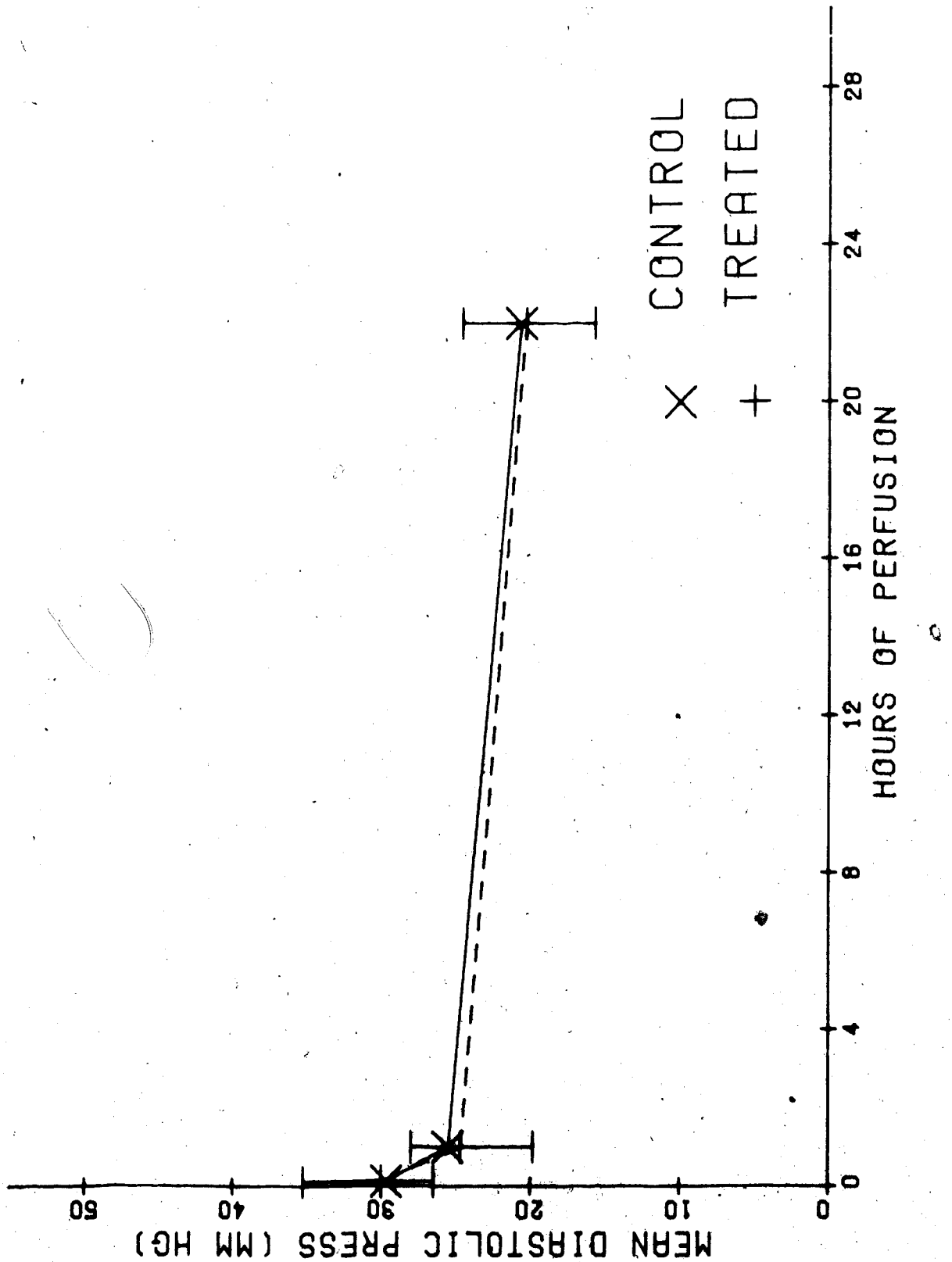

MEAN DIASTOLIC PRESS (MM HG)
0
10
20
30
40
50
HOURS OF PERFUSION
0
4
8
12
16
20
24
28
X CONTROL
+ TREATED

Figure 15 60 Minute Warm Ischemia Group: Mean diastolic pressure (millimeters of mercury) with standard error plotted against hours of perfusion. No significant difference between control and treatment groups.


MEAN DIASTOLIC PRESS (MM HG)
50
40
30
20
10
0
0
4
8
12
16
20
24
28
HOURS OF PERFUSION
X CONTROL
+ TREATED

When mean diastolic pressures were statistically analyzed, comparing them to the zero minutes warm ischemia groups, occasional differences in pressure were found: in the control group pressures were significantly decreased in the 30 minute group at one hour ($p<0.01$) and in the 60 minute group at one hour ($p<0.01$) and at 22 hours ($p<0.05$). In the phentolamine treated kidneys, pressures were significantly increased in the 15 minute group on initial placement ($p<0.02$) and at one hour ($p<0.05$) while the 60 minute group had increased pressure on initial placement only ($p<0.02$). The remaining mean diastolic pressures were not significantly different.

Since these differences are distributed on a random basis, their significance is uncertain, particularly since some are the reverse of what would be expected: i.e. there is a fall rather than a rise in mean diastolic pressure with increasing warm ischemia. However when the poor survival in the 60 minute warm ischemia group is correlated with the apparently satisfactory diastolic perfusion pressures, it is evident that this parameter, contrary to the generally accepted theory, is unreliable as a guide to subsequent function.

Perfusate Flow Studies

Perfusate flow through each kidney was measured at the same time as pressures were recorded - that is, immediately after being placed on the perfusion apparatus, at one hour and at 22 hours. Since systolic pressure and pulse rate were maintained at constant level, flow rates provided an easily measurable variable. Table 5 shows the mean flow rates and standard errors for each group. No significant difference was demonstrated between control and treatment groups and, as in the case of the pressure studies, these figures suggest that phentolamine has no effect on the perfusion characteristics of the isolated canine kidney.

Statistical comparison of the flow rates of the various warm ischemia groups was undertaken and it was found that while initial and one hour flows of those kidneys subjected to 60 minutes of warm ischemia with treatment were significantly lower ($p<0.02$ and <0.05) than those subjected to less ischemic insult, 22 hour flow rates in this group were not significantly different from the other groups. The fact that differing periods of warm ischemia produce comparable flow rates suggests that faith in this parameter as a guide to subsequent renal function may be misplaced, a finding in agreement with other workers (Toledo-Pereyra and Najarian, 1973b; Sterling et al., 1971).

