Hypothermic-Hyperbaric Oxygen Preservation of Kidneys in Dogs

"For they (diving bell) can give respiration to divers equally by letting down a bucket for this does not fill with water, but retains its air".

From Problemata by

Aristotle

" Simple curiosité opératoire aujourd'hui, la transplantation d'une glande pourra peut-etre un jour avoir un certain interet pratique".

Alexis Carrel

1902

" From the greater strength and vivacity of the flame of a candle in this pure air, it may be conjectured, that it might be peculiarly salutary to the lungs in certain morbid cases... But perhaps, we may also infer from these experiments, that though pure dephlogisticated air might be useful as a medicine, it might not be so proper for us in the usual healthy state of the body; for as a candle burns out much faster in dephlogisticated than in common air, so we might, as may be said, live out too fast and the animal powers be too soon exhausted in this pure kind of air; but I fancied that my breath felt peculiarly light and easy for some time afterwards. Who can tell but that, in time, this pure air may become a fashionable article of luxury. Hitherto only two mice and myself have had the privilege of breathing it".

J. Priestly

1775

The Use of Hypothermia and Hyperbaric Oxygen in the Preservation of Kidneys in Dogs

A

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Preface

The early phase of this work commenced in July 1963 at the Tufts Surgical Laboratories, Boston City Hospital, Boston, Massachusetts, U.S.A., under the supervision of Drs. D.C. Nabseth and R. Deterling, Jr.

The initial exploration of the feasibility of using hypothermia combined with hyperbaric oxygen in the preservation of dog kidneys was tried at 3 and 5 atmospheres absolute. The result of this study had been published.

A corollary investigation of the circulatory behaviour of immediately removed and preserved kidneys was carried out as well at that institution.

The remainder and main part of the project was completed at the Surgical Laboratories of the Royal Victoria Hospital, McGill University, Montreal, Canada, under the guidance, advice and support of Dr. Lloyd D. MacLean.

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Dedicated to my wife, children

and parents

List of Tables, Page

List of Figures Page

Contents

III. Experimental Design and Methods

I. Introduction

The limited frequency distribution of genetically compatible donor-recipient combination in the natural population added to the uncertainty in assessing the possible adverse effects occasioned by the loss of one kidney to the donor preclude the general use of living donors as the main sources of kidneys in the treatment of irreversible kidney disease.

The gratifying results of allografts from cadavers reported in recent years (64a, 143a, 166a, 244a, 305a) make organ preservation technique an imperative armamentarium in transplantation, to complement the efforts to modify the homograft response.

Permanent impairment of organ function due to ischemia can occur at the time of transplantation of kidneys from living donors and especially when cadaver donors are used.

Hypothermia alone is effective for short-term preservation.

Boerema, Illingworth and others have suggested that oxygen at elevated pressures might have an ameliorating effect on certain oxygen-deficient clinical states. In this study, a comparison was made of the value of hypothermia alone or combined with hyperbaric oxygen in the preservation of dog kidneys.

An attempt was made to develop a simple technique for organ preservation which would permit salvage of organs that

could be transported to a transplantation center for clinical use.

II. HISTORICAL PERSPECTIVE

A. TRANSPLANTATION

1. Evolution of technique. The first successful attempt at transplanting a kidney in toto was made by Ullmann in 1902 (323). This achievement culminated innumerable failures at transplanting hog's kidney, on account of its thin renal vessels which were torn very easily. Immediately prior to this initial success, several canine renal autografts were tried in the groin which uniformly ended fatally because of the ease with which the dog soiled, licked and subsequently infected the wound.

The successful autograft was grafted in the region of the neck connecting the renal artery and vein to the common carotid artery and external jugular vein respectively by means of Payr's prostheses (magnesium tubes-257, 1900; 258, 1904). Following this rather quick inoscultation, the kidney was buried amongst the strap muscles and the ureter was sutured to the skin. Clear urine apparently issued from the ureter almost immediately following re-establishment of circulation. The animal appeared normal in every way up until the time of sacrifice several weeks later. Although no detailed examination of urine was done, it was thought that it was normal. The implication he drew from this achievement was that the kidney or any other organ could be successfully grafted in any convenient segment of the circulatory system with preservation of its function. Three months later, (324) he tried

to transplant kidneys between two dogs (allograft) and between the dog and goat (xenograft) without evident success understandably. Gross areas of necrosis were noted in the transplanted kidney but neither the detailed urinalysis nor the length of survival of the animal was reported.

In the same year, Carrel (67) performed several kidney transplantation in dogs to the cervical region uniting the renal artery and vein end-to-end to the common carotid artery and external jugular vein respectively with a continuous fine circular suture that he introduced and popularized, ingeniously and skillfully employing three guy sutures equidistant with each other along the entire circumference of the vessels to be sutured, to facilitate approximation. This method turned out to be the answer to the undesirability of Payr's prosthesis and the use of other heat-soluble and therefore blood-soluble substance like caramel which he and others tried as a means of vascular anastomosis, without encouraging outcome. The ureter was sutured to the skin just above the suprasternal notch. Urine was noted shortly after the re-establishment of circulation. No urinalysis was made. Septic complications invariably occurred in every case and no long-term survivors were reported. Although he transplanted the thyroid gland, pancreas and the ovary, no definite report was made on the results. From this significant milestone, a prophetic passage flowed from his prolific pen to summarized and immortalize its significance for generations to come, thus: "Simple curiosité opératoire aujourd'hui, la transplantation d'une glande pourra peut-etre un jour avoir un certain interet pratique".

It took slightly over a half century later to fulfill this prognostication. It was only after a reliable technique of vascular anastomosis was established that whole organ transplantation was placed on a firm scientific basis. Indeed Carrel's technique of continuous anastomosis produced a more consistent result and inevitably paved the way to our greater understanding of the behaviour of renal autografts in particular and transplantation in general. Pari-pasu, Decastelo (102) transplanted the kidney from one dog to the site of a previously extirpated kidney of a recipient animal using Payr's prosthesis for vascular anastomosis. The host lived for 40 hours and died due to fatal hemorrhage from a separated venous anastomosis. While alive, the dog reportedly secreted copious amount of urine containing a great deal of albumin and casts.

In 1903, Carl Beck of Chicago (20), tried renal transplantation employing Murphy's method (245, 1896 - invagination of the proximal end into the distal end) of anastomosing blood vessels. No results were published.

Floresco (130) in 1905, reported a work that he started three year previously in which he transplanted canine kidneys first in the groin and then in the region of the neck. Both experimental models terminated in necrosis of the autografts. Later, he tried in-situ replantation with section of the nerves and lymphatics of the renal pedicle and contralateral nephrectomy without ill-effects on the function of the autograft. He used interrupted sutures of fine silk for vascular anastomosis. Either uretero-cutaneous, ureteroureteral or uretero-vesical anastomosis was utilized for urinary

drainage. From this experience, he concluded that the abdominal location was the only suitable place for renal transplantation. Denervation and section of the lymphatic drainage did not adversely affect kidney function even after contralateral nephrectomy.

The first decade of the century was a very productive one for Carrel. He concluded experiments on transplantation on various organs with very interesting results (67 - 81). In collaboration with Guthrie, he used two methods of transplantation, namely; (1) simple transplantation and (2) transplantation en masse. In the former method, they transferred individual organs. While the immediate results, were satisfactory in most cases, the late results were usually disappointing. They thought that the cause of the failures was technical in nature and primarily due to imperfections at the line of anastomosis. So to obviate this problem, they tried patching technique (72 - 74) of vascular anastomosis and later employed transplantation en masse (76).

