URINE COMPOSITION IN THE ISOLATED DOG KIDNEY DURING PERFUSION WITH BALANCED SALT SOLUTIONS AT LOW TEMPERATURES

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PREFACE

One gap in the field of organ transplantation is viability testing of donor organs. With a long interest in the composition of urine the following work was undertaken as a start to fill this vacuum.

A substantial contribution, not the least to the author's morale, has been the kind and patient guidance from Dr. Margaret Beznak and Dr. G.J. Hetenyi, especially if one considers the capricious circumstances of this study, by a student-turned middle aged voluntary University teacher.

This study would not have been possible without the cooperation of the National Research Council of Canada, notably Mr. N.D. Durie of the Low Temperature Laboratory, and Dr. H. Neil, Head of The Animal Facility of the Division of Biology.

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GLOSSARY OF SYMBOLS

U	Urine
P	Arterial perfusate
RV	Renal vein
PAH	Para-aminohippurate (mg/100 ml)
Cr	Creatinine (mg/100 ml)
K	Potassium (mEq/l)
Na	Sodium (mEq/l)
Osm	Osmolality (mOsm/kg)
$\dot{V}$	Flowrate (ml/min/100 g)
C	Clearance: $U \times \dot{V}_U / P$
RPF	Renal perfusate flow: $\dot{V}_{RV} + \dot{V}_U$
GFR	Glomerular filtration rate : C_{Cr}
FF	Filtration fraction : C_{Cr}/RPF
E	Extraction ratio : (P - RV)/P
$T^{c}H_2O$	Free water clearance : $C_{osm} - \dot{V}_U$
T_{Na}	Reabsorbtion of sodium : $(P_{Na} \times C_{Cr}) - (U_{Na} \times \dot{V}_U)$

INTRODUCTION

The purpose of organ preservation

Preservation of biological material may concern sub-cellular units such as virus particles, cells, tissues, organs and organisms. It is also in that sequence met by a rising order of difficulties. Thus preservation of virus, bacteria, cells and tissue is possible for very long periods of time while whole organs can only be preserved temporarily. Efforts to preserve higher organisms have so far failed altogether. The following treatise will focus on the preservation of a whole organ: the kidney.

Isolated organ preparations have been studied since the middle of the 19th century mainly by physiologists to enlarge their knowledge of these organs' function (16). The pioneering discovery of glycerol as a suitable cryoprotective agent by Polge (55) was used by Smith (65) for permanent storage of blood. This permitted the separation of blood into its sub-components thus facilitating more economical ultilization, for example of platelets for thrombocytopenia, of red cells for anemia and of factor VIII for hemophilia, while eliminating hepatitis associated antigen as a risk to the recipient. It has also been suggested that isolation and storage of an organ, especially at risk during a temporary systemic illness, for example the kidney in glomerulonephritis, might be used as a means of prophylaxis (1). Treatment of a diseased organ with drugs that are toxic to

the whole organism (e.g. cancer chemotherapy), may be possible by the functional isolation, for the treatment period, of that organ (1).

The incentive to the present study came from yet another field of whole organ preservation, i.e. the rising demand for donor organs in surgical kidney transplantation.

Organ preservation in the context of kidney transplantation

The history of organ transplantation goes back to the middle ages (56). Kidney transplantations were performed as early as 1902/1903 in Vienna and Chicago (15,71). The nature of the allograft rejection was not generally appreciated until the studies of Medawar in 1944(47). The first successful kidney allotransplantations in men were done in the 1950's (56) and since then over 5000 operations of this type have been performed the world over (56). This surge in surgery was promoted by the discovery of methods (drugs and radiation) capable of modifying the rejection reaction. The kidney was favoured as a transplant organ for several reasons: the advent of the artificial kidney permitted maintenance of the lives of recipients in their anephric phase; uremia represents a state of suppressed immune competence; the kidney is a paired organ one of which can be spared; it has a simple vascular stalk and its function can readily be monitored.

In spite of the lack of an ideal immunosuppressive technique,

and of the failure to this point of histocompatability testing, kidney allotransplantation has been remarkably successful, undoubtedly to some extent due to an element of luck.

Techniques of Organ Preservation

Whole organ preservation may be practiced "in vivo", e.g. an intermediate host or an "organ xenobank" (51), "ex vivo", e.g. connected to an extracorporeal arteriovenous shunt of another individual who may be the future recipient, or "in vitro" (51,56,67). The latter is by far the commonest procedure.

Whole organ preservation should aim for permanent storage. Deep freezing might achieve this but has been unsuccessful so far (67) with the exception of blood preservation (v.s.). Another means to that end might be, one would assume, the perfusion in vitro with autologous blood, trying to perfectly imitate physiological conditions by using another animal or a pump oxygenator. However, all such efforts have universally failed to maintain organ function (44,62,69). For a great number of reasons, the resistance to perfusion in such organs rises within a matter of hours to prohibitive levels. This problem has been reviewed comprehensively by Belzer (8).

For the moment, in vitro organ preservation has to content itself therefore with a temporary success. Expressed in another way, the process of cellular death is slowed rather than prevented.

Physiologically this means that the ratio between nutritional supply to, and demand by the organ is decreased rather than balanced. In practice this refers essentially to the supply of oxygen in relation to its consumption by basic and work metabolism.

Oxygen may be supplied by saturating the perfusate with pure oxygen at atmospheric or hyperbaric pressure (67). In the absence of hemoglobin or other oxygen carriers, the amount of oxygen dissolved in aqueous or plasma-like perfusate is in linear relation to its pressure (17). In addition,high pressure per se has been said to enhance the diffusion of oxygen to the site of its metabolism and to suppress enzyme activity (56). Pure oxygen on the other hand may cause vasoconstriction and impaired renal cortical flow, due to oxygen toxicity (29,76). Helium (20,28,29,30,37,48,61,72,73,76) and CO_2 (19) have been added to prevent this detrimental effect. Nevertheless, supply of dissolved oxygen alone can barely meet even basic demands as illustrated in Table I. The presence of red blood cells as oxygen carriers is believed to contribute significantly to a prohibitive and early rise in vascular resistance (8). Instead, hemoglobin solutions (52) fluorocarbons (51), safflower oil (51), and hydrogen peroxide (22,58,79), have been used empirically to enhance the oxygen content of perfusates and without much knowledge of whether such bound oxygen under the circumstances

is actually delivered appropriately, e.g. in the absence of catalase. Oxygen gas has also been blown into the artery of cat hearts, in the absence of liquid perfusion (25,26,60). Superior survival was perhaps related to the fact that this technique avoids washing out critical nutrients or other factors.

Fuel, e.g. dextrose, is contained in most perfusates but does not appear critical in the presence of sufficient stores in renal tissue to outlast standard preservation times of one to three days (78). The addition of ATP and other substrate has been used to advantage (39,51).

More important, because of greater leverage, appears the suppression of oxygen demand. To an overwhelming extent that means cooling of the organ (33). This tool has been perfected by hibernating animals in order to allow them to survive the winter season, and by surgeons to allow them to do open heart surgery. Its latter application was facilitated by the advent of the phenothiazine group of drugs. Magnesium sulfate has been added since as a metabolic suppressant, (51).

Freezing, as mentioned earlier, is not applicable yet to kidney preservation even with the aid of cryoprotective agents such as Dimethylsulfoxyde, glycerol and etyhlene glycol (1,51). The kidney uniquely is subject to three other possible approaches to lower metabolic demand. First, 95% of renal metabolism relates to active transport of sodium by tubular lining cells and this

activity is driven by renal blood flow in the physiological range. At lower perfusion pressure however, glomerular filtration rate falls and eventually ceases in the presence of ongoing perfusion. At this point the major metabolic demand for sodium transport suddenly ceases while supply continues to meet the remaining low basal demands (21,40,68). Secondly, the fixed relation of blood flow to oxygen demand of the kidney may be interrupted by a unique group of drugs commonly known as diuretics but more accurately defined as blockers of sodium transport (64). This concept has been used successfuly in the prophylaxis of acute renal ischemia complicating surgery or shock (49). Finally, lowering the sodium content of the perfusate, and thus of filtered load, should suppress metabolic demand. It could be that property of low sodium, intracellular fluid like solutions that might make them superior perfusates, rather than other theoretical considerations (18,59,77).

A few further prerequisites for organ preservation must be considered in addition to supply and demand of oxygen. Perfusion per se may also be important to remove metabolic waste, notably CO_2 and other sources of hydrogen ions (34,35). Mannitol and other osmolites have been added to enhance renal blood flow. Dextran (12,43), denatured plasma (6,7,8) and albumin (75) provide colloid osmotic pressure and in this way prevent tissue swelling. Pulsed perfusion is thought to be more physiological and gives better results (8). Vasodilators (papaverin, procain, phenoxybenzamine),

lysosomal stabilizers (cortison, chloroquin), anticoagulants (heparin), insulin and antibiotics are commonly added to perfusates (7,8,43,45).

Viability testing as the weak link

To meet rising demands for transplant organs, many now being shipped by aircraft across continents, and to permit time for recipient selection and preparation, isolated kidney preservation has been practiced on a large scale. Constant perfusion and cooling with artificial media, or surface cooling after brief initial irrigation (46) are most commonly employed. Details of these techniques have usually been derived by individual workers from considerations such as those discussed in the foregoing chapter, and without concerted efforts to predict if such an organ about to be hooked up surgically to a deserving recipient, is still viable. The physician, confronted with a non-functioning allograft, is therefore often at a loss to decide if the oliguria is related to lack of viability, reversible tubular necrosis due to ischemia, or to immunological rejection. Viability testing therefore represents a weak link in the overall tranplant endeavour.

The definition of viability is a difficult one and is distinct from function. For example, to ensure organ viability during in vitro preservation, its function must efficiently be suppressed by cooling, etc., to a point where it may be unregocnizable. As permanent in vitro preservation, i.e. a

balanced supply/demand ratio, of whole organs is technically not yet possible (v.s.) one may also state that death, or loss of viability, due to a fatal insult, in such organs is merely slowed. Abbot has given this matter a great deal of thought and writes: "... for couldn't viability be rather simply defined as possessing the qualities of life? Certainly this is valid, but it is in the understanding of the qualities of life that the difficulties arise. Many attributes of living cells such as motility, phagocytosis, respiration, metabolism, and the like have been described in definitions of life, but it should be emphasized that these really are only manifestations of this entity of viability and don't really describe the essence of viability itself. It is this inability to say for sure what this process of life is that has hindered sure, reproducible, and reliable measurements of it" (2).

Noting the survival of the recipient upon implantation of a vital organ is the commonest, albeit a crude viability test for whole organs. As this is a retrospective approach, hundreds of human kidney recipients must have been subjected to the risks of surgery, only to receive a dead organ. Many kidney transplants in dogs and other animals have probably been subjected to the same viability test.

Morphological assessment of viability is widely practiced but also crude and of limited usefulness. Macroscopic inspection of the donor kidney as to colour, size and consistency for

example permits a diagnosis of immediate hyperacute rejection due to preformed antibodies in the recipient. Examination by light microscopy appears to offer little additional information but electron microscopic changes correlating with loss of viability have been described yet are obviously of no value for immediate "bedside" decisions (38,51).

Enzymatic investigations of tissue slices and -homogenates have shown a correlation to survival (1-3,20,45,73). Determinations of enzymes in renal venous and urine samples prior to the kidney's implantation, like clearance studies, require perfusion of the organ at least for the test period (5,29,32,42,76). Unlike clearance studies they are useful even if organ function is completely suppressed. Enzyme testing in renal outflow therefore represents perhaps the most logical and practical assessment of isolated organ viability although it tests for "extent of damage" rather than"presence of function". These methods have been comprehensively reviewed by Abbot (1-3). He derived an "in vitro index" of viability from the determination of oxygen consumption, PAH uptake, lactate and potassium accumulation and six enzyme determinations.