Table 5: Mean Perfusate Flows (ml/min) ± Standard Errors

Minutes of Warm Ischemia	Hours of Perfusion					
	Control Group			Treatment Group		
	Initial	1 hour	22 hours	Initial	1 hour	22 hours
0	53.1±16.4	60.6±19.3	57.6±10.6	59.5±9.1	70.5±8.2	65.3±19.3
15	55.0±19.5	58.5±24.3	57.8±17.0	50.9±13.6	61.9±19.5	62.3±11.6
30	52.1±14.6	70.3±19.0	72.6±21.1	61.0±20.7	68.6±19.3	72.1±28.9
60	43.0±9.1	58.3±14.2	67.5±10.0	43.2±9.5	57.3±11.0	73.4±18.0

Light Microscopy

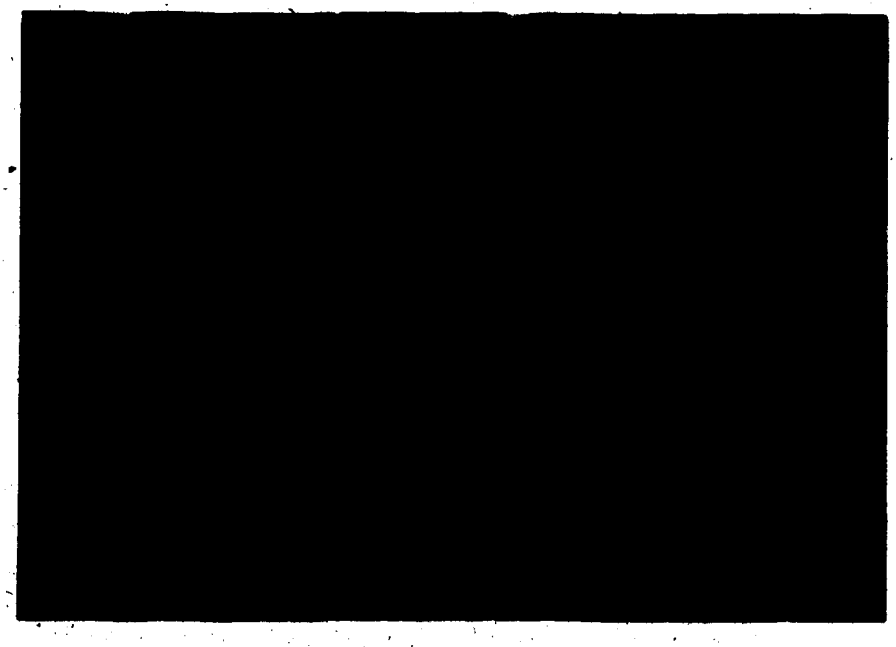
Light microscopy was used to examine sections of all kidneys. In order to avoid observer bias, the sections were evaluated by an independent witness who graded the histopathological changes according to the degree of severity. The majority of kidneys were normal in appearance (Figure 16) but some showed changes which varied from animal to animal, but which were encountered with equal frequency in all groups. It should be pointed out that the optimum time to evaluate ischemic damage is during the first week postoperatively. However, practical considerations and the need for protracted monitoring of renal function precluded an earlier assessment.

Some changes are easier to quantify than others: calcification in the collecting tubules for instance (Figure 17), is readily apparent and can be estimated with reasonable accuracy, while tubular regeneration (Figure 18), which is probably the most sensitive indicator of renal ischemic damage, is more difficult to interpret. This is because the characteristic features of tubular regeneration, namely dilated tubules with heavily basophilic low cuboidal epithelium and little or no brush border, may be difficult to differentiate from the normal appearance of the distal convoluted tubules. Several kidneys showed evidence of mild dilatation of Bowman's capsules and the collecting tubules

Figure 16 Light Microscopy: Normal light microscopic appearance of kidney.

Figure 17 Light Microscopy: Calcification in the collecting tubules.

Figure 18 Light Microscopy: Tubular regeneration.



(Figure 19) and while mild postoperative hydronephrosis is a frequent finding following ureteral implantation, due to edema at the uretero-vesical junction, this finding was unexpected at six weeks post-transplantation.

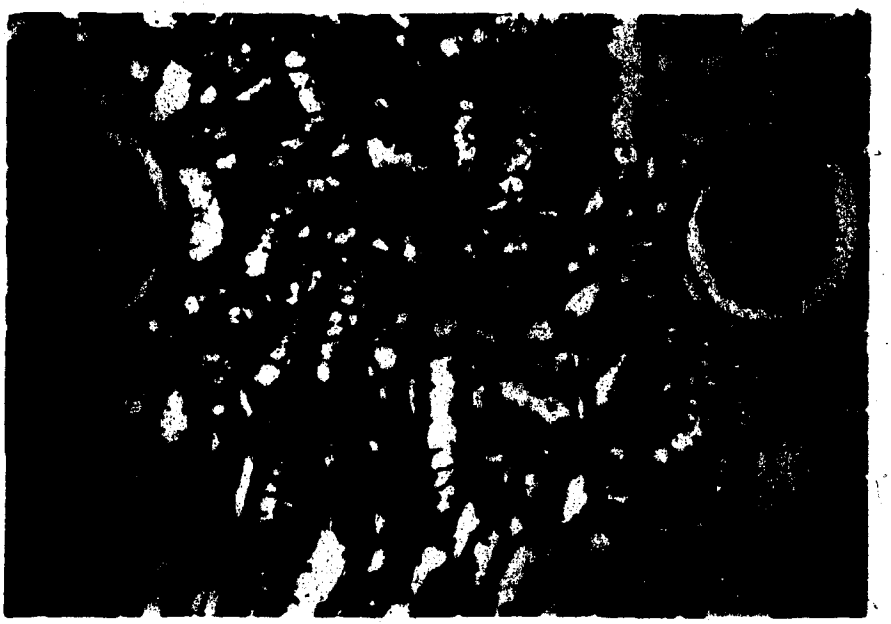
The most prominent lesion found in the non-survivors was tubular necrosis shown in Figure 20 where necrotic cells containing pyknotic nuclei are seen to be separating from the basement membrane. Calcification tended to be a more prominent finding in the non-survivors than in the survivors. Prominence of the juxtaglomerular apparatus (Figure 21) was often seen, and is attributed to the use of exsanguination as the method of sacrifice.

However, within the limitations imposed by such an evaluation there appeared to be no quantitative difference in histopathological changes between the survivors of treatment and non-treatment groups nor between the survivors of different warm ischemia groups.

Electron Microscopy

At sacrifice, an equal number of animals from each group were subjected to renal cortical biopsy. This was immediately diced into small blocks and placed in 6.5% glutaraldehyde for fixation. Definitive conclusions may not be drawn in these circumstances since lesions of a patchy

Figure 19 Light Microscopy: Mild dilatation of Bowman's capsules and collecting tubules.