In one experiment along the lines of simple transplantation with clinical application (68), they removed the thyroid gland of a dog and replaced it in-situ with the peripheral end of the superior thyroid vein anastomosed to the central portion of the superior thyroid artery and the distal superior thyroid artery connected to the proximal segment of the extremity of the superior thyroid vein, thus reversing the circulation. Eleven days after replantation, the wound was opened and the gland was found to be somewhat enlarged but its color and consistency were normal. Twenty-five days later,

apparently no observable change occurred but no histologic section was reported however. This observation was applied in dogs with goiter and they claimed that not only was the gland reduced in size but the hyperthyroid state subsided as well. They ascribed these effects to augmentation of the circulation. They also transplanted the heart, a loop of intestine, the kidney, the thigh, the head and other organs, to the cervical region (75). For some reason or other, the kidney was the only organ that was successfully autografted and one such kidney was replanted into the neck with the renal artery joined to the common carotid artery end-to-end and the renal vein end-to-end to the external jugular vein using Carrel's technique of continuous suture anastomosis. The ureter was sutured to the esophagus for dependent drainage. The cervical and abdominal wounds were opened three days post-transplantation for inspection. The transplanted kidney was somewhat enlarged, the color darker and the arterial pulsations stronger but the consistency seemed normal. The urine formation of the transplanted kidney was five times more than the normal kidney and increased further on intravenous infusion of physiological sodium chloride solution which did not alter the rate of urine flow of the control kidney. The chloride was noted to be increased in the transplanted organ while the organic sulfates, pigments and urea were more abundant in the urine from normal organ. He concluded from this experiment that the function of the transplanted kidney need not be different from the normal. However, he thought

that the neck region was an unfavorable location because of several factors as; the difference in blood pressures, the ease with which the renal vein can be compressed and disturbed due to the mobility of the neck. Therefore he gave up the idea of transplanting to the neck and preferred instead the lumbar region.

Most of the immediate technical difficulties were traced to the area of anastomosis, not realizing of course that the failures in allografts were due to rejection phenomenon. Having decided that the lumbar area was the preferable and only site and having incriminated imperfect technique at the area of vascular anastomosis as the cause of subsequent failures in transplants, a different approach was attempted, that of the patching method (72 - 74), whereby large patches of the wall of the aorta and vena cava were excised with the kidney and transplanted to another recipient animal. With this technique, they reported fourteen experiments on kidney transplantation and one of the ovary. A surviving cat supported by an allografted kidney from another cat was claimed to have been well for four months. This method ended up in failure understandably but again it was attributed to faulty technique and they suggested that for studying the functions of a transplanted kidney, transplantation in mass appeared to be an ideal method because it permitted an almost perfect reconstruction of the urinary apparatus, since the renal vessels and ureter were not disturbed. The first transplantation en masse was performed by Carrel et al in the dog (76). Both kidneys including the upper part of the ureter, nerves, ganglia, supporting connective tissue, the supra-

renal glands, the peritoneum and the corresponding segments of the aorta and vena cava were removed. The mass was then placed in a vessel containing isotonic sodium chloride solution and transplanted into a bitch which was similarly prepared interposing the segments of aorta and vena cava. The circulation was re-established after one and a half hours of ischemia. It was claimed that the immediate behaviour of the kidneys in particular and the animal in general were not unlike the simple autografts. Several days later however, the animal took a progressively downhill course and was sacrificed. The features of primary rejection was found which was not evidently appreciated then, including localized peritonitis, intense bowel and fibrous adhesions and marked enlargement of one kidney but the vasculature was seemingly perfect.

Several experiments were attempted later (77), without getting improvement in their results. The animals were thought to have died rapidly from intestinal, peritoneal and ureteral complications (rejection process).

He concluded after several trials at transplanting feline kidneys that the cat was a much better animal to work upon. In a series of 14 allografts in cats, 2 survived for 4 and 5 weeks respectively. The average interruption of blood flow was one hour. He further suggested that the kidney should be irrigated completely of blood to prevent subsequent thrombosis and that the perfusing fluid should be isotonic with the kidney tissues.

It was considered that the duration of the period of ischemia determined to a large extent the fate of the kidney since all tubular cells lost their brush borders in one hour after death. So to slow down autolysis, it was recommended cooling the organ to 1°C. He commented trivially that maybe the host serum was injurious to the new organ.

Stitch (308) in 1907 published a report on iliac transplantation very much like the technique adopted presently. The ureter was grafted into the bladder but the contra-lateral kidney was not removed and therefore the long-term fate of the transplant could not be determined. The circulation remained excellent and histopathologic examination at sacrifice several weeks later showed that the organ was practically normal. It was thought that the iliac region was certainly a very much better site than the cervical region as a recipient site in contrast to Carrel's view that a perfect functional result can be obtained more easily by putting the kidneys in their normal location in the lumbar region.

Watts (331; 1907) and Guthrie (141, 142; 1908), made very pertinent observations in blood vessels surgery. The latter reported some interesting results of an allografted ovary of a chicken from another chicken which survived for at least 7 months, a male cat's kidney transferred to a female cat with the recipient apparently well a year later and a dog's head transplanted to the neck of another dog with preservation of reflex movement and voluntary activities.

Langlois (204, 1908) xenografted kidneys of dogs and cats employing "en masse" technique of Carrel. One such experiment on a dog survived for 81 days.

Canine kidneys were autografted by Villard and Tavernier (330, 1910) to the cervical, femoral and splenic regions using vaselinized silk for continuous anastomosis. The ischemic time averaged one and a half hours. Almost all of the autografted organs sufferred ascending pyelonephritis at sacrifice.

Carrel in 1910 (78, 79) categorically stated that from a surgical standpoint the problem of grafting of organs could be considered as having been solved. At this later stage of his study, when he considered his surgical technique to be excellent, he had long-term survivors up to 23 months and 2 1/2 years respectively without impairment of its function. The procedure consisted in the removal of the kidney followed by perfusion with Locke's solution to remove the blood and replacement into its normal location. Autopsy of one animal that died in a fight with other dogs 10 weeks after contra-lateral nephrectomy showed patent arterial, venous and ureteral anastomoses without modification of their caliber. The kidney itself was grossly normal. The dog that survived for 2 1/2 years bore several litters on 2 occasions and eventually died of an intercurrent disease unrelated to kidney failure. Autopsy of the animal revealed a normal kidney. At no time in these experiments did the period of ischemia exceed 50 minutes. From this one long-term survivor he made a sweeping but nonetheless correct conclu-

sion that the experiments proved definitely that the extirpation of the kidney in the dog, its perfusion with Locke's solution, the complete interruption of its circulation for fifty minutes and the suture of its vessels and ureter did not interfere with its function, even after a long period of time and it indicated that from a purely surgical standpoint, the grafting of organs was a real possibility. Basically the definitive character of this conclusion remained unchallenged even to this day.

Some modifications to this finding are that the severed organ can be replanted into the iliac region with even better results and the autotransplants suffer varying degrees of transient functional impairment immediately following return of circulations.

It is to the credit to these workers notably Carrel and Guthrie that the mechanical and surgical aspects of transplantation were made on a firm basis and opened wide the ever-fascinating field of experimental and clinical organ substitution. Their blood vessel suturing method had insured a higher percentage of mechanically successful operations. Prior to their munomental studies, the methods of blood vessel suture were largely unsatisfactory and to a great extent this explained the poor outcome of previous experiments.

2. Physiological Studies. After a reliable surgical technique of organ transplantation had been established numerous studies were made on the transplanted kidney. Many were mainly of interest from the physiological standpoint.

In 1913, Lobenhopper (214) transplanted the kidney to the splenic region utilizing the splenic vessels for vascular anastomosis followed by contra-lateral nephrectomy. The autografted kidney responded to the various diuretics like sodium chloride, sodium sulfate and dextrose similar to the normal kidney.

Zaaijer (341; 1914) performed autotransplantation to the iliac region of dogs which kept the host alive for 6 days after removal of the opposite kidney.

The left kidney was transplanted to the neck by Dederer in 1918 (103). He reported compensatory activity of the transplanted kidney following excision of the opposite mate two weeks after replantation. The animal lived for more than four months. Before the contralateral nephrectomy, the transplanted kidney was observed to increase its volume of urine secretion and an intravenous phenolsulfonephthalein was mostly cleared by the transplant and none from the normal un-operated side during the first two hours.