The work of the healthy kidneys is reflected in the composition of urine excreted (54). This fact permits a simple monitoring of function that is unique to that organ and forms a basis for the clearance concept developed by H. Smith in the 1930's (66). Basically, the urine concentration of non-reabsorbable filtered solute such as creatinine indicates active reabsorbtion of sodium,

with chloride and water, while concentration of PAH indicates active tubular secretion. Under appropriate conditions, the clearances of these solutes are an expression of glomerular filtration rate and renal plasma flow, respectively.

It was the purpose of the following investigations to utilize the clearance principle in the assessment of viability of kidneys preserved under conditions commonly employed today in renal transplant surgery.

METHODS

Standard Protocol

Nineteen kidneys of mongrel dogs weighing 11 to 33 kg were studied. The animals were anesthetized with nembutal and acepromazine, intubated and ventilated with the help of a commercial anesthesia machine with a standard mixture of fluothane and oxygen. Nephrectomy was performed by laparotomy. Perirenal vessels were thoroughly ligated to avoid leakage during the following perfusion. It therefore took approximately one hour to remove a kidney. During that period 400 ml of Ringer-Lactate solution, with 100 ml of 25% Mannitol and 25 ml of 75% ethanol added, were given intravenously to induce a brisk diuresis. Immediately after its removal, the kidney's artery, vein and ureter were intubated and gently irrigated with 200 ml of Ringer-Lactate solution, precooled to 8° C. Thereafter the organ was transferred to a pump cooling unit designed at the National Research Council of Canada by N.D. Durie.

This Unit comprised the following components: an automobile fuel pump, the die-cast valve unit being replaced by an acrylic body with ball valves, was used as a perfusion pump. This pump was driven by an electrical pulsing circuit. Ethylene glycol was pumped from a commercial refrigeration unit to a heat exchanger by a constant-temperature circulating pump. Perfusate flow was measured by a variable area flowmeter and organ temperature by a thermistor probe. Perfusion pressure was recorded

by a Statham transducer. The perfusate was contained in a stainless steel reservoir and perfused the kidney only once ("single-pass" perfusion)with a rare exception when venous return was used for lack of sufficient original perfusate toward the end of an experiment. This unit has facilitated maintaining a constant pressure even when resistance rose. It has also provided a constant and adjustable temperature, pressure (up to 115/80) and pulse rate. The assembly has proved to be highly reliable, inexpensive and simple to operate. Technical detail will be published elsewhere (23).

Ischemia at body temperature, i.e. from clamping the renal artery to the start of cooling perfusion ranged from 45 to 155 sec. Subsequent perfusion at a pulse rate of 60 per min and with a constant pressure of 100/60 mm Hg lasted for two to four hours. The perfusate was continuously bubbled with oxygen. Three 10-min urine and renal venous collections were obtained at each 8, 18, 28, 38 and 18° C in that sequence. The change in temperature within the kidney was accomplished within the first 10-min period but only the third of each triplet was used for statistical purposes.

All kidneys were weighed before and after perfusion. A sample of kidney tissue was fixed in formalin, section stained with hematoxillin-phloxin-safranin and examined under the light microscope.

A commercial balanced salt solution (Tis-U-Sol, Baxter Company),

(Table II), was used as standard perfusate to which creatinine and PAH were added. The pH was adjusted to 7.3 to 7.5 using a phosphate buffer. The final composition of this "perfusate A" is shown in Table II. Experiments according to this protocol will be referred to as group A.

In another series, hemoglobin solution prepared from outdated human bank blood was added to the same standard perfusate to give it concentration of 1.5 gm per 100 ml. Methemoglobin content was below 2%. The final composition of this "perfusate B" is shown in Table II. Experiments according to this protocol will be referred to as group B.

A third set of experiments was conducted after deliberate ischemia. These kidneys were removed and flushed as usual but then incubated for one hour at 38° C. After that they were perfused with perfusate A as outlined above. Experiments according to this protocol will be referred to as group C.

Additional experiments

Several additional experiments were designed to study various detail.

One kidney was handled exactly as those in group A except that perfusion temperature was kept constant at 18° C throughout this four hour experiment, to note spontaneous, temperature independent changes of function.

In two experiments 4 g commercial bovine serum albumin was

added per 100 ml of perfusate A. This was calculated to give a colloid osmotic pressure at 8°C and 38°C, of 220 and 240 mm H_2O respectively. (Normal serum: 280-480 mm H_2O) (27).

In one experiment 6% Dextran in 0.9% sodium chloride (Macrodex, Pharmacia Uppsala, Sweden, M.W. 70,000)(53) was used as perfusate.

In one experiment a balanced salt solution resembling intracellular fluid was prepared as perfusate. This solution was prepared according to Collins et al (18) and contained 15 mEq/l KH_2PO_4, 85 mEq/l K_2HPO_4, 50 mEq/l KCl, 10 mEq/l $NaHCO_3$, 60 mEq/l $MgSO_4$ and 1 g per 100 ml dextrose. The pH was adjusted to 7.4 and the total osmolality measured 208 mOsm per kg. This solution, with several drugs added is widely used by surgeons today to preserve human kidneys for transplantation.

One kidney was perfused with isotonic sodium chloride at 8, 18 and 28°C. At each of these temperatures, three collection periods were conducted at mean perfusion pressures of 70/30, 100/60 and 115/80 mm Hg, respectively.

One kidney was cooled to 28°C and then perfused with pure oxygen gas. To obtain a visible gas return from the renal vein while the organ was immersed in isotonic saline, mean perfusion pressure had to be raised to 90 mm Hg.

Two additional experiments were conducted in kidneys of group A after the last standard collection period at 18°C had been completed.

In one of these, 200 mg of Furosemide was added to the remaining 2000 ml perfusate and the experiment continued for three additional 10-min collection periods. In the other, renal venous pressure was raised to 10 and then to 30 cm H_2O during four additional collection periods.

Analytical methods

Sodium and potassium were analyzed in a flame photometer (Instrumentation Laboratories Inc.), calibrated against an internal lithium standard. pH was measured with a pH-meter 27 (Radiometer). Creatinine was measured by the method of Bonsnes and Taussky (13). PAH was determined by the method of Varley (74). In one instance when hemoglobin was used, 20 µc of ^{131}I labelled hippuran was used in addition to PAH to exclude that the hemoglobin pigment interfered with the colour reaction. The results by isotope analysis were virtually identical to those by chemical analysis in this instance. Hemoglobin was measured as cyanmethemoglobin and methemoglobin was determined quantitatively by spectrophotometry. Osmolality was determined by freezing point depression using an Advanced Instruments osmometer. Viscosity was measured by a standard viscometer.

Calculations

Renal venous- and urine flow were expressed per 100 g kidney tissue to permit comparison of individual experiments.

Urine concentrations of PAH, creatinine, sodium, potassium, and osmolality were expressed as their ratio (in %) to the arterial perfusate concentration so as to facilitate comparison of individual experiments. Since these were "single-pass" perfusions, i.e. perfusate concentration was constant for a given experiment, the urine/perfusate (U/P) ratio is a linear expression of urine concentration.

Additional functional parameters were derived as outlined in "Glossary of Symbols" and in Tables III and IV.

RPF was measured in three ways: by a pre-calibrated variable area flowmeter in the arterial line, by the Fick principle as C_{PAH}/E_{PAH} (equal to $(U_{PAH} \times \dot{V}_U)/(P_{PAH} - RV_{PAH})$ (54) and by adding $\dot{V}_U + \dot{V}_{RV}$. In spite of careful ligation of capsular blood vessels during surgery there was often a variable degree of leakage. This was presumed to be predominantly pre-glomerular and therefore to compromise the arterial but not the venous flow measurements. In the absence of a leak arterial and combined venous and urine flow were almost identical. A low PAH extraction and urine flow introduced a substantial error into the utilization of the Fick principle for RPF estimates. For these reasons, $(\dot{V}_U + \dot{V}_{RV})$ was chosen as the best estimate of RPF and all results are based on this definition of RPF.

Vascular resistance was calculated as (arterial pressure -

venous pressure)/RPF. Under the standard experimental protocol the numerator of this equation was a constant (arterial pressure = 100/60 mm Hg, venous pressure = 0). Renal resistance was therefore in direct inverse relation to RPF and will not be reported separately.

Colloid osmotic pressure was calculated as fc (45.2 A + 18.86 G) x T/273.16 (27) where fc = a constant, A and G = albumin and globulin concentration respectively in g/100 ml, or by van't Hoff's equation: $\Pi = RTc/M + AC^2$ where R = gas constant 0.8207 ATM/100 ml, T = absolute temperature in $^{\circ}K$, c = concentration in g/100 ml, M= molecular weight and A = a constant depending on the shape of the molecule, for Dextran = 14 (53).

Differences between populations were estimated by Student's paired t-test using a desk computer (Wang Laboratories Canada Ltd.). Log transformation was applied when indicated, e.g. to estimate differences of ratios from unity.

RESULTS

Standard experiments

Results of the experiments carried out according to standard protocols of group A, B and C are summarized in Tables II and III. Figure 1 shows detail of the experiment where one dog kidney was perfused with perfusate A for four hours while the temperature was kept constant at 18° C. It is to show that the preparation per se was quite stable during this time interval and that a fairly constant U_{PAH}, approximately twice that of P_{PAH}, prevailed throughout.

One typical example of each of the three standard protocols (group A, B and C), is depicted in figures 2-4. Individual values of the more pertinent parameters are illustrated in figures 5-12.

The results of group A and B were qualitatively similar and will therefore be reported together.

At 8° C urine flowrates (figure 5) ranged from 0.26 to 2.40 ml/min/100 g. There was no consistent change when the temperature was raised to 18° C and 28° C but at 38° C urine flowrate was significantly higher than that at 8° C ($p < .05$). By inspection warmer urine samples of group B appeared more red suggesting a higher hemoglobin content.

Renal venous flowrates (figure 5) ranged from 14.2 to 118.9

ml/min/100 g. They were significantly higher in group B ($p < .05$) and only in this group also showed a significant rise at 28° C ($p < .02$). But it was occasionally noticed that an initially small leakage of fluid from the kidney increased with warming.

U_{PAH} (figure 7) was significantly above P_{PAH} at 8° C ($p < .01$) and rose in all experiments of groups A and B at 18° C ($p < .01$), and in all but one at 28° C ($p < .1$). There was no significant change with further temperature rise to 38° C, but U_{PAH} fell significantly when temperature was lowered to 8° C ($p < .05$).

U_{Cr} showed qualitatively the same changes but to a lesser extent.

U_{K} (figure 8) was also significantly above P_{K} at 8° C ($p < .05$) and in all but one experiment of group A and B rose at 18° C ($p < .05$). There were no consistent changes with drug or temperature changes.

U/P ratios of sodium and osmolality (figure 9) were only insignificantly above unity in both groups and at all temperatures except in group B at 28° C when U_{Na} was significantly above unity ($p < .05$).

C_{Cr}, RPF and FF are depicted together in figure 10. All three values were well below those of a (hypothetical) normal dog. C_{Cr} and RPF, but not FF in group B were consistently higher than in group A. In group B, RPF rose significantly at 18° C ($p < .05$)

and at 28° C ($p < .02$), and C_{Cr} rose at 28° C ($p < .05$). In all experiments C_{Cr} was more suppressed than RPF and the filtration fraction was therefore very much below the normal of 20 to 30% of a normal dog, especially at the lower temperature.

The extraction of PAH (figure 11) was the same in groups A and B at 8° C and ranged from 1.4 to 6.8% with one value in group B 18.4%. E_{PAH} rose significantly at 18° C ($p < .05$) but not so beyond that temperature. Even the highest observed values for E_{PAH} were very much below those of a normal dog, viz. 75-80% (54).