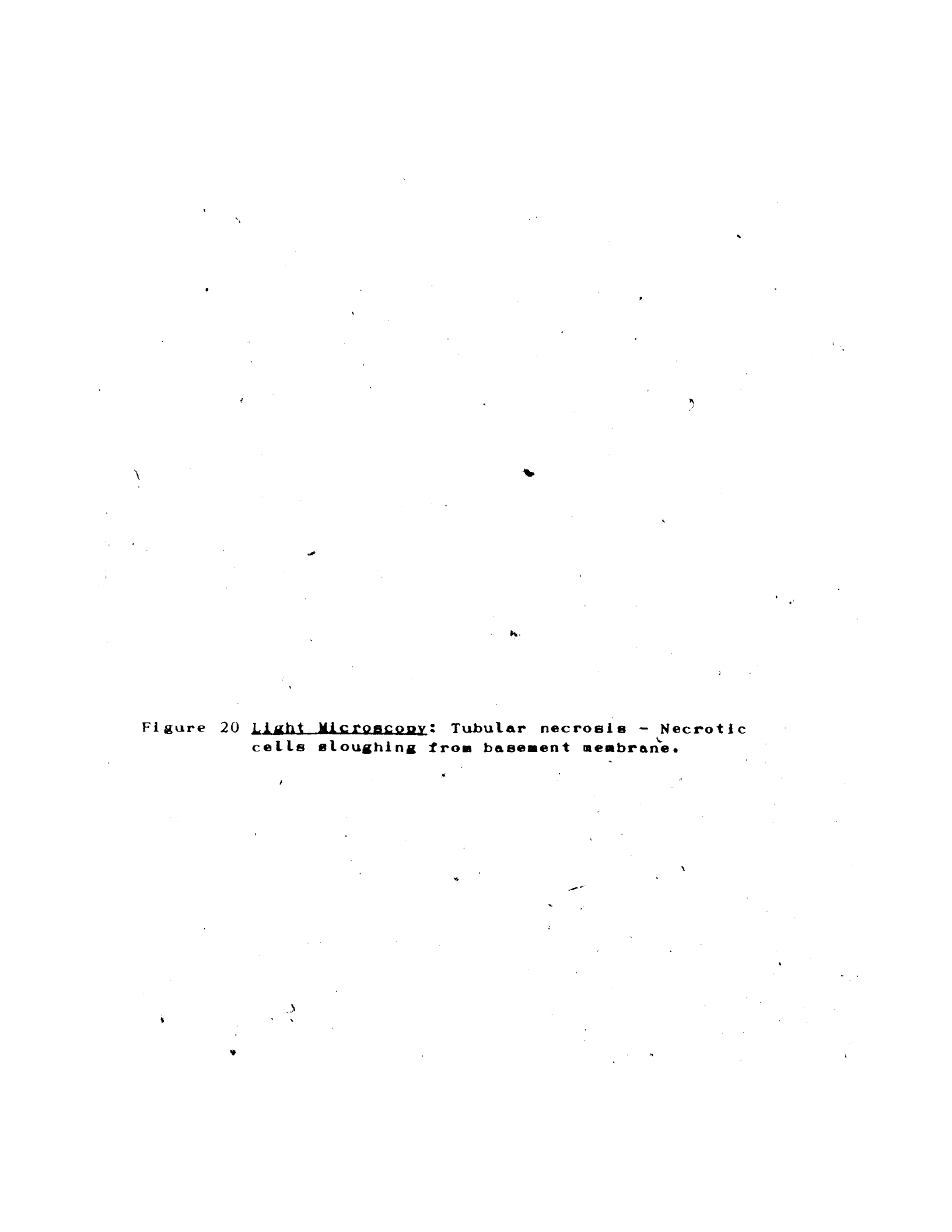


Figure 20 Light Microscopy: Tubular necrosis - Necrotic cells sloughing from basement membrane.

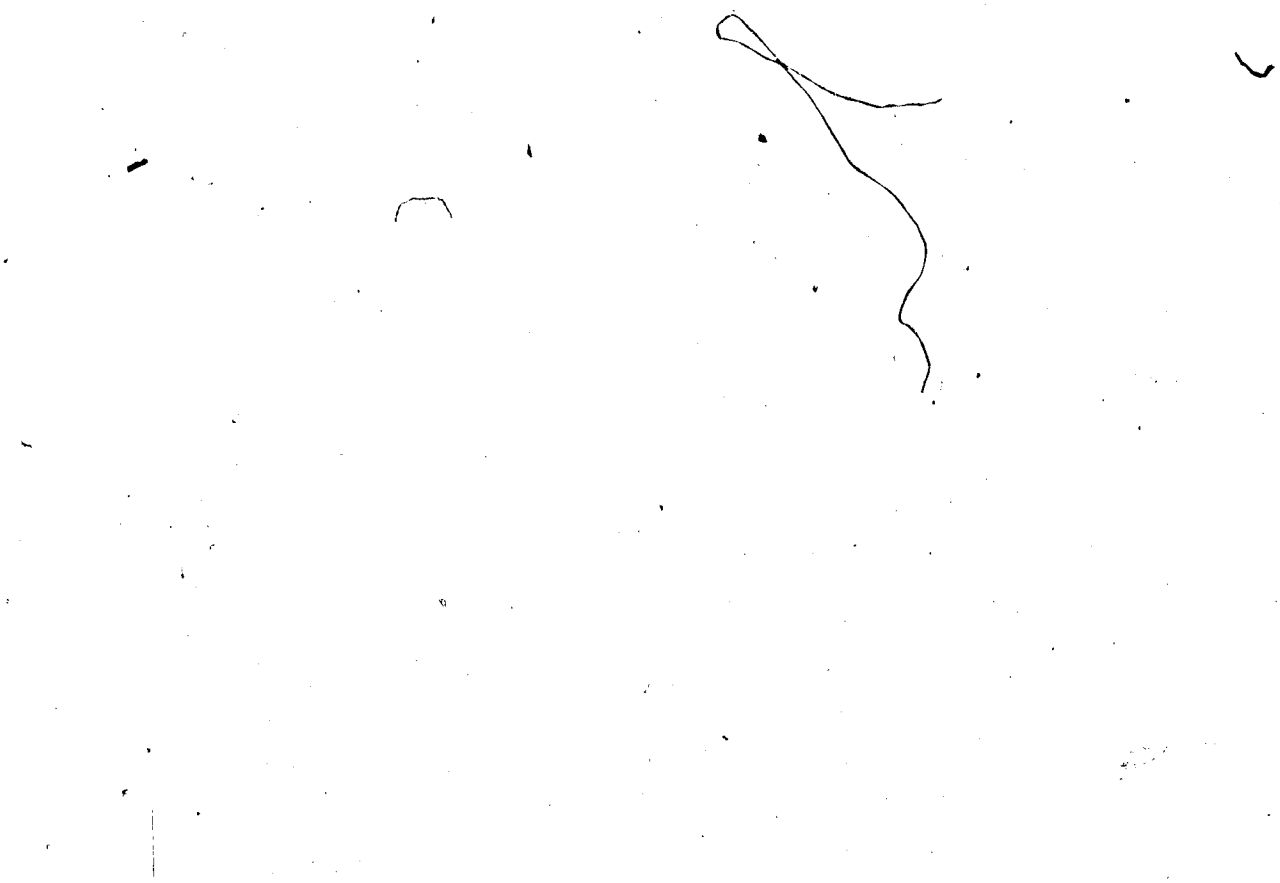


Figure 21 Light Microscopy: Prominence of juxtaglomerular apparatus.

nature are liable to be missed due to the small size of the tissue samples examined. With these provisos in mind, electron microscopic studies on this material revealed no significant differences between the groups, and most appeared normal.

Figures 22, 23 and 24 show respectively the normal electron microscopic appearance of a proximal convoluted tubular cell, distal convoluted tubular cell, and glomerulus. Occasionally however, some randomly distributed, non-specific abnormalities were found.

(a) Intracellular fat droplets. (Figure 25)

Increased numbers of intracellular fat droplets are believed to appear in response to chronic hypoxia in the nephron. One of the effects of ischemia is to inhibit mitochondrial respiration and oxidation of fatty acids. This means that fatty acids presented to the cell are not metabolized sufficiently rapidly and therefore accumulate in the cell as fat droplets. Tubular cell intracellular fat droplets are seen in man in glomerular diseases such as the nephrotic syndrome, and also in some chronic anemias. However their significance in the present study is unknown, because the ease with which fat accumulates in different species varies with the species. For instance, fat droplets are a normal finding in cat renal tubular cells, while dogs