Williamson in 1923 (333) working on a similar experimental model established that the rate of excretion was increased and the urine from the auto-transplanted kidney was alkaline. It was concluded that this experimental model could support life for several months with apparently nearly normal function but the animals generally failed because of hydronephrosis due to ureteral stomal stricture and consequently ascending infection and uremia.

This conclusion was confirmed by Ibuka in 1926 (176) who did 'neck' autografted kidneys using Carrel's technique. The ischemic period varied from 25 to 30 minutes. It was maintained that 'neck'

kidneys can sustain a normal life of the host for an extended period of time (4 months) following the removal of the opposite kidney. Like Dederer and Williamson before him, he noted alkaline urine in the beginning which became acid as time passed by. The concentration of chloride was higher in the transplanted kidney while the excretion of nitrogenous products and sulphonephthalein were less in most cases than the unmolested kidney. The dye excretion was delayed as well. However, improvement was noted to parallel with the recovery from the immediate effects of the operation. Following the excision of the opposite kidney, the volume and rate of excretion of the dye were increased to the normal limits for both kidneys indicating physiological compensation. The response of the autograft to injections of phlorizin, sodium salicylate, water, sodium chloride and glucose overloading either by mouth or by intra-venous injection were fairly normal when the transplant was in good condition. The well-functioning transplants have had a practically normal anatomic appearance of the vascular and tubular elements although some scarring may be found in a few areas. On the other hand, the disabled transplants showed hydronephrosis and pyelonephritis, proliferation of connective tissue and destruction of the nephron. Like most neck autografts, they succumbed with the typical features or uremia. He concluded that the excretory and regulatory functions of the transplanted kidney was sufficient to keep them normal as long as the transplant was in full activity and that the extrinsic nerve supply to the kidney and ureter played a

minor and unessential part in their function.

In 1926, Williamson (334) and Holloway (161) reported that autogenous neck kidneys maintained viability and functional activity indefinitely and death of the animal or disturbance of function was due to extraneous factors causing hydronephrosis and subsequent infection. The functional activity, the secretion and the response to diuretics were apparently similar if not identical to those of a normal kidney.

Nabatoff et al (246; 1951) successfully autografted canine kidneys to the intra-abdominal location.

In 1953 Dempster (104, 105) in his intensive study of the functional activities of the 'neck' kidneys, observed a rapid and permanent impairment of concentrating capacity and acidifying power, a decrease capacity to excrete chloride when presented with an acute load, polyuria after contra-lateral nephrectomy, slight impairment of electrolyte handling under conditions of normal and restricted dietary intake and a decline in water diuretic capacity with the passage of time and was regained on excision of the opposite kidney. It was suggested that this experimental model was not valid in assessing the function of a transplanted kidney.

After having concluded that the neck kidney was not suitable for long-term follow-up Dempster and others (107, 108; 1955) compared the functions of 5 single iliac, normal and neck kidneys. It was observed that the iliac kidney aside from the temporary inability to concentrate urine during the first 2 to

3 weeks, behaved like a single normal kidney in every other respect. In addition, considerable hypertrophy was noted in these transplants 130 to 270 days later. The kidneys before transplantation weighed from 70 to 75 grams and at sacrifice, they ranged from 90 to 120 grams. Histologically all the iliac kidneys appeared normal.

Murray et al (242, 243; 1954) working on iliac kidneys confirmed the previous findings of Dempster and others. He also suggested that it was a practical and feasible experimental and clinical model and dependent vesical drainage was the only physiologic method of ureteral implantation.

Later (244; 1956) he stressed the fact that a renal transplant free of genetic and immunologic incompatibilities totally denervated, revascularized in an anatomic site having the same temperature as the renal fossa and with direct implantation of the ureter into the bladder can survive without detectable impairment of function indefinitely. In this series, the vascular anastomosis was completed in 30 minutes and all functions returned in 4 weeks. It was revealed that there was 60% hypertrophy of the autografted kidneys which 50% was accounted for the cortical hypertrophy.

Bonasso (51) in 1964 reported return of functions within one month after removal of the opposite kidney. However recently (235; 1964) Miller et al re-opened the old controversy and compared the functional and morphologic features of the autotransplants to the neck and the iliac region. These workers did not find any significant difference between these two differently

situated transplants after one year.

3. Factors affecting functions of autografts: Eversince investigators started to assess the results of grafting by kidney functions, the prevalent thought regarding one of the causes of the abnormal functional behaviour of transplants not placed in-situ was the loss of the tonic effect of the renal nerves ablated during surgery. In an attempt to prove or discredit this view, Quinby (268; 1916) methodically proceded to study kidneys replanted in-situ after the nerves had been sectioned and the responses of such denervated kidney to diuretics (269; 1917) and compared them with the normal unmolested kidney. He concluded that there was no secretory nerves to the kidney thereby negating the accepted opinions heretofore.

Marshall and Koll on the other hand (229) in 1919 reported a greater water and chloride excretion from the denervated kidneys in anesthetized dogs which was attributed to greater blood flow secondary to nerve loss.

Kriss (197) in 1948 found that unilateral splanchnicectomy or unilateral adrenalectomy caused greater excretion of water and chloride in the homolateral kidney. He thought that the glomerular filtration and effective renal blood flow was slightly greater than the non-operated side. These changes were ascribed to the effects of nerves on tubular reabsorption.

Kaplan et al (187; 1951) noted an increase in the water and sodium chloride excretions in the denervated kidney and could

not be accounted for by the alteration of blood flow or glomerular filtration rate and suggested that the proximal tubules were under the control of the sectioned splanchnic nerves.

Goldblatt (137; 1937) suggested that sustemic and local renal effects of denervation were due to hormonal factors rather than due to nerves per se. The role of muscular exercise (276; 1938), somatic stimulation (316; 1934) and emotional factors (107, 108; 1955) in inhibiting water diuresis were thought to be hormonal in nature rather than nervous.

Starling (306; 1925), Rhoads (270; 1934), Rhoads et al (271; 1934), Theobald (317; 1934), Smith (300, 301; 1939 & 1951), Hiatt (153; 1942) and Dempster (105, 106; 1953 & 1955) did not find any difference in the blood flows, glomerular filtration rates and renal plasma flows between the denervated and the innervated kidneys in the un-anesthetized and anesthetized dogs.

Before the introduction of Inulin Clearance (285; 1935, 328; 1935, 272; 1936) as a measure of glomerular filtration, differences in the rate of excretion and secretion of the various test substances between the denervated and innervated kidney could not be demonstrated.

Maluf in 1943 (223) advanced the first definite proof that renal nerve supply did not affect renal tubular secretion at least with respect to water, chloride and phenol-red. His work involved transplantation of one kidney to the femoral region with end-to-end vascular anastomosis (average periods of ischemia - 25 to 30 minutes). The ureter including a portion of the bladder was exteriorized. The amounts of water and chloride reabsorbed per unit

volume of glomerular filtrate (Inulin or Creatinine Clearance) were identical in both the transplanted and non-transplanted kidney both during the diuretic and anti-diuretic phase. The ratios for inulin and phenol red clearances were practically identical for both kidneys indicating that the fraction of phenol red outwardly secreted by the tubules of both kidneys was the same. However like other workers preceding him, he found dissimilarities in the rate of excretion of water and other urinary solutes between the normal fully innervated and transplanted kidney. He attributed these to differences of glomerular functions.