T_{Na} (figure 12) was insignificant at 8° C, became measureable in some experiments at 18° C and rose significantly at 28° C ($p < .01$). Values in group B were usually higher than those in group A.

Free water clearance was insignificant in group A but attained a measurable magnitude at 28° C in group B ($p < .05$).

There was no correlation between urine- and perfusate flowrates and any one of the above clearance parameters.

Results in group C were fundamentally different and below those of groups A and B. Urine flow was 0.02 to 0.08 ml/min/100 g. Renal venous flow was 11.0 to 13.9 ml/min/100 g. Neither changed with temperature. Significant U/P ratios were not recorded at any one point and for any solute. Urine PAH and creatinine were at times below perfusate values as noticed in figure 4. Urine samples in these experiments were very small and had to be diluted for analysis.

All deviations from perfusate concentrations are therefore believed to be artifactual.

Additional experiments

In standard experiments all kidneys gained weight during the perfusion. This weight gain was of the same magnitude in groups A and B but if anything a little less in group C and on average amounted to 11.4 ± 4.2%. As this was believed to introduce an artifact into the hemodynamic studies (see discussion) additional experiments were undertaken to prevent the kidneys from gaining weight but as will be seen these were unsuccessful.

With the addition of albumin to the perfusate there was in fact no measurable weight gain. But technical difficulties precluded the use of albumin in standard experiments. The albumin proved difficult to dissolve. After overnight stirring and passage twice through a 0.5 micron millipore filter, a clear perfusate was obtained which became cloudy again on cooling. During perfusion renal venous flow fell within one hour from initial 49 ml per min to 9 ml/min, even when perfusion pressure was raised to a mean of 90 mm Hg. Urine flow fell from an initial 0.33 ml/min to 0.22 ml/min. Raising the perfusate temperature from 8 to 28° C led only to a minimal and insignificant rise in U/P ratios for creatinine and PAH.

When Macrodex in isotonic saline was used as a perfusate, renal venous flow rose throughout the study from 37 to 61 ml/min. In spite of the presence of an 8 micron millipore filter in the arterial circuit, no urine flow was obtained at any time thus defeating the

purpose of the experiment. This kidney, interestingly enough, lost 11% in weight and one wonders if all filtrate was reabsorbed passively, by this strong hypertonic solution, together with some interstitial or intracellular fluid.

Balanced salt solution resembling intracellular fluid is said to prevent weight gain (18). But "Collins solution" proved an unsuitable perfusate because crystallization was noted during cooling on three attempts at 10, 13 and 18° C respectively.

Urine and renal venous flow fell from 0.6 ml/min before to 0.2 ml/min after and from 14 ml/min before to 7 ml/min after gas perfusion, respectively. It was concluded that this technique did not contribute sufficiently to warrant further pursual.

Thus a certain amount of weight gain had to be accepted as unavoidable, in order to obtain satisfactory urine collections. Stripping the capsule of the kidney to avoid the rising tissue pressure associated with weight gain was attempted once in a preliminary experiment and led to an outpouring of fluid from the renal surface precluding timed flow observations altogether.

In one kidney, perfused at three different pressures, the U/P ratios of PAH showed no significant differences. RPF tended to rise, but the available data are insufficient to state whether or not autoregulation of RPF was present.

Furosemide, or a raised venous pressure, had also no effect on renal hemodynamics or urine composition. Immediately prior to Furosemide U/P_{PAH} was 176, U/P_{Cr} 108 and urine flow 3.1 ml/min. Thirty minutes later these values were 122, 107 and 3.5 ml/min respectively, not notably different from the pre-drug figures.

Viscosity of perfusate A was 1.34 and 0.77 centipoise at 8° C and 38° C respectively. The corresponding figures for perfusate B were very similar: 1.39 and 0.80 centipoise. This amounts to a 74% rise on cooling to 8° C in both cases. In contrast Dextran perfusate had a higher viscosity than perfusates A and B. This was shown by the observation that the arterial line flowmeter had to be recalibrated with this solution.

The light microscopic appearance of a normal dog kidney (control) and of samples obtained at the end of experiments A, B and C respectively, are shown in figures 13 to 16. There was a considerable degree of interstitial edema in group A. This was less noticeable when albumin or dextran had been used. The vessels and glomeruli of the latter kidneys showed no visible signs that could account for the low urine flowrates in these experiments. The kidneys in group B were almost indistinguishable histologically from the control. The ischemic kidney (group C) showed signs of autolysis.

DISCUSSION

Review of pertinent literature

Prolonged preservation of the isolated kidney by perfusion with blood is met with considerable difficulties (8,44,62,69). Nevertheless some very relevant observations have been made when using this basic approach:

During the 1930's Winton in Cambridge with various collaborators (11) perfused isolated dog kidneys with defibrinated blood, to which creatinine (20-90 mg%), urea (200-400 mg%) and glucose (100 mg%) were added, at temperatures varying between 38° C and 3° C. He used a double pump-lung preparation to facilitate prompt changes in temperature. Cooling led to an increase in urine flow from two to tenfold. A fall of 10° C below body temperature produced about 15% of the maximum change in urine flow produced by cooling.

Cooling caused blood flow decrements that were if anything higher than values predicted by a glass viscometer corrected for blood. Intrarenal pressure rose invariably on cooling to below 27° C, by an average of 13 mm Hg. This was considered a constant, unaffected by perfusion pressure, but affecting blood flow analogous to a constant venous pressure increment.

The plasma/urine concentration ratio of chloride fell on cooling to reach unity at 80° C. 80% of this was achieved by cooling to 10° C below body temperature.

The U/P ratio of creatinine fell also on cooling to reach its lowest value of 1.5 at 12° C. 50% of this was accomplished by cooling to 10° C below body temperature.

Concentration ratios of both creatinine and chloride were inversely proportional to perfusion pressure at 36 to 38° C but not at 3 to 13° C.

If equal changes in urine flow were produced by changes in arterial pressure or by change in temperature, the change in composition of the urine, though qualitatively in the same direction were quantitatively much greater with the latter than with the former manoeuvre.

One important reason in Winton's mind for conducting these experiments was his search for further support of the filtration/reabsorbtion theory of urine formation. Previous experiments by Bayliss and Lindsgaard and by Starling and Verney had yielded similar data with cyanide. But these remained open to criticism because of poor survival properties of their preparations (early proteinuria) and the fact that cyanide rendered the tubular membrane abnormally permeable,

One observation made both in cyanide poisoned and cooled kidneys was a pronounced fall of creatinine clearance to approximately one-fourth of its original value. Leaking from the tubular lumen, abolition of a hypothetical active secretion into the tubule, and lowered glomerular capillary pressure were all considered

unlikely explanations for the low creatinine clearance. A part of the low creatinine clearance was believed related to a low glomerular filtration rate, the remainder by an assumed inactivity of a proportion of glomeruli. It was noted that creatinine clearance fell twice as much as renal blood flow.

Autotransplantation of a kidney to a neck or another site creates somewhat more physiological conditions. Such "ex vivo" kidneys have been intensively studied by several groups of authors (4, 57). Their results are of some marginal interest to our observations:

In 1969 Bartha et al (4) in Budapest studied 24 ex vivo perfused (38° C) dog kidneys under osmotic diuresis. Their aim was to show that the "impaired function" of such preparations recorded by others was not so much evidence for injury to tubular lining cells, but rather reflected short-circuiting of peri-tubular capillaries by true post-glomerular arterio-venous anastomosis. Thus, directly measured RBF, vascular resistance, oxygen consumption and maximum PAH transport of the ex vivo kidney were all equal to those of the in situ kidney whereas PAH clearance, and -extraction, creatinine clearance and nutritive blood flow (^{42}K) were below in-situ values. "Extraction deficits" of PAH and ^{42}K matched closely and suggested a shunt of approximately 20%.

Reinhard et al in Munich in 1967 conducted extensive studies on the "neck kidney" (57). They noted that 7 hours after re-implantation, and for the following day, the ex vivo kidney's function

was comparable to that of its mate in vivo.

Hamamoto et al. in Osaka in 1969 (31) perfused 22 human cadaver kidneys that were collected during autopsy, three to 23 hours after death, with outdated human blood bank at 97% oxygen, 37° C, and 110 mm Hg mean pressure for 2 to 5 hours. RBF ranged from approximately 10 to 20 ml/min/100 g and urine flow from 0 to 27 ml/h per 100 g. All kidneys gained weight. Histological examination showed evidence of autolysis and in most cases azotemia was documented ante mortem. Nevertheless the authors concluded that their observation permitted them to predict which organs were suitable for transplantation, a notion that is in no way supported by their recorded data.

Virtually thousands of animal and human kidneys have now been transplanted after isolated bloodless perfusion/preservation. Only very few of these organs have been studied during the preservation period for evidence of function and viability.

Some of the most extensive studies along these lines have been conducted since 1967 on baboon kidneys by a group active at the University of Stellenbosch in South Africa collaborating with various centers in the United States.

Initially (29,76) they used 5% invertsugar in a balanced extracellular fluid-like salt solution with added dextran, PAH and creatinine and a pulsatile pump oxygenator. In 13 experiments oxygen and in seven experiments helium gas was used. Perfusion

was at 37° C and lasted for three hours at the end of which renal tissue blocks were sampled. The helium experiments gave a higher renal blood flow, PAH extraction, effective renal cortical flow and filtration fraction. Urine glutamic pyruvate transaminase and glutamic oxalate transaminase levels remained normal and stable through both series. Assuming from the latter a maintained organ viability, the authors concluded that the use of helium prevented oxygen induced cortical vasoconstriction while the organ was capable to thrive on anaerobic metabolism. Although urine concentration is not given one can derive from the reported results that U/P ratios of creatinine were in the vicinity of 10, and PAH clearance was one-half to one-fourth of directly measured renal blood flow. Compared with 21 intact control animals, PAH clearance and creatinine clearance were approximately one-third in the experimental baboons.

Elaborate in vitro studies of metabolism were performed in kidney slices (cortex and medulla) of four unperfused-, six oxygen- and five helium-perfused animals of the same series (48). Oxygen uptake and oxydative phosphorylation were impaired after both oxygen and helium perfusion, and lactate/pyruvate ratios were raised.

In another series (20,72) isolated perfused baboon kidneys were studied for one hour at 37° C, 100% oxygen and ambient pressure using the same perfusate, after storage for 24 hours at three atmospheres, low flow pulsed perfusion and exposure to 100% O_2 (9 experiments), 100% helium (6 experiments), 50% of each

oxygen and helium (9 experiments) or 95% helium and 5% oxygen (7 experiments). Cortical and medullary slices of these kidneys were afterwards again subjected to metabolic studies. A higher rate of renal blood flow and less weight gain were again noted with helium while sodium handling and tissue metabolism were demonstrable in all experiments in vitro.

Studies with microspheres and with ^{203}Hg Chlormerodrin under comparable experimental conditions failed to confirm the beneficial effects of helium on effective renal cortical flow. The authors believed that this was related to washout of the former and lack of protein for binding by the latter compound (37). ^{133}Xe-studies on the other hand appeared to confirm the beneficial effects of Helium (61).

Some criticism of interpretation of these results, however, appears warranted. Of the many parameters studied (76), some, e.g. changes in sodium transport are of equivocal significance considering that almost all filtered sodium is reabsorbed. It is also somewhat doubtful that an organ can remain viable after 3 hours of total hypoxia at 37° C. The "improved effective renal cortical flow, derived as renal perfusate flow X E_{PAH}, in the Helium series related predominantly to a higher RPF but this may also be explained by assuming a totally dead (and flaccid) organ. U/P ratios of PAH are not given in their papers. They can be derived approximatively and are not obviously above 1, suggesting that their "urine" was merely a transudate.