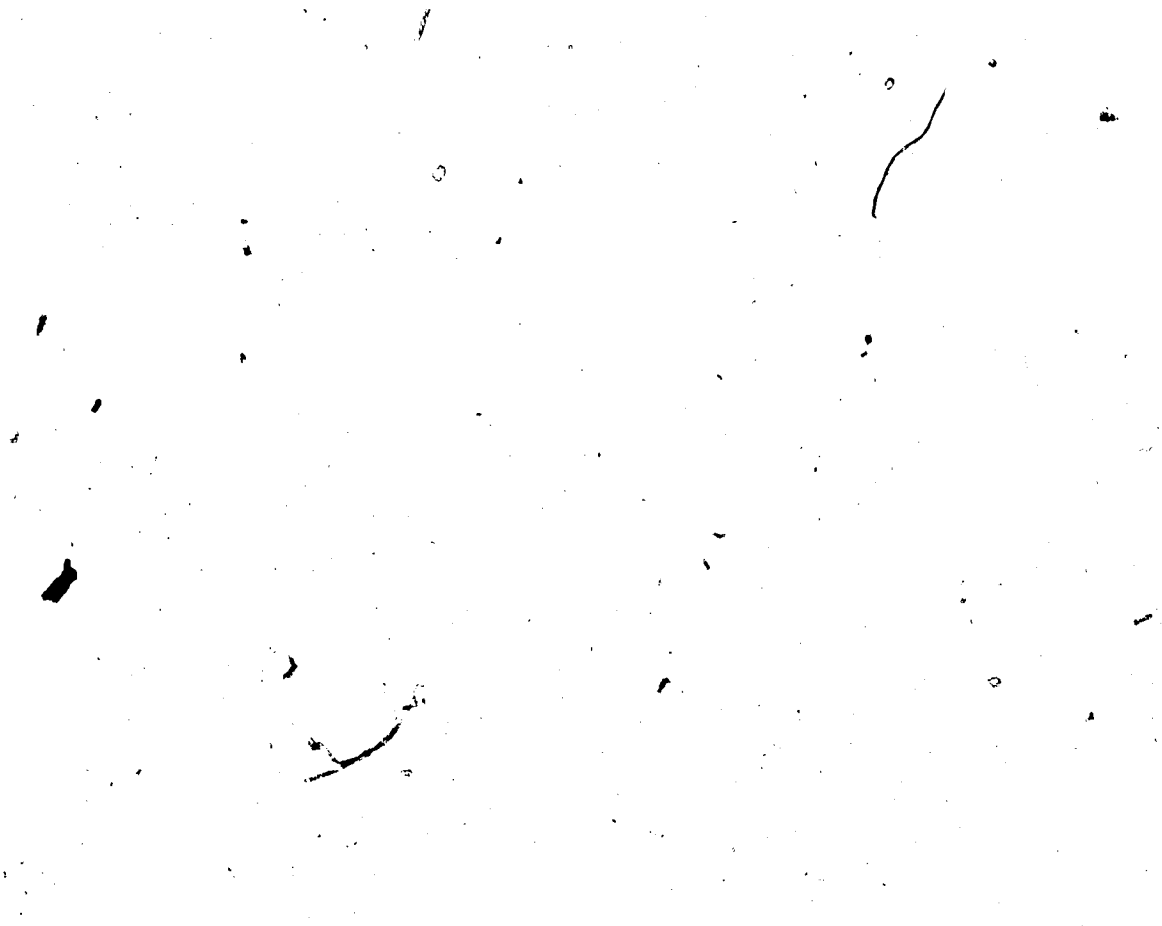


Figure 22 Electron Microscopy: Normal proximal convoluted tubular cell.

BM	= basement membrane
Lum	= lumen
Lys	= lysosomes
Mit	= mitochondria
MV	= microvilli
N	= nucleus

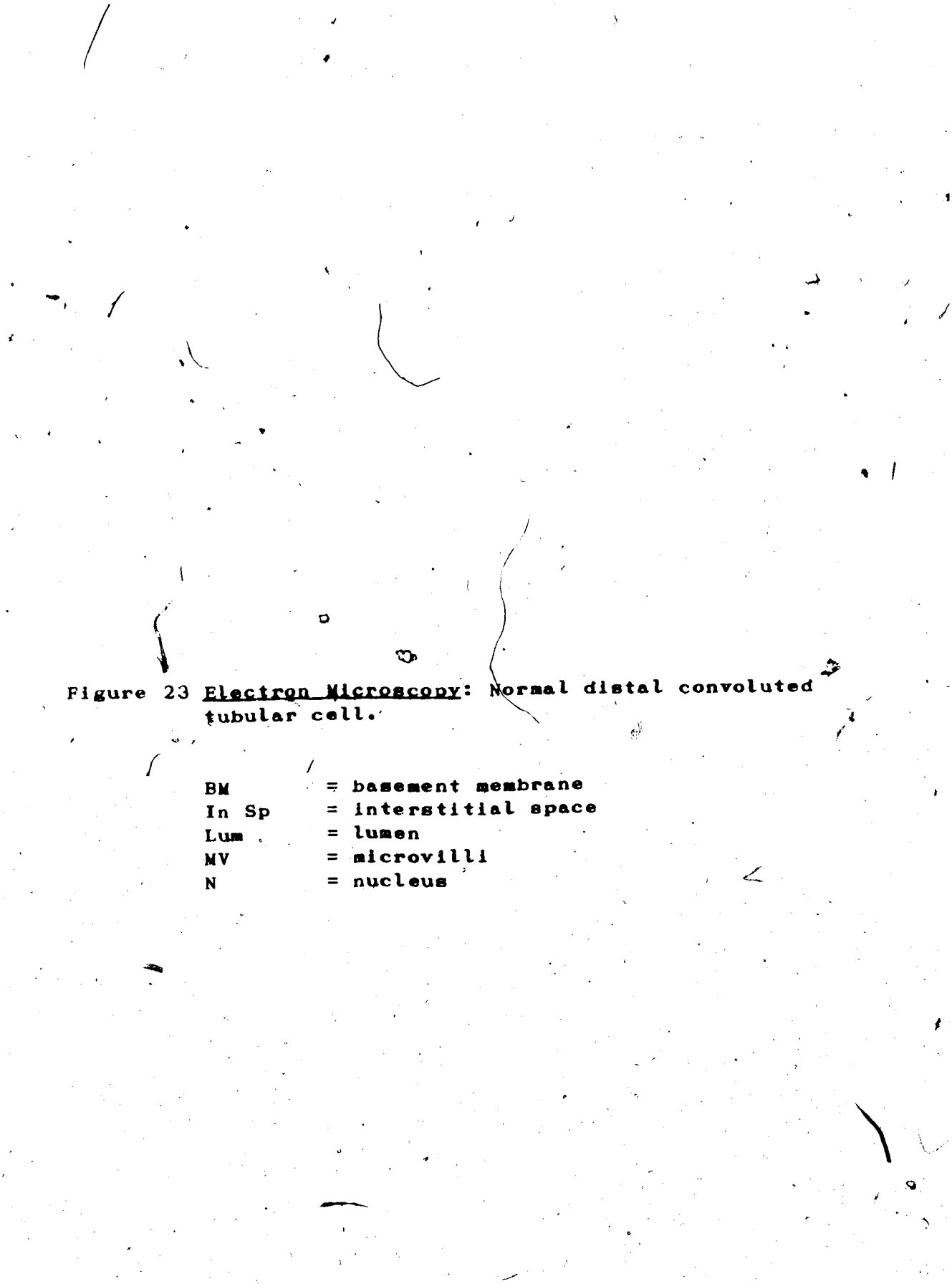


Figure 23 Electron Microscopy: Normal distal convoluted tubular cell.

BM = basement membrane
In Sp = interstitial space
Lum = lumen
MV = microvilli
N = nucleus

Figure 24 Electron Microscopy: Normal glomerulus.