Berne in 1952 (28) compared the renal function in the normal and denervated kidney in anesthetized and unanesthetized dogs in an effort to elucidate the mechanism responsible for the discrepency. The left kidney was denervated 6 to 49 days prior to the test. He showed a statistically significantly greater renal plasma flow, glomerular filtration rates and sodium excretion in the denervated kidney of the anesthetized dog as compared to the unanesthetized animal in which they were essentially equal. The maximum tubular reabsorption of para-aminohippurate sodium (T_m pah) and glucose (T_m glucose) were unaffected by denervation. Administration of colloid diuretics (Mannitol and Glucose) did not result in a significant difference in urine sodium concentration of the two kidneys. Pentobarbital anesthesia in one case produced a considerable reduction on plasma flow, glomerular filtration rate and urine sodium concentration in the innervated kidney with-

out affecting the function of the denervated kidney. Emotional stress brought about a similar but less marked depression of the renal plasma flow, glomerular filtration rate and sodium excretion of the normal kidney. He concluded that sodium appeared to be dependent upon glomerular filtration rate and there was no evidence that chronic denervation per se affected tubular reabsorption of Sodium. The difference in function of the normal and denervated kidneys in anesthetized dogs and the decrease in the renal plasma flow, glomerular filtration rate and sodium excretion observed in the control kidney of unanesthetized dogs subjected to stress was believed to be due to reflex vasoconstriction of the normal innervated kidney.

Dempster (104; 1952) found no significant differences existed on the glomerular filtration and effective renal blood flow as measured by clearances of creatinine and p-aminohippurate between the adrenalectomized and the non-operated side. He also noted an increase in water, chloride and sodium excretion and the urine had low specific gravity. This was attributed to pituitary-adrenal-renal hormone relationship (106; 1954).

B. Normothermic Ischemia

1. Pathologic Changes and Survival.

While the previously mentioned investigators were perfecting their techniques of transplantation, another group of workers concerned themselves with the relation of the period of ischemia in kidneys to the subsequent renal damage as measured by function tests and pathology.

Litten (213, 1880) performed experiments in dogs in which he ligated the renal vascular pedicle temporarily and claimed that interruption of blood flow in this manner for one and a half hour was enough to effect damage to the kidney, and extending the period of ligation to 3-4 hours caused more extensive damage. No functional evaluation in terms of being able to support life could be made however since the opposite untouched normal kidney was not taken out.

Similar experiments in dogs were performed by Jaboulay (178) in 1906 and Carrel (77) in 1909 and they reported damage to the kidney after variable periods of ischemia.

Eisendrath and Strauss (120, 1910) and Scarff and Keele (278, 1948) compressed the renal vessels of rabbit kidney for varying periods of time and observed characteristic sequential pathologic changes related to the duration of blood flow interruption. Histopathologic studies were made either at 48 hours or one month. If the occlusion was applied in 30 minutes or less, very slight changes in the kidney were seen, if continued for 45 minutes, it caused parenchymatous degeneration of the tubules and interstitial round cell infiltration and if extended from 1 to 2 hours or longer, he observed marked permanent degenerative changes in the parenchma consisting of marked interstitial round cell infiltration and extensive coagulation necrosis of the tubular epithelium and later by deposition of calcium in and about the destroyed epitherlium.

Identical experimental model was carried out by Bradenoch (52, 1947) in which temporary partial occlusion of the remaining

renal artery in the unilaterally nephrectomized rabbits led to renal changes comparable to human traumatic uremia (Crush Syndrome) as described by Beal et al (15, 1941), Bywaters and Beal (63, 1941), Mayon-White (232, 1941), Tomb (320, 1942), Lucke (215, 1946), Cournand and Colleagues (94, 1943), Maegraith and Findlay (221, 1944), Trueta (322, 1946), Bull and co-workers (62, 1950) and Oliver (248, 1951). The length of time during which there was occlusion of the renal artery bore a direct relationship to the mortality and survival rates of these animals. The first lesion to occur in these circumstances was degeneration of the proximal and distal convoluted tubules which are the parts of the nephron supplied by blood last in these animals.

Very interesting findings were made in the necrotic zone by MacNider (220, 1911) of cat's kidney supplied by the permanently ligated posterior branch of the renal artery. The necrosis affected a greater portion of the cortex and the superficial portion of the medulla of approximately one third of the posterior aspect of the kidney and that imperfect anastomosis existed between the anterior and posterior vascular zone. With the development of vascular anastomosis, an ingrowth of connective tissue followed by capillary budding which subsequently canalized and contained blood to form regenerated glomeruli. Then secondary fibrosis set in, which produced atrophy of the tubules, fibrosis of the glomeruli and obliterative changes of the vessels. The whole pathologic picture simulated chronic interstitial nephritis.

Rowntree and co-investigators (275, 1913) induced chronic passive congestion of the kidney in dogs by partial or temporary total interruption of venous return to the heart above the renal vessels and noted impairment of renal function depending upon the duration and degree of obstruction as evidenced by a delay in the excretion of Potassium Iodide and a decrease in Sodium Chloride excretion.

Marshall and Crane (230, 1923) investigated the influence of temporary occlusion of the renal artery in the dog. It was observed that one to two minutes of occlusion produced no effect on excretion of the kidney except proteinuria and anemia of longer duration (20 to 25 minutes) produced definite changes in the urine. The elimination of sodium chloride and bicarbonate might be unchanged or increased while those of urea phosphates, sulfates, creatinine and ammonia were distinctly decreased. He ascribed the altered urine or anuria to reflex arteriolar vasoconstriction and suggested that the functional changes can be prevented by avoiding vasoconstriction.

On the other hand Stoll and Carlson (309, 1923) by ligating the renal artery for 5, 15 and 20 minutes and the renal vein for 10 minutes, reported anuria for as long as 2 to 3 hours during the whole extent of the operation. Anuria caused by venous clamping lasted longer than the one due to arterial obstruction. The specific gravity of the initial urine following anuria was decreased. The arterial spasm that ensued the release of the arterial clamp could explain the anuria.

The need for an experimental model for acute parenchmatous

nephritis led McEnery and co-workers (222, 1926) to render the kidney temporarily ischemic from 30 minutes or more found a histopathologic picture simulating acute parenchmatous nephritis. If both kidneys were ligated from 30 to 60 minutes, the dog may or may not survived. The majority of animals however would lived after 45 minutes of occlusion of one kidney. Different degrees of renal insufficiencies were seen. The blood urea nitrogen was always elevated.

Scheibe and others (279, 1949) investigated the results of the ligation of either the renal artery, renal vein or both upon the renal function in relation to the duration of ischemia. Their findings indicated that the renal vein can be occluded for 30 minutes and the renal pedicle for 60 minutes in the rat or twice these periods of time in the dog without significant renal damage. Occlusion of the whole renal pedicle was strikingly less damaging to the kidney in rats and dog than occlusion of the vein alone. They attributed this more rapid appearance of kidney damage to the presence of greater capillary pressure associated with venous obstruction as suggested by Hinman (1945) unlike the cause of death in ureteral ligation (Hoff, 160, 1941) which is hyperkalemia. The characteristic lesion produced by temporary occlusion of the renal vein or renal artery was degeneration of the epithelim of the proximal convoluted tubules. The distal convoluted tubules and glomeruli remained relatively unaffected except in more prolonged occlusion. This finding of greater sensitivity to anoxia of the proximal tubular cells was in contradiction with Treuta's shunt theory of the lower nephron nephrosis where the distal con-

voluted tubule was considered to be affected selectively by cortical ischemia.

Creech et al (97, 1956) and Morris (239, 1956) reported the protective effect of subfiltration arterial pressure on the kidney. Using similar model, Moyer (240, 1957) compared the effects of occlusion of the aorta above the renal vessels, clamping of the renal artery alone and clamping of both for variable intervals of time up to 3 hours. It was found out that occlusion of one renal artery even for one hour produced damaged in some dogs and some animals withstood occlusion for 2 hours without manifesting significant damage to the kidney, however after 3 hours of occlusion, severe renal damage resulted from all animals studied. Occlusion of the aorta above the renal arteries in the other hand which resulted in a maintained arterial pressure distally of 30 mm.Hg or less, apparently prevented renal damage for periods of occlusion up to three hours.