In 1969 Husemann in Erlangen perfused 21 isolated dog kidneys using a pulsatile heart-lung machine (pressure 100/70 mm Hg) (36). The kidneys were cooled by perfusing a Mannitol containing Ringer solution and maintained up to 24 hours at 15° C by perfusing a solution consisting of 40 parts of 6% dextran (M.W. 60,000), 25 parts 3.5% gelatin and 35 parts heparinized autologous blood. Average weight gain amounted to 22.1%. Perfusion flow was 22 to 120 ml/min per 100 g, urine flow 0.36 to 1.73 ml/min/100 g. At 15°C U/P for phosphate was below-, for sodium and glucose equal to-, and for potassium, calcium, inulin, PAH, creatinine and urea above unity. Lack of detail precludes further interpreation of these otherwise interesting data.

Scott et al. in Newcastle upon Tyne in 1969 perfused 10 dog kidneys for 24 h at 10°C and 1 atmosphere oxygen with cryoprecipitated human plasma (63). Urine formation during perfusion was 0-4.5 ml/h. This urine was isotonic with respect to osmolality and "electrolytes" but all kidneys functioned after reimplantation.

Lotke et al. in Bethesda in 1970 perfused dog kidneys for 48 to 72 hours at 2°C and 1-3 atmospheres of oxygen with homologous serum and Ringer-lactate solution (1:1) (45). In some experiments cortisol or chloroquin were added. Tissue slices and homogenate were subsequently studied in vitro. Oxygen consumption and PAH uptake rose with the addition of either one of these two drugs, and with hyperbaria but acid phosphatase and acid ribonuclease did not change with these variables. The authors concluded that the effect

of lysosomal stabilizing drugs was too small to be noticed in the presence of other, more effective preservation manoeuvres.

Liebau et al. in Munich in 1971 studied 16 dog kidneys perfused for 10 to 52 h with cryoprecipitated plasma at 4°C (42). The kidneys were thereafter reimplanted to the neck of another dog. Renal function after reimplantation was demonstrable if during perfusion weight gain was below 10%, if renal venous effluent potassium, lactate dehydrogenase and glutamic oxalic transaminase were not raised and if urine flow was absent. The latter related to the concept that urine formation during isolated perfusion reflected excessive and harmful perfusion pressure.

Benjamin and Sell (10) of the U.S. Navy in 1971 perfused isolated dog kidneys at 24 to 25°C and systolic pressures of 75 to 95 mm Hg for four hours using the method of Belzer. The perfusate consisted of cryoprecipitated plasma bubbled with 95% oxygen and 5% CO_2 to which cortisol, penicillin, insulin, magnesium sulfate, dextrose, mannitol and PAH were added. On 30 minute urine collections in "7 to 9 dogs" they recorded venous flow rates of 1.5 to 3.5 ml/g/min, and creatinine clearance of about 2 (ml/g/min ? -units not given), PAH clearance of 4.2 to 12.6 (ml/g/min ? -units not given) and filtration fraction of 20 to 30%. Urine flow was not given and some of the values do not seem to agree. Nevertheless U/P ratios for PAH were as high as 11, for creatinine around 2, for sodium 0.6 and for potassium 4.4 to 7.9. These were all taken as evidence for viability, the electrolyte ratios being

related to the presence of cortisol. These kidneys gained 0 to 20% in weight but RPF, renal resistance and plasma lactate values remained constant throughout the 4 h run.

Evidence for renal function

A nosological note appears appropriate at the onset of this chapter. The results in our ischemic kidneys (group C), and other published data (20,31) suggest that even a dead kidney is temporarily capable of being perfused and possibly of forming a glomerular filtrate. These of course are not energy-requiring mechanisms, and may be referred to as "mechanical function".

Evidence for active, energy dependent, cellular transport work may be distinguished as physiological function or briefly "function".

The author's experiments have shown a higher concentration of some solutes in urine when compared to perfusate. This is in agreement with observations by others (10,11,29,36,76). It can only mean that solute (i.e. PAH) was actively secreted into the tubular lumen, or that filtered solvent was reabsorbed along with active reabsorbtion of sodium, thus concentrating non-reabsorbable filtered solute, i.e. creatinine. That significant U/P gradients should be demonstrable at a temperature as low as 8° C is perhaps somewhat unexpected but consistent with Winton's observation who noted a constant U/P creatinine at temperatures below 15° C (11). The demonstration of such active tubular work is taken as evidence for cellular function. The greater urine concentrations in the

oxygen richer group B and their lack after two hours of warm ischemia support this notion, as well as the rise of these U/P ratios with temperature as metabolism is released from the cold spell. The lack of further rise of U/P gradients of PAH and creatinine when the temperature was raised from 28 to 38° C may indicate that at this point oxygen supply available for tubular work had reached its limit. The constant U/P_{PAH} of approximately two, over four hours, when temperature was stable at 18° C rules out that results in group A and B were artifactual and related to organ deterioration. The lack of correlation of $\dot{V}_U$, $\dot{V}_{RV}$ and perfusion pressure with U/P_{PAH} and U/P_{Cr} makes it improbable that the latter reflect mere pressure diuresis (50).

The lack of U/P gradients of sodium and osmolality are quite consistent with this interpretation and show that reabsorbtion was isotonic, i.e. taking place in the proximal tubule. PAH secretion is also known to be limited to the proximal tubule. In fact there was no evidence that the (distal) countercurrent multiplier mechanism was in operation. Urine was isotonic and free water clearance remained essentially zero at all temperatures. These findings also corroborate previous published data (10,36,63). It is therefore also not surprising that Furosemide had not effect on U/P_{PAH} and U/P_{Cr} because this drug's action is limited to the distal nephron (9).

Evidence for renal damage

While the observations discussed in the foregoing chapter

furnish strong evidence for renal function in groups A and B, there was on the other hand also evidence that these kidneys were damaged.

The potassium diuresis probably reflected intracellular fluid leakage from damaged tubular lining cells (10,36). Such potassium leakage has also been noted in damaged or aged erythrocytes and represents a dissipation of intracellular/extracellular electrolyte gradients due to a lack of ATP (70). It follows from Table I that even in the presence of some hemoglobin the oxygen balance becomes negative between 8° C and 38° C at a point which escapes more precise definition, But there is, in addition, a question if hemoglobin carried oxygen can be released in hypothermia and in the absence of CO_2, due to a left shift of the oxygen dissociation curve under these conditions (14). Lack of 2,3 diphosphoglycerate in stored blood, and the aquous solution of hemoglobin, promote this left shift.

In a consideration of nutritional balance the question of fuel supply should also be raised. Our perfusate contained 100 mg/ 100 ml of Dextrose but no insulin and may therefore have been inadequate.

All kidneys gained weight in standard experiments and none of the additional studies succeeded in abolishing this weight gain with one exception: with 6% Dextran 70,000 in isotonic sodium chloride there was actually a weight loss, and no urine formation. It is possible that this hypertonic perfusate not only dehydrates the kidney but "sucked back" all filtrate. This is an interesting observation in itself and may have some bearing on the understanding of sodium diuresis in volume expanded dogs, a phenomenon not

mediated by changes in GFR or mineralocorticoid hormone activity (24).

RPF was low in comparison to a hypothetical normal dog. This may have been related to an increased viscosity of the perfusate at low temperatures or to a raised vascular resistance due to tissue swelling or experimental trauma (41). Since RPF did not significantly rise on warming it is probable that a raised vascular resistance accounted for its low values.

GFR was even more suppressed than RPF, as indicated by the very low FF. It seems reasonable to assume that C_{Cr} was an approximate measure of GFR because only abnormal reabsorbtion of creatinine by tubular lining cells could have led to a false low GFR and this is an unlikely event. The main pressure gradient in a normal kidney is post-glomerular (54). It is probable that a rise of pre-glomerular resistance (due to tissue swelling?), or pre-glomerular arterio-venous anastomosis were responsible for the disproportionately strong suppression of GFR. This interpretation agrees with the findings of Winton (11) and of Bartha (4) although their experimental protocols were not strictly comparable. As a result, the filtered load of sodium must have been markedly lowered. This observation may be of the greatest significance insofar as it represents a most efficient relief of active tubular work and thus acts as an important contribution to suppression of oxygen demand and metabolism in these kidneys. It is comparable to, but much more efficient than, efforts to

protect the kidney during hypoxia by the prophylactic use of sodium transport blocking drugs (49,64).

The occasionally noted drop in urine flow at the onset of an experiment may in part relate to the weight gain of these kidneys with an increase in tissue tension and vascular resistance. The following rise of urine flow may represent a different mechanism. This is opposite to Bickford and Winton's observations (11) and to the concomitantly rising U/P gradients which would lead one to expect a lesser flow as temperature rises and urine becomes more concentrated. If the rise in urine flow at this phase reflected a decreased viscosity it should have been reversable on dropping temperatures from 38 to 18° C which it was not in all instances. In the presence of a considerable suppression of tubular reabsorbtion it can be assumed that urine flow reflects to a large measure GFR.

E_{PAH} may be determined by at least five variables: species (in dogs lower than in man), blood redistribution within renal cortex and medulla, arterio-venous anastomosis, filtration fraction and suppressed active secretion. The last one of these presumably offers the best explanation for the very low E_{PAH} in all experiments with the former four contributing to some additional extent. Even at a low P_{PAH} arterio-venous PAH differences remain very small under such circumstances. This introduces a certain error into the calculation of E_{PAH}, and into a calculation of RPF by the Fick principle (C_{PAH}/E_{PAH}). The somewhat erratic extraction

ratios of PAH noted in our experiments were probably related to this error. Most certainly E_{PAH} suppression of such magnitude precludes the otherwise valid assumption that C_{PAH} is a reasonable estimate of RPF. Nevertheless this has been done by some authors (76).

Conclusions

It is concluded that this study has revealed data in evidence of cellular function in the isolated bloodless perfused dog kidney. This has been documented for the first time utilizing clearance techniques. It is reasonable to suppose that the demonstration of such function is an estimate of organ viability but this point requires confirmation by showing a correlation with post-reimplantation survival. If successful, a simple viability test of transplant kidneys is conceivable using e.g. the U/P ratio of ^{131}Iodine hippuran after rewarming to room temperature and immediately prior to reimplantation.

Under the experimental conditions of this study a marked fall of GFR, and of tubular sodium load, was regularly noted. This was out of proportion to the fall of RPF. It is proposed that this inadvertent phenomenon represents a most efficient way to suppress cellular metabolism and is second only to cooling in facilitating organ preservation. The role of pharmacological metabolic suppressants in this context is probably of a lesser order of magnitude (45). These considerations may well apply to

human organ preservation if one considers that the experimental protocol was designed to mimic going surgical practices.

Concurrent evidence also for cellular damage even during short term experiments such as ours may lead the way to rational improvements of existing organ preservation techniques.

SUMMARY

Isolated kidneys of mongrel dogs were subjected to pulsed perfusion with a balanced salt solution using a pump cooling unit, at a constant pressure of 100/60 mm Hg and at 8, 18, 28 and 38 and 18° C, 30 min each and in that sequence. Kidneys were studied following ischemia (group C), without preceding ischemia (group A) and with addition of hemoglobin to the perfusate (group B).

Urine/perfusate ratios of PAH and creatinine rose with temperature and in group A reached a peak mean value at 28° C of 300 ± 67% and 153 ± 17% (mean ± 1 S.D.), respectively. These values were even higher in group B but were at unity in group C. They are believed to reflect active proximal tubular function. Urine remained essentially isotonic in respect to sodium and osmolality in all three groups and at all temperatures and Furosemide had no effect on existing PAH and creatinine gradients. These findings failed to demonstrate any distal tubular function.