BM	= basement membrane
Endo	= endothelial cell
Epith	= epithelial cell
Ft Pr	= foot processes
Mes	= mesangium
RBC	= red blood cell

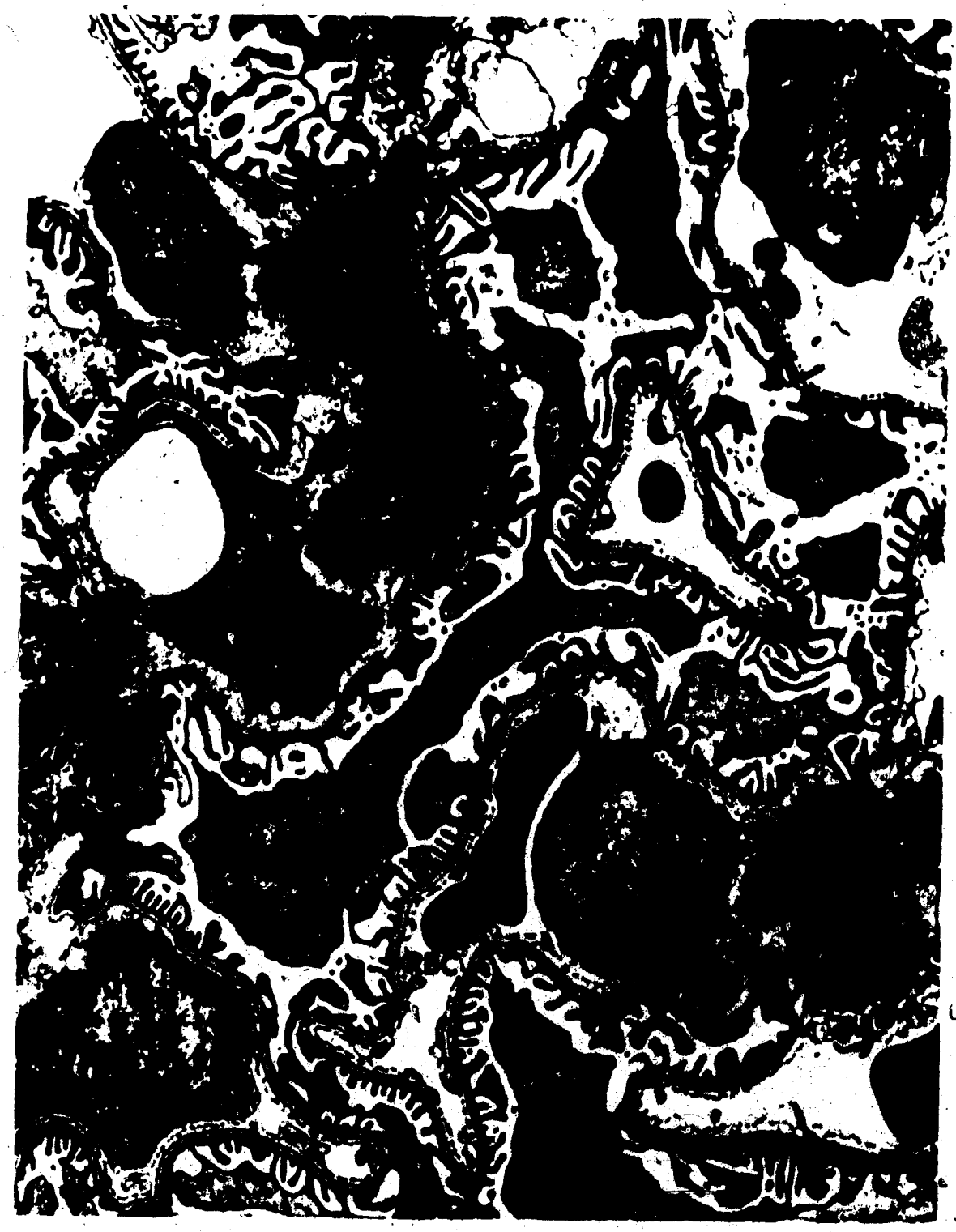


Figure 25 Electron Microscopy: Intracellular fat droplets.

BM = basement membrane
Fat Dr = fat droplet
In Sp = interstitial space
N = nucleus


In
Fat Dr

may well occupy an intermediate position between cat and man in this respect. In support of this, it is unlikely that an ischemic insult produced six weeks previously would result in the persistence of a finding which is essentially reversible, once the ischemic insult is removed.

(b) Intranuclear protein crystals. (Figure 26)

These were found quite frequently in the nuclei of the proximal convoluted tubular cells, and bore no quantitative relationship to the duration of warm ischemia. It appears that this may be a species peculiarity of the dog (T.K. Shnitka - personal communication).

(c) Cell necrosis and regeneration. (Figures 27 and 28)

Figure 27, from a kidney subjected to 60 minutes of warm ischemia, represents the only biopsy with unequivocally compromised ultrastructure. Proximal tubyles are lined by regenerating epithelium, while some tubular basement membranes are split and frayed. Necrotic inflammatory cells are seen in the stroma. Figure 28 shows a regenerating cell characterized by numerous mitochondria and containing a dead cell as an intracellular inclusion.

(d) Cloudy swelling. (Figure 29)

Some tubular cells displayed evidence of cloudy swelling shown in Figure 29 as electron-lucent areas of

Figure 26 Electron Microscopy: Intranuclear protein crystals.

Mit = mitochondria
MV = microvilli
N = nucleus
Pr Cryst = protein crystals


N

Figure 27 Electron Microscopy: Cell necrosis and regeneration.

BM = basement membrane
Necr = necrotic cell
Reg Epith = regenerating epithelium
St = stroma

Figure 28 Electron Microscopy: Regenerating cell containing dead cell as an intracellular inclusion.

Incl = intracellular inclusion body
Mit = mitochondria
N = nucleus

Figure 29 Electron Microscopy: Cloudy swelling.

BM	= basement membrane
CS	= cloudy swelling
In Sp	= interstitial space
Lum	= lumen
Mit	= mitochondria
N	= nucleus


Lum

apical cytoplasm with abundant hyaloplasm and scant organelles. This also reflects cell hypoxia and is caused by an influx of water into the cell secondary to a decrease in the rate of sodium extrusion - the latter of course being ATP dependent. Again, no explanation can be offered as to why this finding is present so long after transplantation.

(e) Thickening of stalk regions of the glomeruli. (Figure 30)

Several glomeruli showed an increase in mesangial matrix with variable thickening of stalk regions of glomerular tufts. This finding is difficult to evaluate apart from the known responsiveness of mesangial cells and matrix to a variety of injurious stimuli.

Renin and Lactic Acid Assays

Although these results did not constitute a major part of the study, it was felt that perfusate assays of these two substances might provide valuable information in the prediction of organ viability. Only a small number of results were available for inclusion since assays could only be done during the perfusion of a single kidney. It soon became evident that the 22 hour value for both lactic acid and renin bore little relationship to subsequent renal function. Lactic acid values tended to be decreased at the

Figure 30 Electron Microscopy: Thickening of glomerular stalk region.

BM	= basement membrane
Endo	= endothelial cell
Epith	= epithelial cell
Ft Pr	= foot processes
Mes	= mesangium
N	= nucleus

end of perfusion, while renin values tended to be at extremely high levels, regardless of ultimate function. The reason for the latter is not easy to understand, unless it is evidence for a cumulative effect of renin in a closed system which lacks a metabolic pathway for the breakdown of this enzyme. It appeared that changes in the first hour might be of value in predicting organ viability, and this figure was therefore analyzed.