The most severe damage occurred from combined aortic and renal occlusion which even after 2 hours of occlusion produced marked renal damage in all animals. Certainly after 3 hours, renal function was completely destroyed equivalent to the degree of damage sustained at 2 hours of occlusion of one renal artery suggesting that an adequate amount of blood reached the renal parenchma via the cortex to prevent consistent severe renal damage during the period of occlusion.

2. Renal Function

Since Van Slyke and associates (325, 1916; 326, 1927; 327, 1932), Peters and others (261, 1931), Winton (335, 336, 1934)

introduced urea clearance technique in assessing kidney function and other diagnostic refinements (Owens et al, 250; Smith et al 302), subtle changes in the functional activities had been unmasked which hitherto have been left undetected. With these elaborate techniques, more precise and meaningful informations had been gathered with respect to renal functions in health and disease.

Van Slyke et al (329, 1944) clamped the renal artery and capsular vessels for 3 hours of one kidney after having removed the opposite mate. The urea clearance and other excretory products were diminished which recovered in 2 to 3 weeks. Only one half of the animal studied recovered after 4 hours of occlusion and there was no recovery after 6 hours of complete interruption of blood supply.

Selkurt (283, 1945) compared the renal clearances with direct renal blood flow under control donditions and following renal ischemia. He concluded that following a twenty-minute period of complete renal ischemia there occurred a prolonged period of increased vascular resistance evidenced by a reduction in the volume of direct blood flow. The final periods of observation after an average interval of 85 minutes following release of arterial clamps averaged 55% of the control mean. Since no significant change in systemic mean blood pressure occurred which could be related to the change in renal blood flow, it was suggested that the increased vascular resistance was largely the result of arteriolar vasoconstriction. Glomerular and tubular impairment resulted from anoxemia created by renal arterial

occlusion as evidenced by the reduction in the plasma extraction ratio of p-aminohippuric acid and by decrease in the concentration ratio (u/p) of creatinine at reduced urine flow. These changes were reflected in a greater percentile reduction in the clearances of p-aminohippuric acid and creatinine than the simultanemous direct blood flow, resulting in discrepancies of renal blood flow values based on the clearance of p-aminohippuric acid and hematocrit. A factor contributing to the disparity between direct blood flow and the renal clearances might be a reduction in glomerular filtration pressure created by the arteriolar vasoconstriction which followed the period of ischemia.

3. Hemodynamics in shock

The renal hemodynamics in shock was investigated by Lauson et al (206, 1944) It was observed that the glomerular filtration rate and renal plasma flow were reduced in every shock and roughly paralleled the degree of shock. In most cases the decrease in renal blood flow and glomerular filtration was greater than can be accounted for solely on the basis of reduced arterial pressure suggesting vasoconstriction.

Selkurt (283) stated that anesthetized dogs showed changes in renal function analogous to those seen in hypotension and shock resulting from temporary complete ischemia produced by clamping the renal artery. The reduction of the clearances of creatinine and p-aminohippurate as a measure of glomerular filtration and renal plasma flow respectively reflected the underlying vasoconstriction and tubular damage.

A comprehensive study of the effects of acute hemorrhagic

and traumatic shock on renal function of dogs was made by Phillips in 1946 (263). Sudden hemorrhage of 20 to 30 milliliter per kilogram body weight triggered a chain of events in the dog under nembutal anesthesia starting with a drop in blood pressure to 50 to 60 mm.Hg. with cessation of measurable blood flow and excretion of urine followed quickly by partial or complete restitution of central blood pressure and of renal function. This appeared to be due to extrarenal vasoconstriction. Acute blood losses over the approximate range of 30 to 40 milliliter per kilogram body weight was accompanied by diminution of renal blood flow although the central blood pressure might be maintained above 110 mm.Hg. During this period, the glomerular filtration rate tended to increase thereby upholding the volume flow despite the shrinkage of renal blood flow. The circulatory phenomena apparently included a partial loss of afferent arteriolar pressure while the efferent arterioles constricted still more than the afferent arterioles to produce the compensatory increase in the extraction of plasma water filtered. When the hemorrhage exceeded the 40 to 50 ml/kgm. bdy wt. range, anuria followed.

4. Pattern of Recovery of Kidney Function.

Hamilton (145, 1948) and Roof and others (274, 1951) reported that dogs with the right kidney removed uniformly survived clamping of the left renal artery for 2 hours and some dogs survived after clamping the renal artery for 2 to 3 hours. Deaths were associated with environmental high humidity and temperature during

the summer months and poor nutritional state of the animals. Death in uremia regularly ensued with longer clamping of the renal artery. After the clamp was released from the renal artery, the renal clearance as measured by urea clearance was extremely low, of the order of 10% associated with a rapid rise in the blood urea nitrogen. The clearances eventually returned to pre-ischemic levels during the next 2 to 3 weeks and certainly recovery was complete at one month. The clearance in those that terminated in progressive uremia remained at a low level.

A more detailed studies conducted by Phillips and Hamilton (264, 1948) in the same laboratory indicated that renal blood flow was quickly resumed at a nearly pre-ischemic level after removal of the arterial clamps after 20, 60 and 120 minutes of occlusion. The proportions of para-amino-hippurate and creatinine extracted from the plasma were not markedly affected after a 20-minute period of ischemia but after 2 hours of clamping, they were reduced for p-aminohippuric acid to 37, 14, 10 and for creatinine to 63, 26 and 9 per cent respectively. They suggested that with tubular injury such as caused by ischemia, it appeared probable that the decreases in extracted fractions were due to tubular injury which decreased the proportions of plasma excreted by the tubules and increased tubular reabsorption of creatinine from the glomerular filtrate and doubted in the presence of such injury that p-aminohippurate clearance served as a measure of blood flow and clearances of creatinine and other diffusable substances could be interpreted as measures of glomerular filtra-

to

tion rate.

Friedman (133, 1954) reporting on the pattern of recovery of renal function following renal artery occlusion in the dog for 2 hours, claimed that uninephrectomized explainted kidney (Rhoads-270, 271; 1934) underwent functional compensation by 50% compared with the pre-operative value. The renal function after release of arterial clamp following 2 hours of renal ischemia was depressed to negligible levels for almost 24 hours and then slowly improved over the following 2 weeks. The functional depression was apparently referrable to tubular damage which was partly reversible (62, 1950) (248, 1951).

C. Hypothermia

1. Usage in the Past.

Bert(31), the eminent French Physiologist demonstrated a century ago that a rat's tail preserved for several days in a small quantity of confined air at a temperature not higher than 12°C was autografted successfully.

At the turn of the century Jolly (182, 1903) observed indirect cell division of triton's blood which had been preserved for 15 days in cold storage when put back to normal temperature. Several years later, Ehrlich (119, 1906) showed that pieces of tumor grew again when transplanted after being frozen for a long period of time. Carrel (75) in 1907, grafted segments of common carotid artery preserved for several days outside the body in the cold and noted that the segment did not suffer from degeneration of its muscle fiber when examined several months later. This

investigation was pursued further (78, 79; 1910) and he came to the conclusion that the segment of the carotid artery could be preserved best in cold storage above freezing, immersed in either defibrinated blood or vaseline. The preservation time was extended for as long as 6 months and this preserved segment continued to be incorporated into the vascular system for a long period of time.

The drawback of the blood vessel as a test organ for the preserving influence of hypothermia was apparent from the start and that was, there was no test to ascertain its viability. Nonetheless the experience generated an interest in hypothermia with regards to preservation of organs, parts of the body and the entire organism in the decades that followed.

Hypothermia had been employed as an agent to supress tumor growth (301, 1939), to allay pain (313, 1941), to alleviate albeit transient, mental disorders (314, 1942) and as a means of increasing patient's tolerance submitted to cardiac surgery (90, 1952).

The greatest stimulus to the renewed interest in hypothermia with regards to the preservation of organs was largely provided by the initial success of clinical transplantation by Murray (242, 1954) and associates.