Renal perfusate flow was low but stable at all temperatures and in group A measured 20 to 25 ml/min/100 g. It was more than twice that high in group B but much lower in group C. Its degree of suppression reflected a raised intrarenal resistance.

Creatinine clearance was taken as a measure of glomerular filtration rate and was suppressed out of proportion to RPF, presumably due to a high pre-glomerular vascular resistance or pre-glomerular arterio-venous anastomosis. Whatever its cause, the

resultant drop of filtered load of sodium is proposed to represent a most effective contribution to the suppression of tubular metabolic work and to successful isolated kidney preservation.

Urine was formed in all experiments of group A, B and C but not if Dextran was added to the perfusate. In group A it rose from 0.6 ± 0.5 at 18° C to 1.9 ± 1.4 ml/min per 100 g at 38° C and was higher in group B and lower in group C.

The average weight gain of these kidneys during perfusion amounted to 11.4 ± 4.2%. Together with a potassium diuresis and a markedly suppressed PAH extraction this was taken to reflect renal cellular damage due to hypoxia.

Histological examination of the kidneys showed interstitial edema in group A but not if the perfusate contained hemoglobin (group B) or Dextran. There was nothing that could have explained the lack of urine formation in Dextran containing perfusate. The ischemic kidney (group C) showed evidence of beginning cellular autolysis.

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Temp.	α	Supply			Demand			Balance
		Dissolved[a]	in Hb[b]	Total[c]	Basic[d]	T_{Na}[e]	Total[f]	
8°C	0.03983	3.7	2.0	2.9	0	0.1	0.1	+2.8
18°C	0.03220	3.0	2.0	2.5				
28°C	0.02691	2.5	2.0	2.3				
38°C	0.02350	2.2	2.0	2.1	2.2	0.9	3.1	-1.0

TABLE I. Estimate, in round figures, of oxygen balance under the prevailing experimental conditions. Even if one assumes that the total oxygen content of the perfusate is actually made available there is a deficit at 38°C, whereas the balance is positive at 8°C.

a) Oxygen dissolved in perfusate equilibrated with 100% oxygen gas as $pO_2 \times \alpha/760$ (ml/100 ml).

b) Oxygen bound to 1.5 g of perfusate hemoglobin as 1.34 x 1.5 (ml/100 ml).

c) Total oxygen delivery by perfusate B assuming RPF of 50 ml/min/100 g ((a+b)/2).

d) Basic oxygen consumption of 100 g dog kidney (no glomerular filtration)(14)(ml/min/100 g).

e) Oxygen demand to reabsorb 0.1 (8°C) and 0.9 (38°C) mEq Na/min/100 g assuming that 1 mM (22.4 ml) O_2 reabsorbs 26 mEq Na.

f) (d + e).

	Tis-U-Sol	Perfusate A,C	Perfusate B
Sodium (mEq/l)	138	148 ± 9	165 ± 15
Potassium (mEq/l)	5.8	5.8 ± 0.1	8.3 ± 0.6
Magnesium (mEq/l)	1.6	not measured again in final perfusates.	
Phosphate (mEq/l)	1.1		
Chloride (mEq/l)	142.3		
Sulfate (mEq/l)	1.6		
Dextrose (mg/100 ml)	100.0		
Osmolality (mOsm/l)	273	286 ± 13	305 ± 8
Creatinine (mg/100 ml)	-	10.2 ± 0.6	6.3 ± 0.5
PAH (mg/100 ml)	-	2.0 ± 0.2	2.2 ± 0.2
pH	6.0	7.43 ± 0.09	7.48 ± 0.03
Hb(g/100 ml)	-	-	1.5 ± 0.4

TABLE II showing composition of original Tis-U-Sol (Baxter Co.), of perfusate A,C (N=6) and of perfusate B (N = 4) as mean ± 1 S.D. Except for hemoglobin, differences in solute concentrations between perfusate A,C and B were inadvertent.

	Group	8°C	18° C	28°C	38°C	18°C
V_U (ml/min/100g)	A	1.4 ± 0.8	0.6 ± 0.5	0.9 ± 0.9	1.9 ± 1.4	1.9 ± 1.0
	B	1.1 ± 0.7	2.2 ± 1.2	2.8 ± 2.1	3.2 ± 0.7	2.6 ± 2.7
	C	<0.1	0.1	<0.1		
V_{RV} (ml/min/100g)	A	24 ± 8	22 ± 14	22 ± 13	23 ± 13	18 ± 10
	B	67 ± 37	84 ± 41	106 ± 54	74 ± 3	81 ± 54
	C	12 ± 2	13 ± 1	14		
U_{PAH}/P_{PAH} (%)	A	149 ± 13	220 ± 34	300 ± 67	260 ± 57	156 ± 24
	B	126 ± 27	370 ± 128	1072 ± 1053	222 ± 37	162 ± 61
	C	104 ± 10	99 ± 13	98		
U_{Cr}/P_{Cr} (%)	A	113 ± 9	132 ± 18	153 ± 17	139 ± 11	119 ± 9
	B	171 ± 10	216 ± 37	308 ± 118	189 ± 14	136 ± 86
	C	92 ± 8	101 ± 1	91		
U_K/P_K (%)	A	143 ± 55	158 ± 35	166 ± 43	157 ± 47	144 ± 60
	B	121 ± 7	201 ± 63	204 ± 90	123 ± 13	121 ± 24
	C	101 ± 1	100 ± 0	100		
U_{Na}/P_{Na} (%)	A	102 ± 1	103 ± 3	102 ± 2	99 ± 2	100 ± 1
	B	98 ± 3	100 ± 5	109 ± 1	104 ± 3	102 ± 5
	C	95 ± 4	97 ± 3	98		
U_{osm}/P_{osm} (%)	A	103 ± 1	104 ± 2	104 ± 1	101 ± 3	102 ± 2
	B	104 ± 5	105 ± 2	110 ± 5	104 ± 1	102 ± 1
	C	103 ± 1	106 ± 5	104		

TABLE III showing results of primary parameters of function in the isolated perfused dog kidney as mean ± 1 S.D. As this was a "single pass" perfusion, P is a constant for a given solute and U/P therefore a linear expresion of U. N(A) = 4, N(B = 4, N(C) = 2.

	Group	8°C	18°C	28°C	38°C	18°C
C_{Cr} (ml/min/100g)	A	1.5 ± 0.8	0.9 ± 0.8	1.5 ± 1.5	2.8 ± 2.4	2.2 ± 1.3
	B	1.9 ± 1.2	4.8 ± 2.5	8.9 ± 7.4	6.0 ± 1.7	5.1 ± 5.9
	C	<0.1	0.1	<0.1		
RPF (ml/min/100g)	A	25 ± 9	23 ± 15	23 ± 14	25 ± 14	20 ± 10
	B	68 ± 37	87 ± 42	109 ± 56	77 ± 3	84 ± 57
	C	12 ± 2	13 ± 1	14		
FF (%)	A	6 ± 1	4 ± 2	6 ± 5	10 ± 7	12 ± 8
	B	3 ± 1	6 ± 3	7 ± 3	8 ± 2	5 ± 3
	C	<1	<1	<1		
E_{PAH} (%)	A	4 ± 2	10 ± 3	9 ± 3	6 ± 6	4 ± 3
	B	8 ± 8	15 ± 6	24 ± 10	12 ± 2	14 ± 5
	C	4 ± 3	3 ± 1	5		
$T^{C}_{H_2O}$ (ml/min/100g)	A	.03 ± .01	.03 ± .03	.03 ± .03	.01 ± .04	.02 ± .02
	B	.05 ± .09	.12 ± .08	.27 ± .19	.13 ± .03	.06 ± .06
	C	<.01	<.01	<.01		
T_{Na} (mEq/min/100g)	A	.011± .005	.035± .042	.082± .096	.139± .128	.051± .044
	B	.139± .100	.429± .245	.991± .964	.436± .176	.451± .591
	C	.000	.000	.000		

TABLE IV showing results of derived parameters of function in the isolated perfused dog kidney as mean ± 1 S.D.

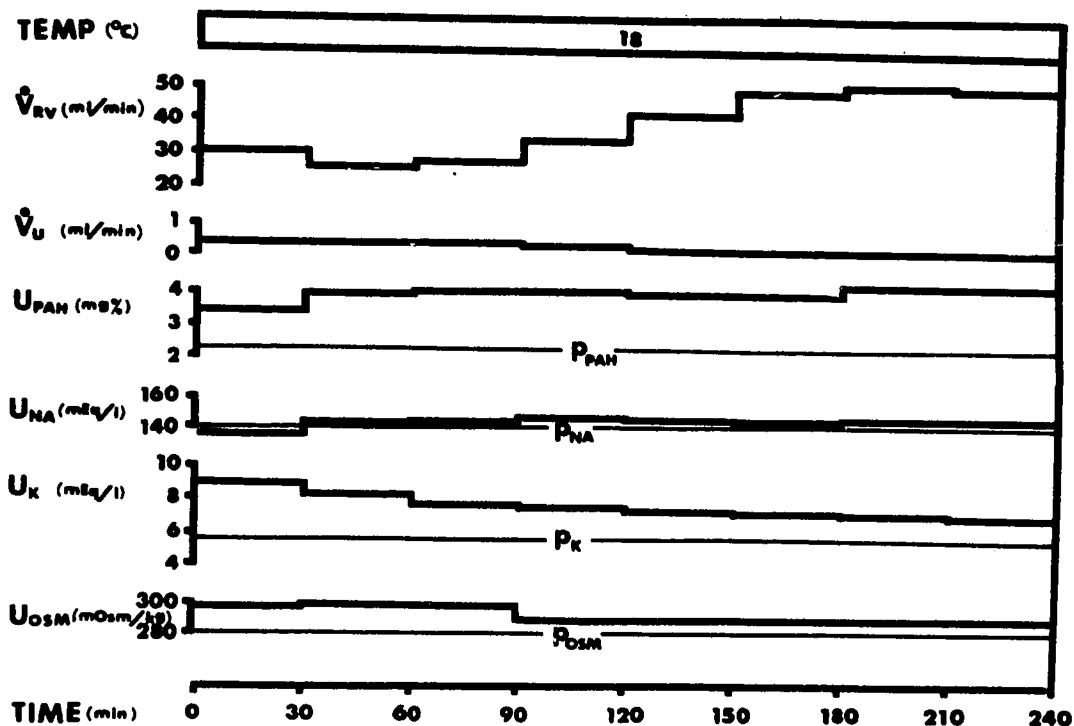

Figure 1 showing perfusion of one dog kidney with perfusate A for 4 hours at a constant temperature of 18° C to show that the preparation is stable during this time interval.