On the assumption that post-operative peak serum creatinine reflects the quality of preservation and the degree of isthemic damage sustained prior to implantation, perfusate lactic acid and renin have been plotted against this figure for each dog (Figures 31 and 32). The random scatter of points when lactic acid is plotted against maximum serum creatinine does not confirm the usefulness of this test to predict organ viability; and although at first sight there appears to be a linear relationship between the majority of renin results and the peak serum creatinines, the failure of the perfusate renin assay to "pick out" the three kidneys which did not sustain life suggests that this test is also inadequate for the prediction of organ viability.

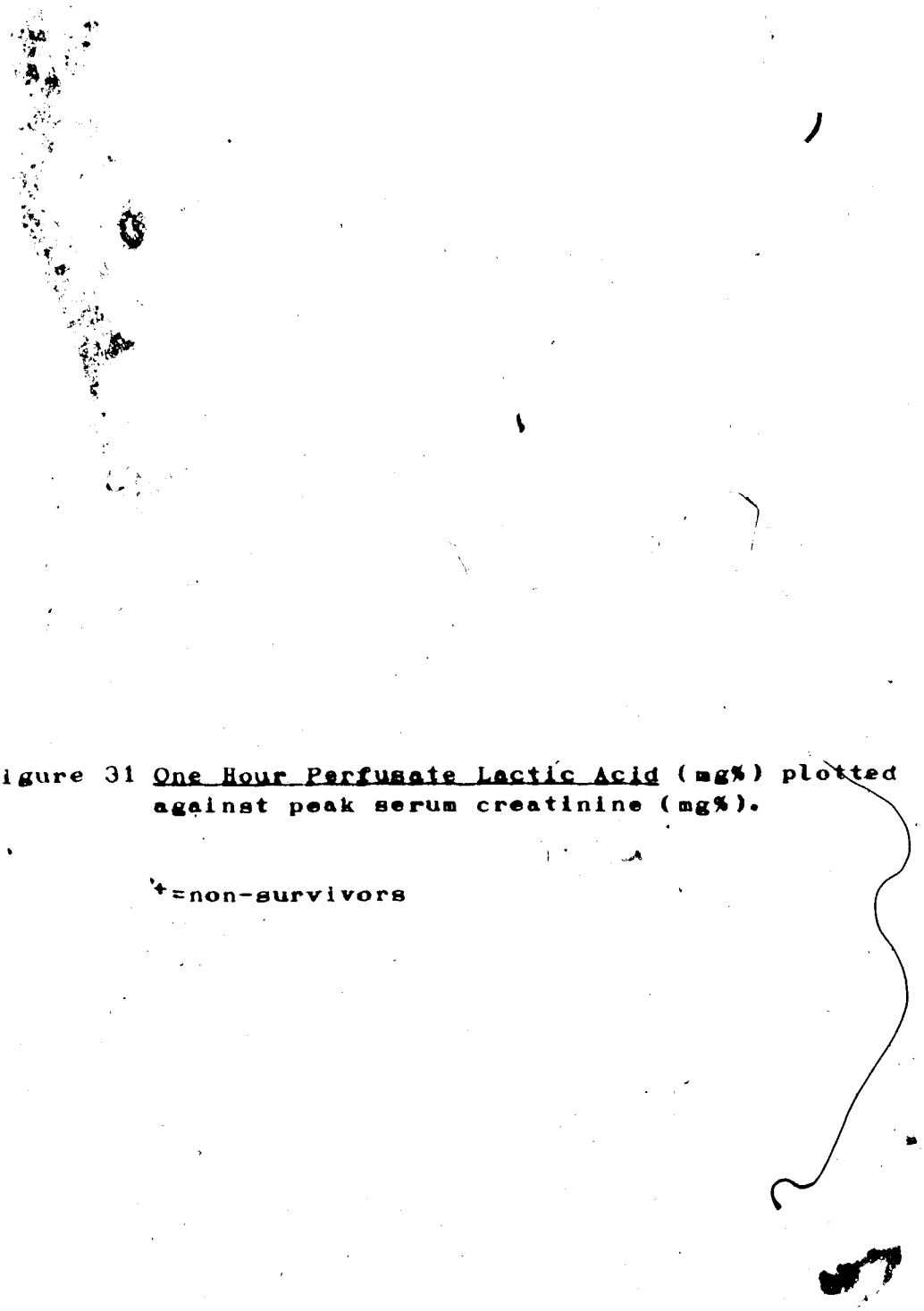


Figure 31 One Hour Perfusate Lactic Acid (mg%) plotted against peak serum creatinine (mg%).