2. Systemic Effects on Homeothermic Animals.

It had been known and determined that non-hibernating animals do not survive if their body temperatures dropped below 15 to 20 degrees centigrade. Lethal hypothermic temperature for different homoiothermic animals had been reported as circa 10° to

15°C (7, 1943; 144, 1937) for the rabbit; 16°C for the cat (288, 1905; 55, 1922); 14°C for the monkey (287, 1902) and 22°C for the dog (339, 1941; 310, 1953).

These various homeothermic animals lose their self heat-regulating mechanisms when exposed below their respective temperature range. They soon assumed the temperature of the environment and thus behave like a cold-blooded animal. Artificial external heat has to be provided to return them to their usual body temperature.

Fleischman (129, 1929) was the first one to draw attention to the fact that the brown adipose tissue of hibernating animals had a higher oxygen consumption than the ordinary white adipose tissue.

Hook and Barron (164, 1941) compared the oxygen consumption of kidney slices of ground squirrel at non-hibernation temperature (38°C) and hibernating temperature (5°C). While the respiration of the kidney at the temperature of hibernation was only 15% and approximately 3-10% during deep hibernation (260, 1896) (25, 1938), that of the respiration at 38°C, the respiration of the brown adipo tissue was still 36.2%. In hibernation therefore while all the other tissues reduce their metabolism to a minimum, the brown adipose tissue still retains one third of its optimum activity.

3. Effect on Enzymatic Activity.

The rat kidney which has one of the highest metabolic rate of the organs of the body (128, 1939) was studied by Fuhrman (134, 1943) and concluded that the rate of oxygen consumption of rat kidney slices measured for an hour at 37.7°C, then for an hour

at 0.2°C, was restored to the initial level on rewarming to 37.7°C. The oxygen consumption at 0.2°C after 15 minutes of thermo-equilibration was immeasurably low. The rates of oxygen consumption and of anaerobic glycolysis of rat cerebral cortex slices, determined under the same conditions, also showed recovery on rewarming. When the duration of the period of exposure of brain slices to 0.2°C was increased to 3, 5, 7 and 24 hours, there was progressive decrease in the rates of oxygen consumption regained on rewarming. At 24 hours the oxygen consumption was reduced to 50% of the control value. This recovery compared favorably with the data of Himwick (154, 1940) on minced cerebral tissue in which he found that the increase of respiration from 30°C to 37°C was 90% in accordance with expectation of chemical reaction. There were no data however on kidney slices at different periods of hypothermic storage. The authors opined that the cause of death of intact animals under hypothermia was probably due to failure of the supply mechanisms; circulation and respiration rather than caused by a direct and irreversible action of cold on the brain since the respiration and anaerobic glycolysis were preserved at a temperature (0.2°C) far below the lethal temperature of the animal (16°C).

In their experimental model the periods of cooling and rewarming did not exceed 15 minutes. This is in keeping with the findings of Hoagland in mammaliam spermatozoa (159, 1942) that 67% of living sperm survived sudden plunging to -195°C in liquid nitrogen followed by sudden rewarming at 35°C. The length of

vitrification did not affect the percentage of survival on rewarming.

In contrast Chang and Walton (85, 1940) stated that when the reduction of temperature to 1 or 3 degrees C. was accomplished in a stepwise fashion for 5°C at 2-hour intervals, 74% of the spermatozoa retained its original activities when preserved for 6 days.

4. Systemic Responses in Dogs.

Several investigators (2, 1965; 5, 1955; 38, 1950; 111, 1955; 115, 1953; 127, 1959; 135, 1956; 146, 1961; 147, 1959; 162, 1957; 163, 1959; 165, 1953; 185, 1954; 186, 1965; 239, 1956; 240, 1957; 253, 1955; 304, 1954) working on intact dogs by inducing hypothermia down to 18°C demonstrated generalized and renal vasoconstriction and increase of blood viscosity. There was no demonstrable evidence of oxygen deficit of the tissue except in the presence of marked muscular activities (Diel and Forbes, 112, 1941; Deterling and others, 111, 1955) which was reversed by rewarming but prevented by hyperventilation. The oxygen dissociation curve was shifted to the left drastically so that a near saturation of the blood was attained around 10 degrees centigrade. Although the blood volume, hematocrit and red cell volume diminished linearly with temperature fall there was essentially no fluid shifts.

5. Hemodynamics, Oxygen Extraction and Function Tests.

On account of the relative ease, convenience and the availability of renal function tests, the kidney as been extensive investigated with reference to its tolerance to ischemia and the influence of hypothermia on its subsequent function. While other

research workers set out to determine the metabolic effects of hypothermia on the kidney at the tissue level, others investigated the general behavior of the intact organ either left in situ or isolated.

Conway (89, 1925) lowered the body temperature in dogs from 38°C to 29°C and reported an increase in the glucose and chloride excretion in the urine which amounted to 10% per degree fall in temperature. The increase in urine volume was greater than either the glucose or chloride concentration in the urine. If there was no salt injected there was dilution of the concentration of the solutes studies. This effect was similar to the effect of cyanide on the kidney (Bayliss et al - 14, 1922).

The role of a diminution in intrarenal pressure as the cause of the increase of urine flow was investigated by Winton (35, 1933) who found no relation as brought about by cold inspite of the fact that hypothermia down to 3°C produced well marked and reversible swelling and increase in the tenseness of the kidney which suggested an accompanying increase in intrarenal pressure and cause diminution in glomerular filtration. Instead the urine volume and the solute concentration was increased until the urine plasma ratio reached infinity when urine formation eventually stopped.

Bergstrand and Stersky (27, 1954) noted decrease of glomerular filtration and renal plasma flow in dogs in which the body temperature was decreased to 18°C to 20°C. These changes in renal function roughly parallels to the decreased in temperature in centigrade and was reversible even t.. ough the recovery of renal

plasma flow lagged behind during the period of observation (50 minutes). The blood pressure fell in a linear fashion as the degree of drop in temperature. There was no consistent decrease in the arterio-venous oxygen saturation although the oxygen consumption exponentially decreased with the falling body temperature.

Semb et al (284, 1960) and Harper and others (146, 1961) investigated renal metabolism and blood flow during local cooling of the kidney by in-situ perfusion. Both groups of workers noticed exponential relation of oxygen consumption with reduction of kidney temperature to 15°C. The general metabolism of the dog kidney was reduced to one-half by cooling to 29°C or reduced to two-thirds at a temperature of 22°C.

A similar study conducted by Levy (210, 1959) revealed confirmatory results with respect to the relation between oxygen consumption and falling kidney temperature and it was in keeping with the Von't Hoff Arrhenius rule*, with a difference that the degree of oxygen extraction varied directly to the decrease in the fall of body temperature and varied inversely with the normothermic arterio-venous oxygen saturation difference at reduced renal blood flow rates (300, 1951; 152, 1965).

They concluded that the hemodynamic changes in the kidney were due to vasoconstriction and to the increase in the viscosity of the blood. The depression of the reversible oxidative metabolism of the kidney was greater than that of the body as a whole.

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* The rate of chemical reaction is doubled for each 10°C rise in temperature and halved for each 10°C reduction.