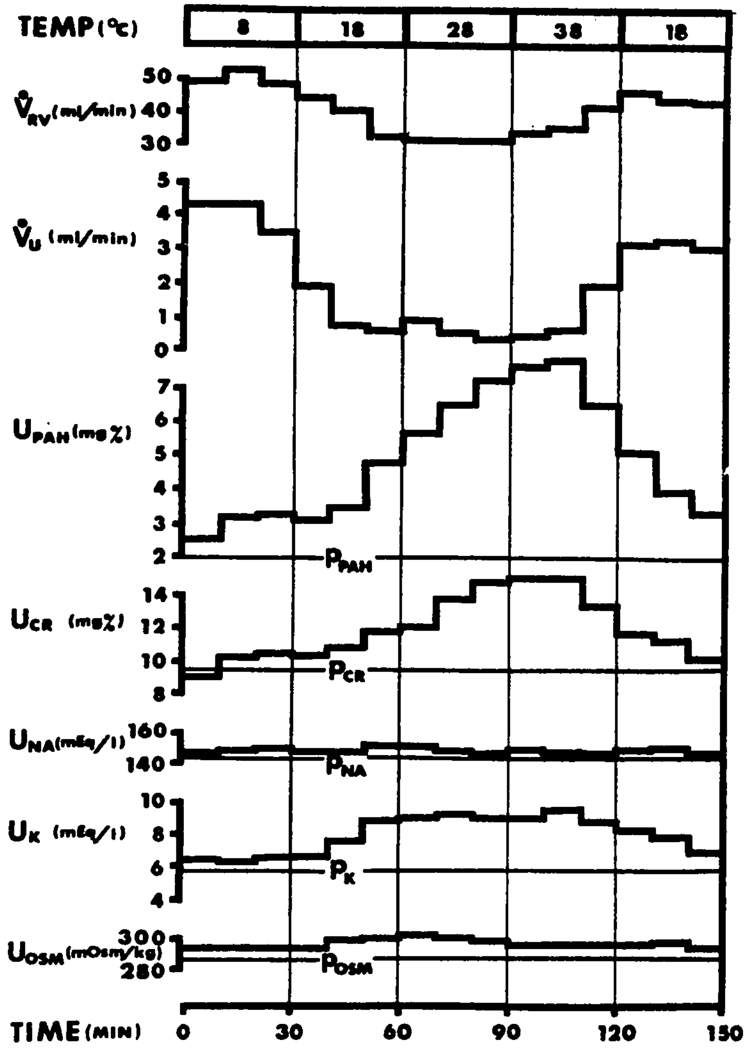

Figure 2 showing detail of a representative experiment, group A (dog kidney perfused with a balanced salt solution immediately after removal).

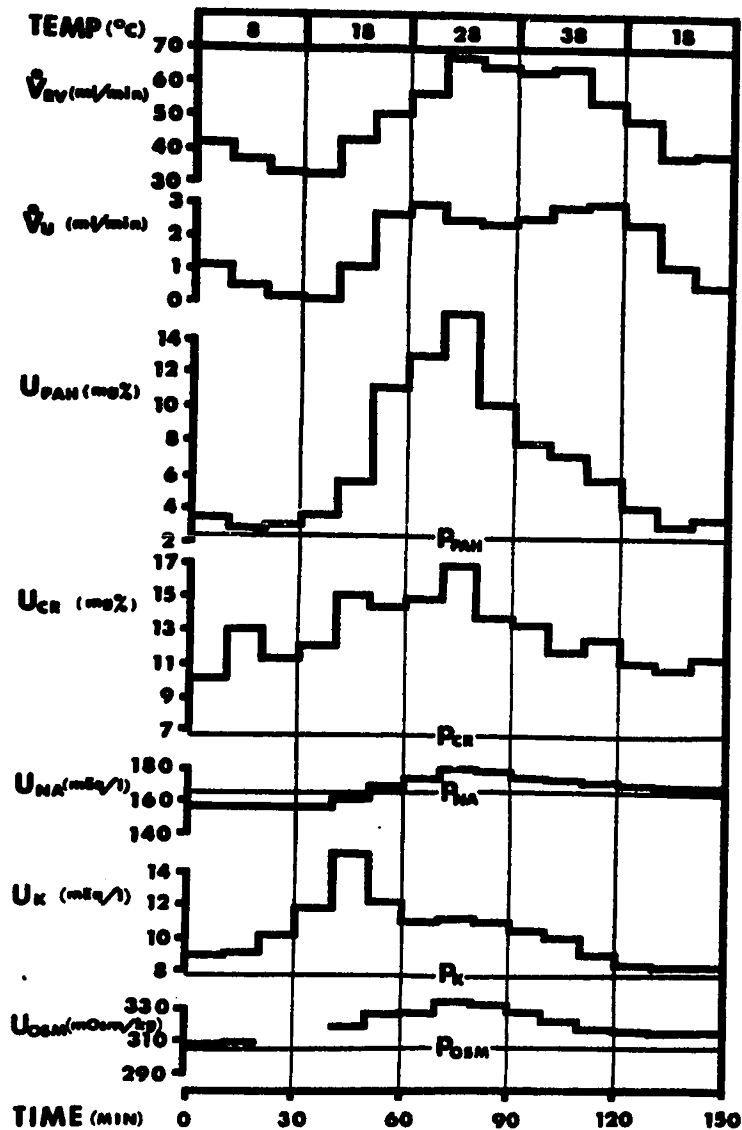

Figure 3 showing detail of a representative experiment, group B, (hemoglobin added to standard perfusate). Note that U_{PAH} rose to 7-fold P_{PAH}. U_{PAH} is therefore shown at one-half scale as compared to all other figures.

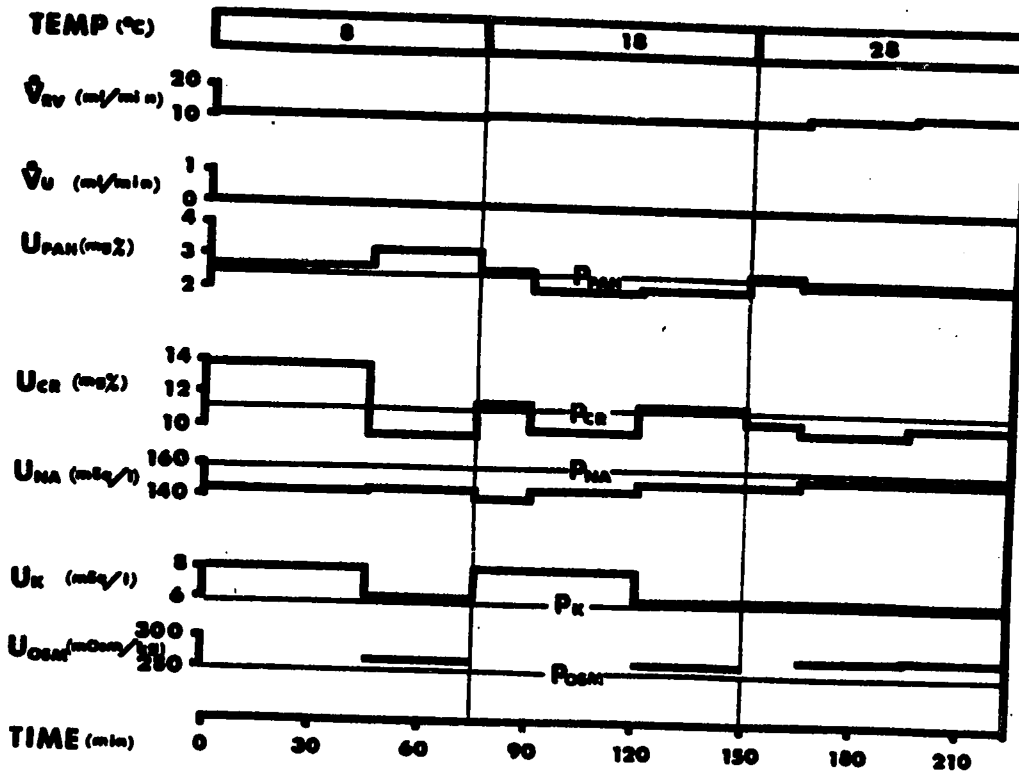

Figure 4 showing detail of a representative experiment, group C, (perfusion after 1 hour of incubation at 38° C). Urine concentration of PAH and creatinine are somewhat erratic and are not consistently different from perfusate concentrations.

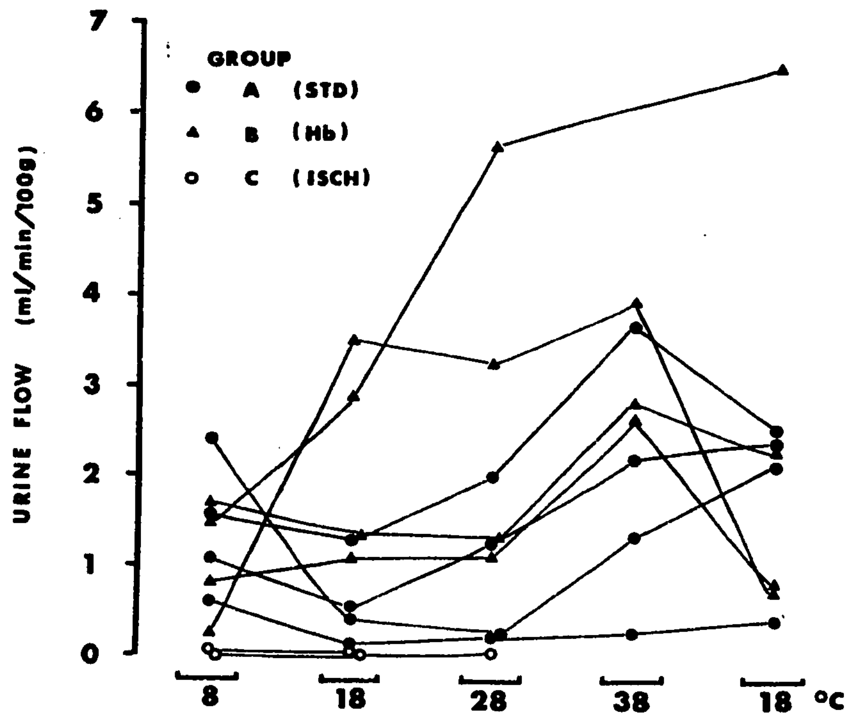

Figure 5 showing urine flowrates of all experiments, group A, B and C with changing temperature.

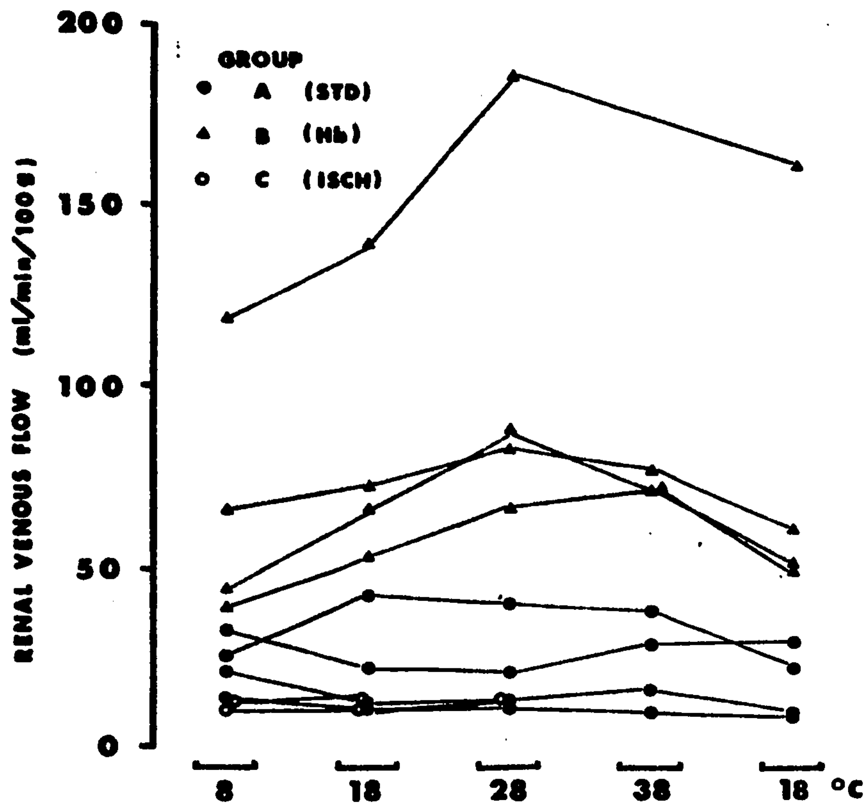

Figure 6 showing renal venous flowrate of all experiments, group A, B and C, with changing temperature.