+=non-survivors

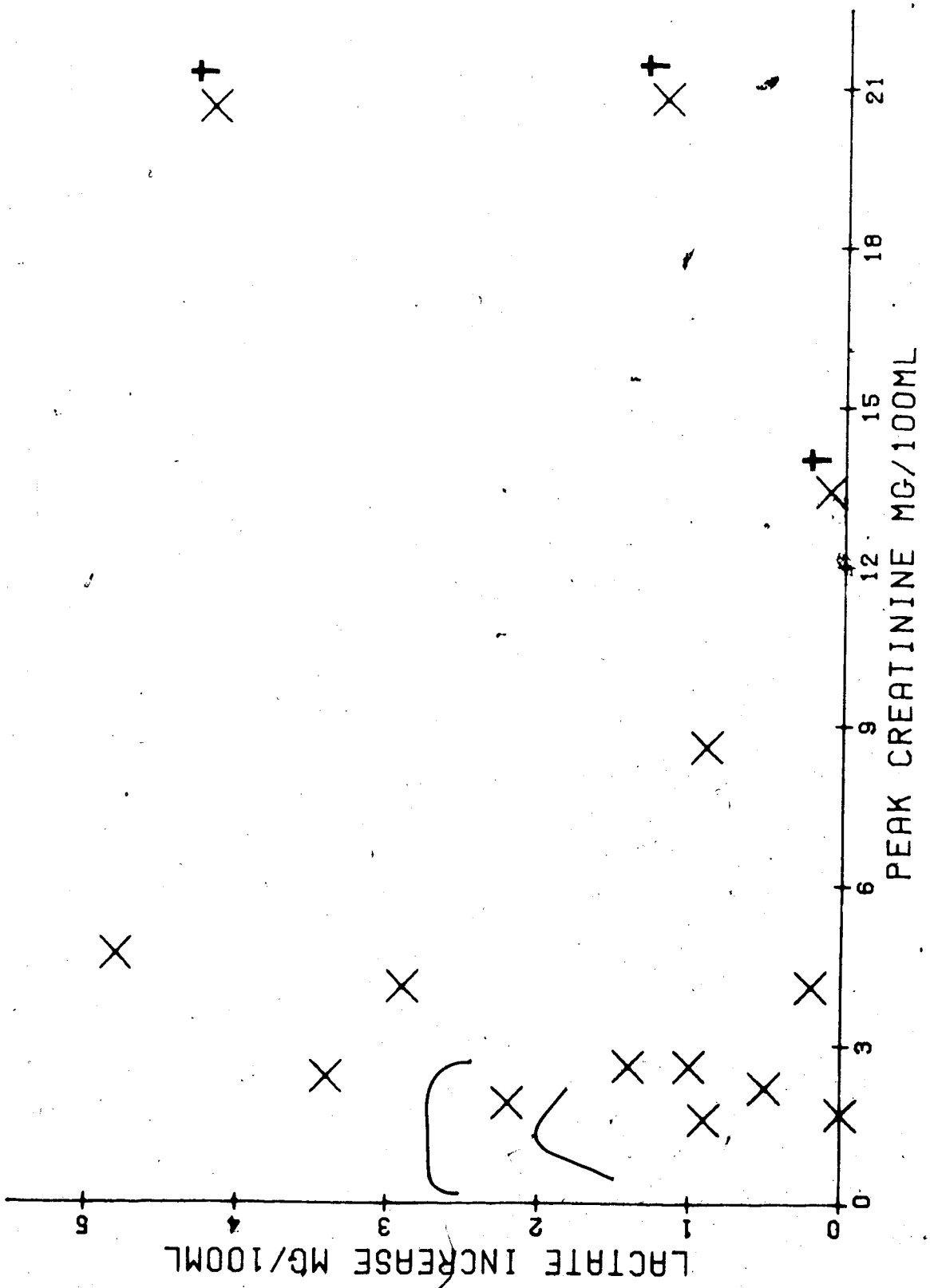
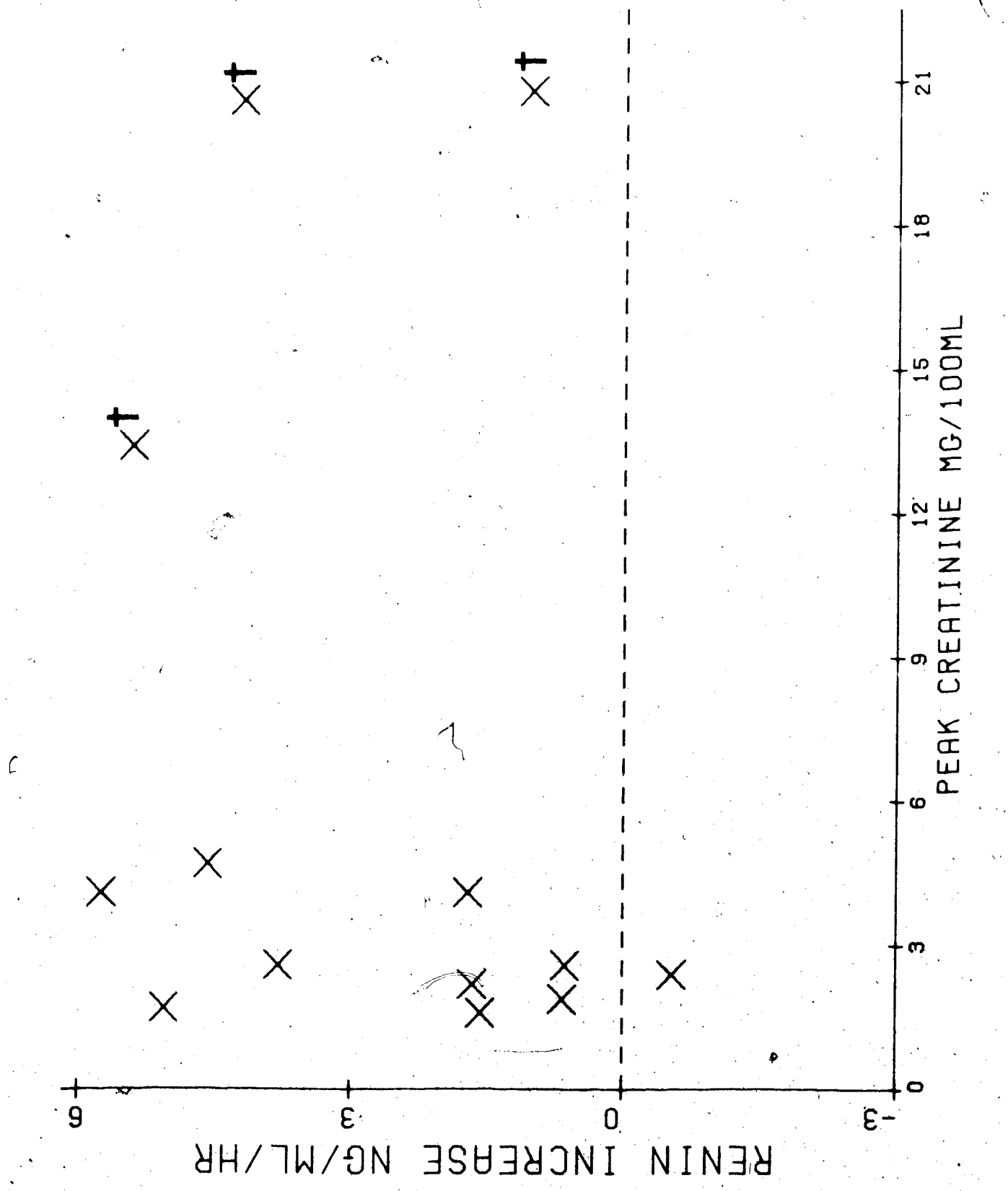

LACTATE INCREASE MG/100ML
0
1
2
3
4
5
PEAK CREATININE MG/100ML
0
3
6
9
12
15
18
21

Figure 32 <u>One Hour Perfusate Renin</u> (mg/ml/hour) plotted against peak serum creatinine (mg%).

+=non-survivors


RENIN INCREASE NG/ML/HR
6
3
0
-3
PEAK CREATININE MG/100ML
0
3
6
9
12
15
18
21

CHAPTER IV

DISCUSSION

This study was designed to evaluate the effect of intraarterial phentolamine during hypothermic pulsatile preservation on the perfusion parameters and postoperative renal function of ischemically damaged canine kidneys. In addition, serial renin and lactic acid assays have been performed, where circumstances allow, in an attempt to find a satisfactory ex vivo viability test. Light and electron microscopy studies have been performed at the end of six weeks or at autopsy to determine if there is any difference between control and treated groups.

Phentolamine (Rogitine) is an alpha blocker with a rapid onset of action chiefly used as an aid in the diagnosis of phaeochromocytoma, although it is also used to prevent tissue necrosis from the accidental leakage of intravenous epinephrine. The rapidity of onset of its anti-hypertensive activity suggested its use in the reversal of the established vasospasm of ischemically damaged cadaver kidneys: and one study (Miller et al., 1974) demonstrated improvement both in perfusion parameters and in postoperative renal function when this drug was given intraarterially during hypothermic pulsatile perfusion.

The present study using an autotransplantation model and employing different lengths of warm ischemia to simulate agonal or post mortem situations, was unable to confirm the findings of Miller and his colleagues; neither in survival nor in postoperative renal function could a statistically significant improvement be demonstrated. Similarly, no significant differences could be found when perfusion parameters and light and electron microscopic data were evaluated.