During the drop of kidney temperature of whatever modality of cooling employed the surface pH shifted to the acid side which was decreased further by hypothermia and reverted to normal on revascularization (114, 1966)

6. Ischemic Episode and Local Cooling.

By perfusing the isolated kidney with cold blood to anywhere within the range 3° to 13°C, Bickford (36, 37) reported that the composition of urine changed substantially to that of a serum transudate. The urine flow increase reached a maximum at about 10°C. The maximum increase varied from about two to tenfold or more according as the initial urine flow was high or low. At a normal flow, the increase was tenfold. A fall of 10°C below body temperature produced about 15% of the maximum change. The minimum arterial pressure which produced urine was reduced to 40 to 50 mm.Hg. from 70 to 80 in warm kidney uninfluenced by diuretics. The blood flow was reduced proportionately greater at low than at higher arterial pressure. The chloride concentration in the urine increased progressively with cooling to approximate the serum value about 18°C. A fall of 10°C below body temperature accounted 80% of the maximum increase. The creatinine concentration in the urine could only be reduced to about one and a half times that of the serum and it reached this maximum value by about 12°C. A fall of 10°C belcw body temperature produced about 50% of the maximum reduction. The creatinine clearance was reduced at normal arterial pressure by 75%. This work could be summarized as follows; reduction of kidney temperature to within

3°C produced both physical and chemical alterations in the kidney. The latter resulted in large changes of urine composition and small changes in urine and blood flow. The temperature characteristics of tubular reabsorption or secretion were found to be different for each substances. The urine composition thus assumed the composition of plasma ultrafiltrate where u/p ratios approached unity as cooling progressed and these were reversible on rewarming. The clearances of substances normally reabsorbed by the kidney tubules, such as glucose, water, urea, sodium and chloride were increased while clearances of non-reabsorbable solutes such as endogenous or exogenous creatinine were reduced. The reduction in the maximum tubular reabsorption of glucose was greater than water. The clearance of substances believed to be secreted by the tubular cells were reduced also. The influence of Pituitrin was nullified by cold resulting in the loss of concentrating operations of the kidney. The tubular function involved was suggested to be active in nature and was related to a high energy transfer mechanism.

Inspite of the fact that less fluid was filtered in the glomeruli as indicated by the progressively falling glomerular filtration rate, urine volume and threshold-substance concentrations increased and eventually urine formation fell due to the marked fall of the glomerular filtration which could not offset the tubular injury.

With the advent of cardiovascular surgery necessitating occlusion and/or replacement of a segment of the aorta and the surgery of renovascular hypertension, the fear of paralysis and

renal shotdown was a deterent factor. Hypothermia was then employ in the expectant hope of avoiding these harmful sequelae since hypothermia is known to reduce general metabolic activities, thus increasing the permissible period of ischemia.

Owens (250, 1955) clamped the thoracic aorta in for dogs periods up to three hours with the rectal temperature reduced to within the range of 23° to 26°C. without demonstrable renal injury determined 24 hours after the release of the clamp.

Bogardus (47) occluded the renal pedicle for 2 hours while the kidney was packed locally with ice. The renal core temperature range varied from 16°C to 30°C resulted in a less rise of creatinir compared to the normothermic kidney occluded for the same length of time. With one hour of clamping of the renal pedicle of the chilled kidney, each of two canine kidneys excreted phenosulfone-phtalein more quickly and in larger volume than the kidneys not chilled during the period of occlusion.

The sheep's kidney which does not tolerate more than one hour of ischemia was studied by Mitchell (36, 37; 1957). The best protection he found was after 2 hours of ischemia with the rectal temperature between 10 to 20°C. All animals survived with normal function. The kidneys at sacrifice 4 weeks later appeared small indicating lack of hypertrophy.'

Steuber et al (306, 1958) lengthened the pedicle clamping to 6 hours. At room temperature 100% of the dogs succumbed in uremia and in the experimental group with regional hypothermia applied 30% of the animals survived at 20°C and 75% survival was achieved at 10°C. Renal function measured by blood urea nitrogen levels

showed significant protection afforded by hypothermia during the period of anoxia. Histological studies showed proximal tubular damage with less severe degrees of injury in the cooled group. Long-term follow-up did not reveal renal functional deterioration (307, 1965).

This study was pursued further by Schloerb and co-workers (1959) by extending the period of ischemia to 24 hours and the in-situ cooling was brought down to 0° to 5°C. Survival and restoration of normal kidney function followed ischemia-cooling for up to 8 hours with immediate contra-lateral nephrectomy. None survived at 12 and 24 hours of ischemia even with delayed contra-lateral nephrectomy in the latter group.

Birkeland and others (39, 1959) observed that beyond one hour of clamping of the renal pedicle at normothermia resulted in some irreversible renal damage. Whereas cooling the kidney locally to 5°C permitted the kidney to withstand the effects of ischemia to 7 hours and to 12 hours if only the renal artery was temporarily occluded.

The group of Kerr (190, 1960) and Yoon and Landsteiner (340, 1965) arrived at similar conclusion working on the same experimental model.

7. Simple Cooling of Isolated Kidneys.

Oudot (249, 1948) preserved autologous kidneys by simple cooling technique for 8 hours and transplanted to the neck region of dogs with good preservation of function and sustained life after a delayed contra-lateral nephrectomy.

Identical method was used by Lefebre (208, 1951). He had

one canine kidney preserved for 24 hours closed to 0°C but the opposite mate was not removed.

Autografts selectively refrigerated for 7 to 8 hours at 1°C to 5°C with good results were accomplished by Kiser and others (191, 1960; 192, 1961). A mixture of Heparin, Procaine, low molecular weight dextran and autologous blood was utilized as perfusate.

Knight et al (195, 1963) preserved kidneys for 6 hours at 4°C with delayed contra-lateral nephrectomy.

Sub-zero (-5° to -6°C) preservation had been shown to be possible by Desphande et al (110, 1963) immersed in 15% Di-methyl Sulfoxide (DMSO) for 8 hours. Two out of 15 survived for at least 10 and 12 months with normal blood urea nitrogen and blood pressure.

The group of Calne (64, 1963) were able to keep kidney autotransplants alive which had been preserved for 12 hours below 5°C with immediate contra-lateral nephrectomy employing simple surface cooling technique.

Simso (289, 1963) and others perfused 2 baboon kidneys for 4 minutes and stored at 4°C for 21 1/2 and 22 1/2 hours respectively. The opposite kidneys were removed 10 months later. Long-term survivors subsequently developed hypertension which did not correlate with the length of preservation. Biopsies of these animals showed the features of benigh nephresclerosis. Kidneys were kept viable by Dempster et al (109, 1964) by simple storage technique at 4°C for 6 hours. He outlined the various factors that underlay

some of the phenomena associated with failing grafts.

Dormont et al (116, 1964) and Cukier (98, 1964) attained better results by cooling kidneys to 4°C either by immersion in saline solution or by perfusion. Good results were achieved if the preservation time was 10 hours or less. Majority survived and at biopsy or sacrifice exhibited normal histologic pattern. Some animals showed tubular and interstitial changes similar to pyelonephritis. Three out of 16 animals preserved for 17, 19 and 24 hours survived. From the moment of revascularization, the kidneys became abnormal in color and consistency. Their renal functions were impaired. The histopathologic features were characterized by extensive tubular and interstitial changes, partial or total necrosis.

Pegg and others (259) reported 2 survivors in which the only resident kidney was perfused with cold heparinized blood for 24 hours. The longest period of ischemia endured by the kidney by means of simple surface cooling technique to 4°C was 17 hours in one case.

8. Perfusion Combined with Refrigeration.

Telander was able to perfuse baboon kidneys for 7 hours under normothermic condition with encouraging results (315, 1962).

Couch and associates (93, 1958) perfused continuously excised canine kidneys with heparinized oxygenated autologous blood diluted either with saline or Lockes solution at 25°C and reported continued urine formation for 7 hours. Diminution of perfusion flow was consistently noted as an inherent problem in perfusing whole organ. The effects of cold injury in the

form of decreasing glomerular filtration rate and water diuresis were observed as well.

The effectiveness of this preservation method on excised kidneys was tested by Cassie and colleagues (83, 1959) by replanting the kidney to the original host. The period of perfusion was 6 hours and at normothermic condition. Apparently there was no difference in the outcome when perfusion was done at 38°C or 25°C. Kidneys perfused with autologous blood or by an intact animal were able to sustain life indefinitely.

When perfusion was performed under hypothermic setting longer periods of preservation was achieved (Lapchinsky, 205, 1961) by Lapchinsky of Russia. He perfused kidneys for one hour with cold blood followed by storage of the perfused kidney at 2 to 4 degrees centigrade up to 27 hours. The kidney was rewarmed for one hour before replantation to the neck area using vascular stapler. Sixteen animals survived out of 52 experiments after contralateral nephrectomy at 8 weeks which survived longer than 3 years. At sacrifice compensatory hypertrophy was observed ranging from 25 to 100 per cent of the original kidney weight. Histologic sections revealed hypertrophy of the glomerular tufts and regeneration of tubular epithelium.