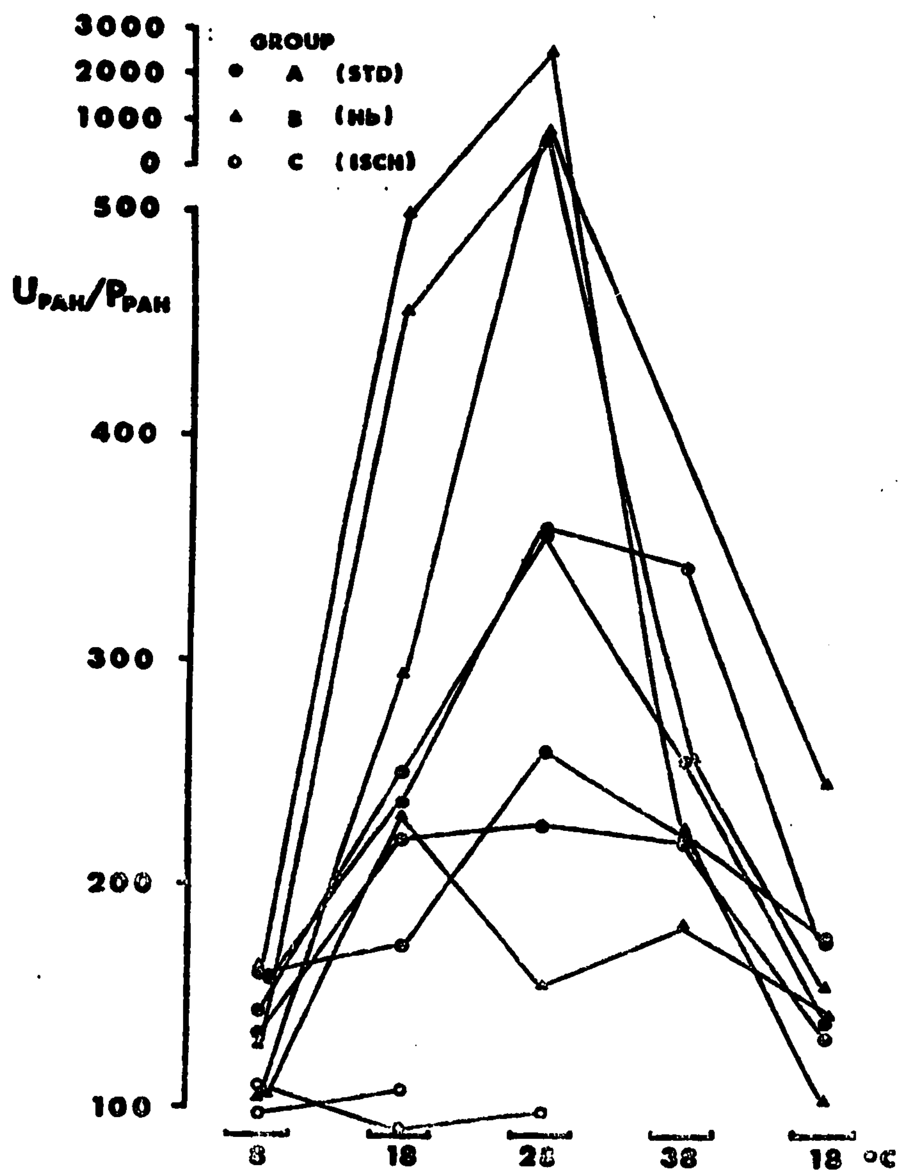

Figure 7 showing urine/plasma concentration ratios of PAH (in %) of all experiments, Group A, B and C,with changing temperature.

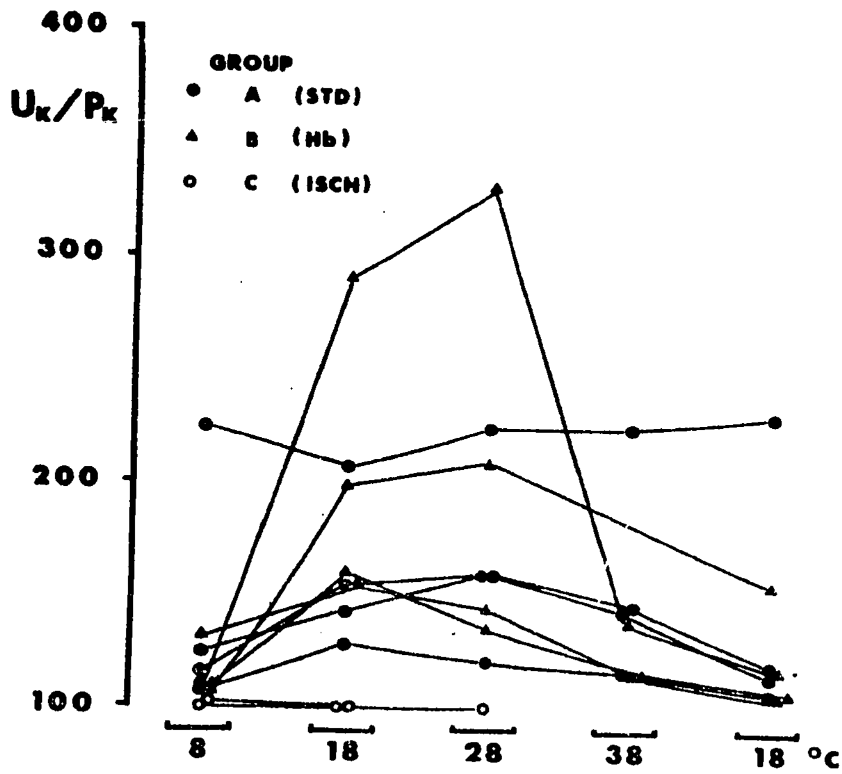

Figure 8 showing urine/plasma concentration ratios of potassium (in %) of all experiments, Group A, B and C, with changing temperature.

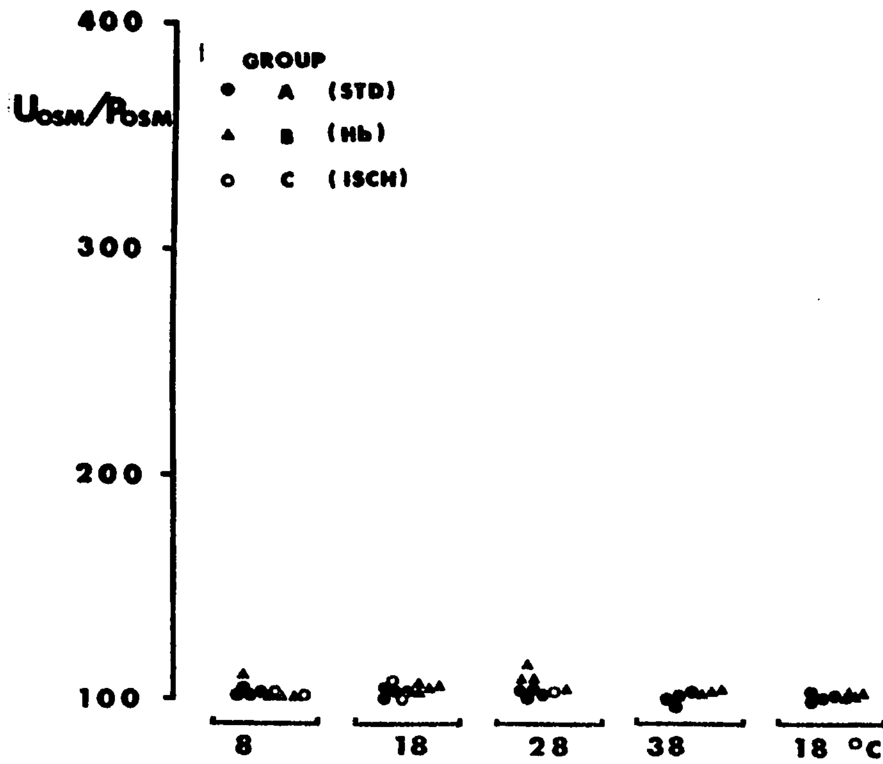

Figure 9 showing urine/plasma concentration ratios of osmolalities (in %) of all experiments, group A, B and C, with changing temperature.

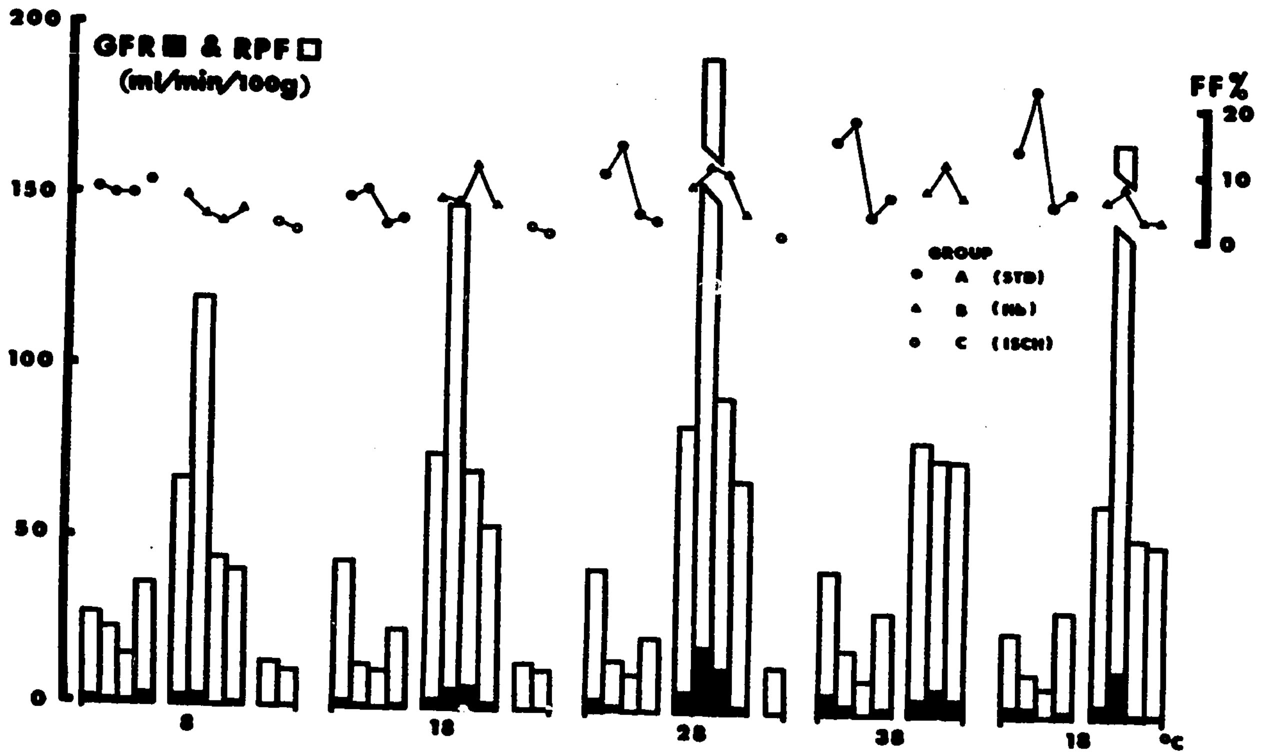

Figure 10 showing creatinine clearance (for glomerular filtration rate), renal perfusate flow and filtration fraction of all experiments, group A, B and C, with changing temperatures.