In considering the reasons for this, it is instructive to discuss the mechanism of ischemic injury to the cadaver donor kidney. Keaveny et al. (1971) in elaborating on the theory that agonal vasospasm is more important than warm ischemia time (Pryor et al., 1971), found that agonal blood pressure remained stable or actually increased following respiratory arrest due to generalized vasoconstriction, but also noted a profound drop in renal blood flow. They felt that severe vasospasm was caused by a combination of anoxia and hypercapnea which produce their effects either by release of catecholamines, by a local effect on the vessels, or more likely by a combination of both. It was also found that renal vasconstriction could be totally abolished by phenoxybenzamine and partially by adrenalectomy. Flores et al. (1972) have drawn attention to the role of cell swelling in the pathogenesis of vascular occlusion. The regulation of

cell volume is dependent upon the supply of metabolic energy; and hypoxia, for whatever reason, is considered to compromise the continuous active extrusion of sodium ions from cell interior to exterior: this allows sodium along with chloride ions to accumulate passively in the cells, resulting in an osmotic indrawing of water and consequent swelling of these cells. After 60 minutes of renal artery occlusion in rats, these workers found a diffuse patchy ischemia affecting all zones of the kidney: these findings were confirmed electronmicroscopically and the vascular occlusion found to be due to cell swelling which obstructed flow in the renal blood vessels. Ischemic damage to the kidneys was thus felt to be self sustaining through a failure of cell volume regulation, but hypertonic solutes such as Mannitol were found to disrupt the cycle. Downes et al. (1973) found that washout solutions such as Collins C3 solution owed their beneficial effect during renal storage more to inhibition of cellular swelling by non-permeable solutes such as glucose, sulphate and magnesium than to conservation of intracellular potassium. It is possible that phentolamine does not reverse ischemic damage caused by longer periods of warm ischemia because the role of cell swelling assumes greater importance at a later stage, a factor upon which an alpha blocker would have little influence. Miller et al. (1974) found that response to intraarterial phentolamine was only obtained if the interval

of warm ischemia was less than 20 to 25 minutes, a finding which might be explained by cell swelling and permanently altered intrarenal flow patterns. It does not, however, explain why no response was seen in the groups with lesser periods of warm ischemia.

Several workers have attempted to reverse established vasospasm after removal of the ischemically damaged kidney, and the findings of these workers have already been reviewed in this thesis. While Miller et al. (1974) reported success in this regard with intraarterial phentolamine during hypothermic perfusion, this study has been unable to confirm this benefit. Several reasons may be postulated as to why this drug is ineffective in the ex vivo perfusion situation. Firstly, it may be that at 8 to 10 degrees centigrade phentolamine cannot exert its vasodilator effect, which might be readily apparent at 37 degrees centigrade. Secondly, some hematologic factor not present in cryoprecipitated plasma may be required to interact with phentolamine to produce its effect. Thirdly, the isolated perfused kidney is, of course, denervated: while Miller and associates claim that phentolamine had a direct action on vascular smooth muscle and also suggested a possible interaction with the alpha adrenergic receptor, it is quite possible that phentolamine may only act in the presence of an intact sympathetic renal innervation. Finally, the hour

of Collins preservation prior to placing the kidney on the Belzer apparatus may render the kidney refractory to vasodilator treatment.

It is noteworthy that the group of kidneys subjected to 60 minutes of warm ischemia had perfusion parameters which, except for initial mean diastolic pressure with treatment and initial mean flow without treatment, were statistically comparable or better than those of the zero minutes warm ischemia group. This certainly suggests that good perfusion characteristics may be maintained in the presence of extensive ischemic damage. This may constitute indirect evidence for the shunting of perfusate from the cortical to the medullary circulation in such circumstances, and would explain the paradoxically excellent perfusion parameters. As has been suggested before, these results undermine the value of perfusion parameters as an index of subsequent function.

In evaluating these findings, the question arises as to whether an animal model employing cross-clamping of the renal artery is a satisfactory one to mimic the agonal situation; for if asphyxia and cardiac arrest are employed, autotransplantation and subsequent <u>in vivo</u> evaluation of renal function could not be undertaken. Løkkegaard and Bilde (1972) found that the pattern of vascular resistance produced by clamping of the renal artery was nearly identical to that produced by suffocation or exsanguination,

the latter situations providing an agonal phase prior to cardiac arrest. Moreover Corica et al. (1975) demonstrated angiographically that vasoconstriction of the main renal artery and its branches, together with decreased cortical perfusion was unequivocally present in kidneys subjected to 30 minutes of warm ischemia by surgical interruption of the renal artery. These studies suggest that the model employed is indeed satisfactory for the purpose of evaluating ischemic damage caused by vasospasm.

CHAPTER V

SUMMARY AND CONCLUSIONS

The major objective of this thesis was to evaluate the effect of intraarterial phentolamine on ischemically damaged canine kidneys during hypothermic pulsatile perfusion. Four main groups were formed in which kidneys were subjected to zero, 15, 30 and 60 minutes of warm ischemia prior to the requisite preservation period. Each experiment was then randomly assigned to the control or treatment groups, the latter consisting of a 15 milligram bolus dose of phentolamine given within 10 minutes of placement of the kidney on perfusion.

Primary parameters to be assessed were those of perfusate flow and pressure, together with postoperative renal function which was followed with serial blood urea nitrogen and creatinine studies for six weeks. At the end of this time the dogs were sacrificed and the kidneys subjected to light and electron microscopy studies.

A number of dogs were excluded because of technical failure, or because death was found to be due to causes other than failure of the transplant: moreover, exclusions

were found to be unrelated to the duration of warm ischemia or to treatment. After the exclusion of these animals, 54 dogs remained in the series, and analysis of the survivors revealed no significant difference between control and treatment groups in terms of survival. Postoperative renal function, measured by serial blood urea nitrogen and creatinine estimations was found to be more compromised with increasing warm ischemic insult, but only one statistically significant difference was found between the control and treatment groups. This was on the first postoperative day of the zero minutes warm ischemia group, when treatment actually resulted in a higher mean serum creatinine.

When perfusion parameters were analyzed a similar pattern emerged, in that no significant difference could be established between flows of control and treated kidneys. Only the mean diastolic pressures were found to be significantly different, those of the initial and one hour readings of the zero minutes warm ischemia group: the significance of this is debatable, since the first reading is taken prior to injection of phentolamine when there should in theory be no difference between the groups.

The differences between perfusion parameters of the various warm ischemia groups were also studied and while occasional significant differences were noted, there was no

consistent pattern to indicate that flow and diastolic pressures are a reliable guide to subsequent transplant function.

Light and electron microscopic studies on the survivors of each group at six weeks post-transplantation showed that there were no differences between control and treatment groups, though a number of interesting but non-specific abnormalities were found.

Perfusate lactic acid and renin assays were obtained during several perfusions. When the increase in these substances during the first hour was plotted against maximum serum creatinine after implantation, it was found that neither provided a satisfactory correlation with subsequent renal function. However, only a small number of studies were available for inclusion.

The conclusions that may be drawn from this study are as follows:

1. Intraarterial phentolamine given at commencement of hypothermic pulsatile preservation has no effect on survival or post-transplant renal function of ischemically damaged canine kidneys.

2. No benefit to the perfusion parameters of flow and diastolic pressure accrued from the use of intraarterial

phentolamine.

3. Perfusate flow and diastolic pressures of the isolated kidney are unreliable as indices to subsequent renal function, since kidneys subjected to extensive warm ischemia and which subsequently failed to support life had excellent flow and pressure characteristics.

4. Within the limitations imposed by the small number of results available, assays of perfusate lactic acid and renin appear to be of little value as an ex vivo viability test.

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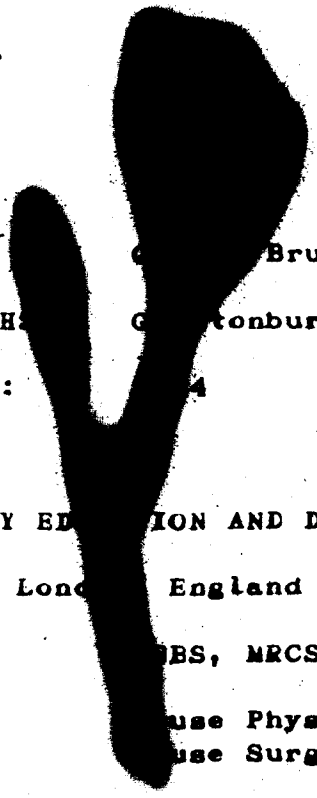

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