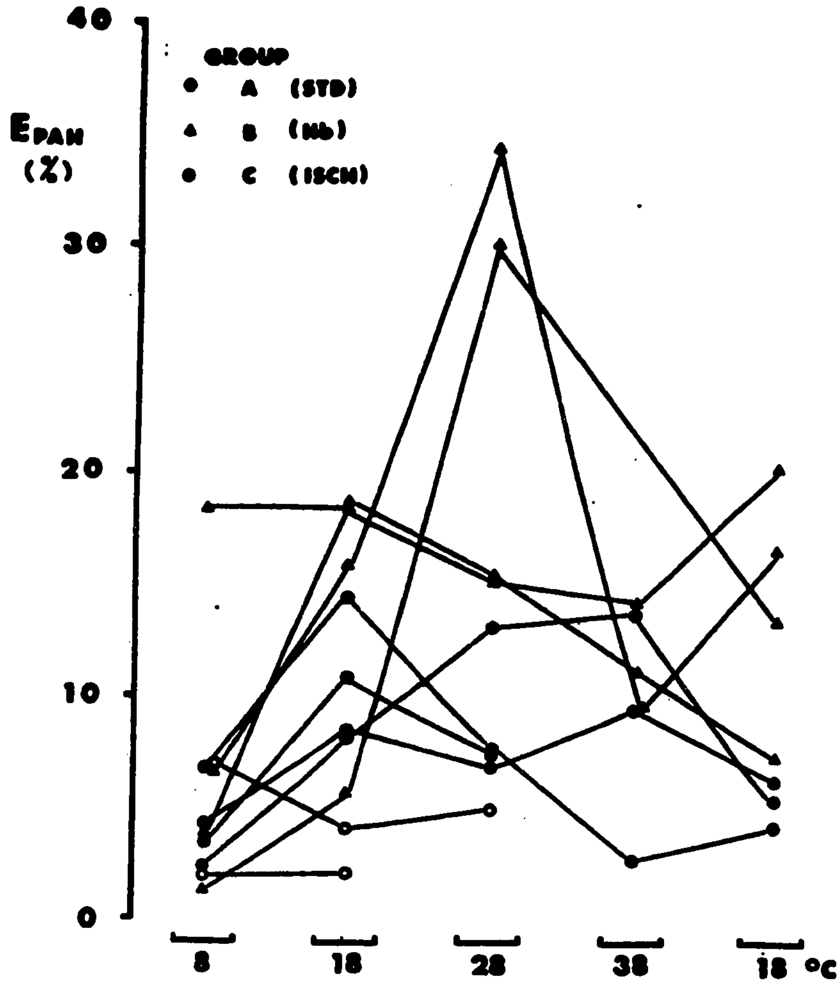

Figure 11 showing extraction of PAH of all experiments, group A, B and C, with changing temperatures.

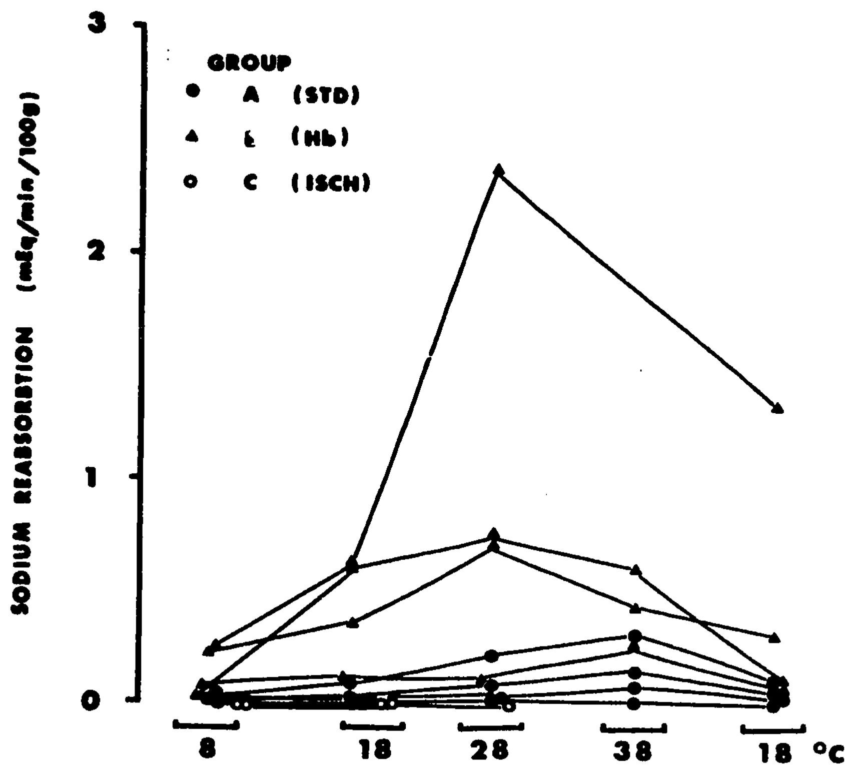

Figure 12 showing sodium reabsorbtion of all experiments, group A, B and C, with changing temperatures.

Figure 13 showing light microscopic appearance of kidney in group A, (hematoxylin, phloxine and saffron stain, x 450). Note interestitial edema. Glomerular tufts and tubular lining cells are remarkably intact.

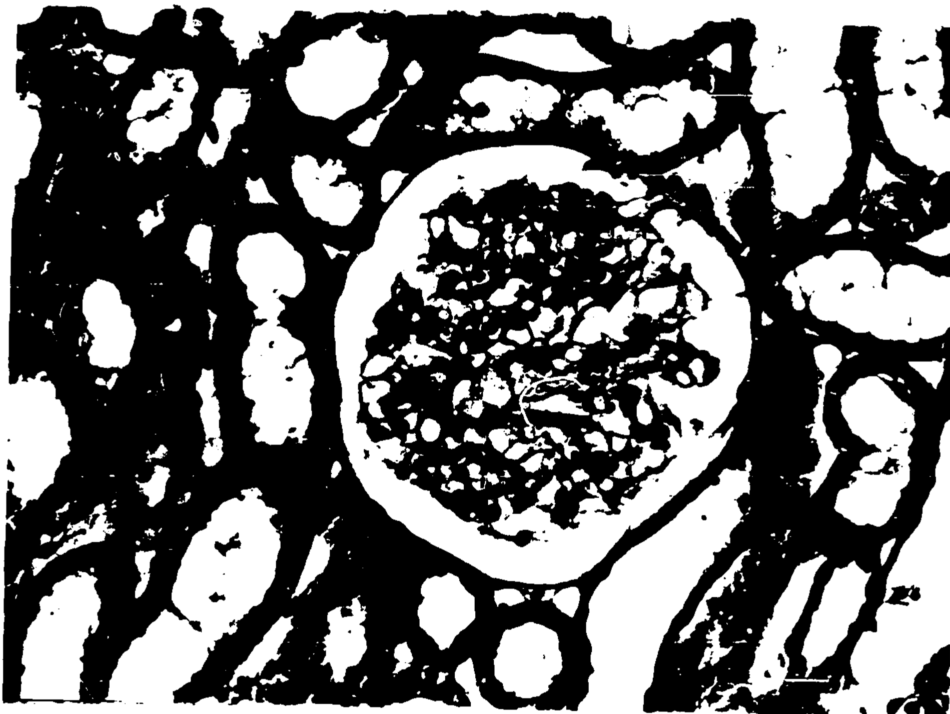

Figure 14 showing light microscopic appearance of kidney in group B (hematoxylin, phloxine and saffron stain x 450) there are no apparent pathological changes.

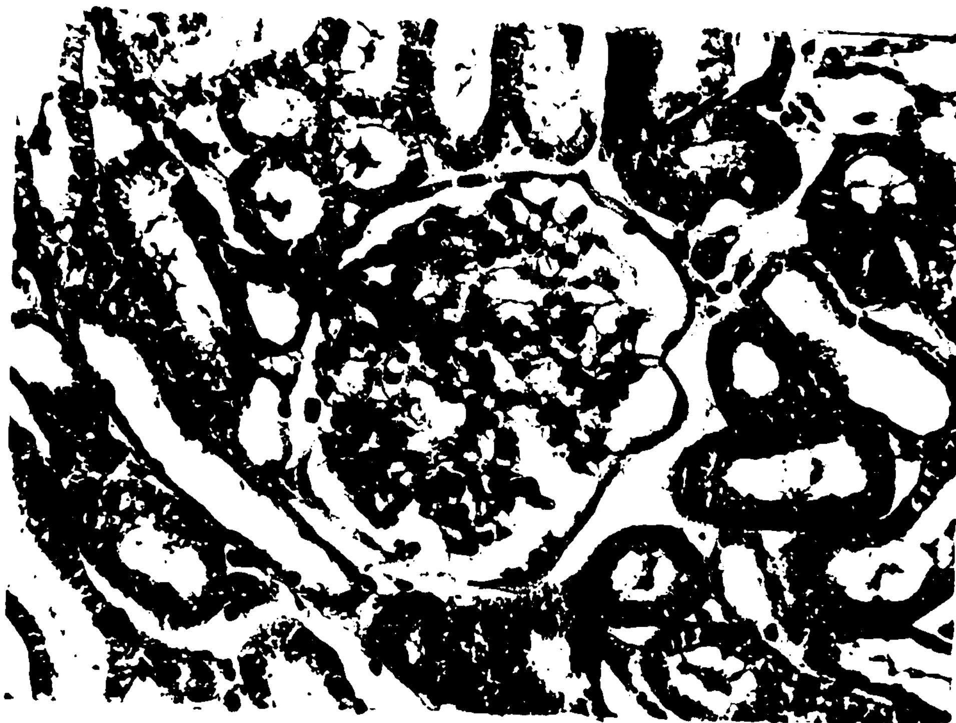

Figure 15 showing light microscopic appearance of kidney in group C (hematoxylin, phloxine and saffron stain x 450). Note mild interstitial edema and beginning necrobiotic changes of tubular lining cells.

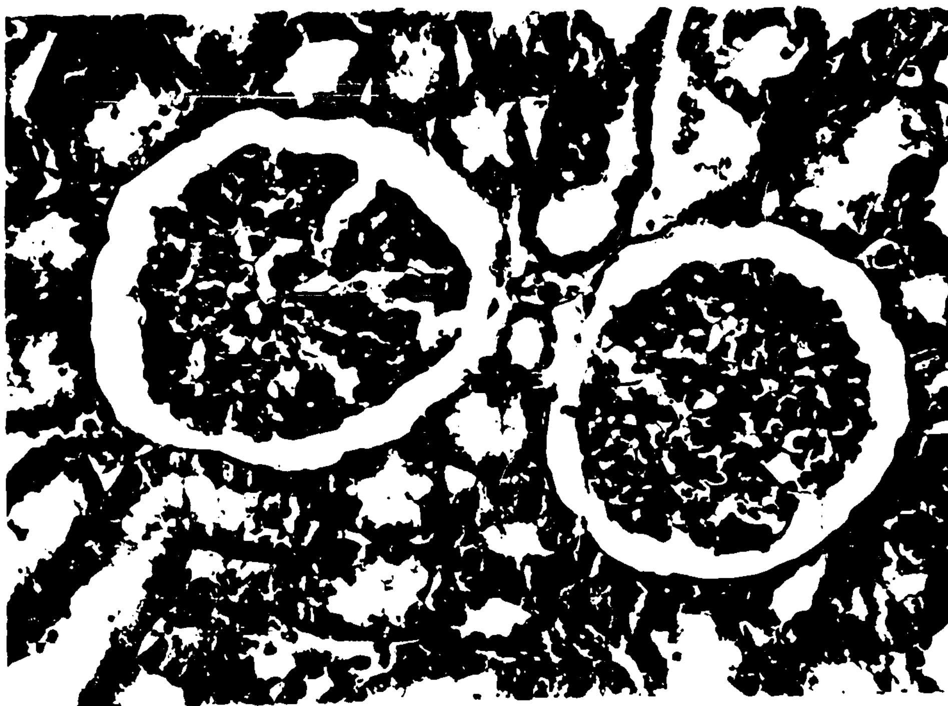

Figure 16 showing light microscopic appearance of a control kidney. A section of this kidney was put into formalin immediately after nephrectomy.