Longer periods of kidney preservation has been achieved also by Simso and Hitchcock (289, 1962) for 22 1/2 hours. They employed hypothermia and low pressure perfusion of variable length of time using diluted blood and non-sanguinous perfusate. Delayed contralateral nephrectomies were done from 2 to 10 months.

Humphries et al (167, 1962) making use of similar principle were able to store successfully 2 kidneys out of 41 cases for 24 hours by a continuous low pressure perfusion between 4° to 10°C using perfusate consisting of a mixture of Ringer's Solution, cadaver plasma, Procaine, Heparin and bubbled oxygen. Removal of the opposite kidney was delayed 3 weeks later. The glomerular filtration rate was reduced by at least 50% and the renal plasma flow in the order of 75%. Biopsies taken from these preserved kidneys demonstrated moderate interstitial fibrosis and tubular atrophy.

Later (168, 1963), the perfusate was substituted with diluted serum or plasma with comparable results but the percentage survival was not improved (3 of 63). In view of this fact, they introduced some more changes including autologous whole blood in the perfusate diluted up to 50%, slightly higher perfusion pressure, a glass wool filter and a membrane lung. (169, 1964)

This new modification produced 5 life-sustained kidneys out of 7 autografts. In four of these animals the normal opposite kidneys were removed 3 weeks after reimplantation and in one the contra-lateral kidney was taken out just before reimplantation of the preserved kidney.

Recently the same group of workers used homologous instead of autologous blood and they reported 8 survivors out of 11 experiments. All dogs were bilaterally nephrectomized. Despite these improvements, it did not prevent cytonecrosis during the period of perfusion.

In 1964, (171) they were able to store 4 kidneys out of 7

for 48 hours with delayed contra-lateral nephrectomy from 1 to 2 weeks post-re-implantation. The kidney functions determined 3 to 8 weeks after contra-lateral nephrectomy revealed at least 50% reduction.

D. The Pressure Chamber and Hyperbaric Oxygenation

1. Early Use.

The first recorded attempt at clinical application of the hyperbaric chamber was made by Henshaw (286, 1857) a British physician in 1662 although the use of air at elevated pressures and the pressure chamber dated back to antiquity. Henshaw's chamber or 'domicilium' was provided with valved organ bellows to allow rerefaction and compression of the chamber. The rationale was to use elevated pressure for acute and low pressures for chronic disease. "In time of good health this domicilium is proposed as a good expedient to help digestion, to promote insensible respiration, to facilitate breathing and expectoration, and consequently, of excellent use for the prevention of most affections of the lungs".

The dividing bell was mentioned by Aristotle in his work - Problemata: "For they can give respiration to divers equally by letting down a bucket for this does not fill with water, but retains its air". The diving bell was also used by Alexander the Great in his siege of Tyre (332 B.C.) (101, 1934).

Of course it was not until after Priestley discovered oxygen in 1775, (Priestley, 267) was the use of hyperpressure system placed on a scientific basis. His classic description thus: "From the greater strength and vivacity of the flame of a candle in

this pure air, it may be conjectured, that it might be peculiarly salutary to the lungs in certain morbid cases...But perhaps, we may also infer from these experiments, that though pure dephlogisticated air might be useful as a medicine, it might not be so proper for us in the usual healthy state of the body; for as a candle burns out much faster in dephlogisticated than in common air, so we might, as may be said, live out too fast and the animal powers be too soon exhausted in this pure kind of air. A moralist, at least, may say that the air which nature has provided for us is as good as we deserve...The feeling of it to my lungs was not sensibly different from that of common air; but I fancied that my breath felt peculiarly light and easy to some time afterwards. Who can tell but that, in time, this pure air may become a fashionable article of luxury. Hitherto only two mice and myself have had the privilege of breathing it".

Renewal of interest was fostered in 1782 by the Dutch Academy of Sciences in Haarlem, The Netherlands which attempted to promote interest in the design and use of the hyperbaric state in biology (Arntzenius, (9). No productive results came out during this movement. However half a century later resurging enthusiasm was kindled in France by the work of Junod (184, 1834), Tabarie (311, 1838; 312, 1840) and Pravaz (265, 1837; 266, 1840).

Pressures of 2 to 4 atmospheres were employed during this investigation.

Tabarie claimed salutary effects in 49 cases of respiratory disease, He emphasized the extreme need of raising and lowering

the pressures within the chamber very gradually. The largest chamber during the decade was built by Pravaz in Lyon which accomodated 12 patients at a time. He felt that "Le bain d'air comprimé" would dilate the bronchi which was considered beneficial in cases of pulmonary tuberculosis, capillary hemorrhages, deafness, cholera, chest deformities, rickets, metrorrhagia, acute conjunctivities, chronic laryngitis, tracheitis and pertussis.

From these early French studies a suggestion to use compressed air in caisson as we know today came to being.

Triger in 1841 (321) reported his experience with the first caisson for excavating the bed of the Loire River. The caisson was sunk to 65 feet. Laborers working for as long as 7 hours complained of severe pains in the arms and knees shortly after emerging into the open air. They also noted pain in their ears during compression and that candles burned out much more rapidly. This apparently is the first recorded account of Caisson's disease or "the bends".

The evolution of the industrial applications of compressed air chambers is well reviewd by Singstad (290).

From the 1850's up to the first quarter of the present century, the vogue of the compression chamber spread far and wide across the civilized parts of western Europe and North America for anything and everything (4, 33, 34, 35, 92, 99, 140, 207, 277, 291, 332).

2. Physiological Observations.

Physiologists on the other hand were interested in defining

the ill-effects of oxygen deficiency encountered by mountain climbers as well as the complications of hyperpressure as occasioned by submerged diving. These needs arose in the quest for riches and adventure and as a necessity of war machines.

Bert (32, 1878), the French plastic surgeon better remembered as the "father of pressure physiology" pursued the problems of mountain sickness during lofty ascents with particular reference to mountain climbing and aerial baloon experiments. No doubt he was influenced by his long association with Jourdanet (183). It was concluded that mountain sickness was due to hypoxemia. His prodigious mind ventured into the field of plant and animal enzymes, thus he reported that oxygen at high tension perfectly preserved fruits for as long as three years. The enzymatic activities of salivary diastase, pepsin, inversive ferments and other tissue juices were retained after prolonged storage under oxygen at high pressure at normothermic condition. These biologic materials were spared from the deteriorating influences of bacteria and molds indicating that the vital processes of simple forms of plant and animal lives were also shown to be inhibited by oxygen at hyperbaric tensions.

The convulsive and anoxemic phenomena observed in laboratory animals and man when subjected for prolonged periods of time under high oxygen tensions and the adverse effects associated with sudden changes of ambient pressures were investigated as well. It was believed then that man and animals could not withstand 20 atmospheres for 3 hours.

This line of investigation was continued and carried further by Behnke et al (22, 1935). They observed that healthy volunteers can breathe pure oxygen with comparative safety as follows: 4 hours at 1 atmosphere; 3 hours at 2 atmospheres; and 2 hours at 3 atmospheres. The symptoms induced by oxygen were referable mainly to the central nervous system. The irritative effects on the lungs were chest pain and dry cough.

Attempts at prolonging endurance of otherwise healthy subject: (Behnke and Motley, 23, 1936) exposed to 3 atmospheres of oxygen pressure were characterized by complaints of progressive contraction of visual fields with dilatation of the pupils and some impairment in central vision in the fourth hour. This visual disturbance was associated with abrupt rise in systolic and diastolic blood pressure, increase in pulse rate and later extreme pallor of the face indicative of peripheral vascular constriction.

In 1940 Behnke (24) summarized his experience concerning the effects of temporary existence in a pressure chamber, diving suit and at high altitude. It was concluded that 2 physiological effects of increased pressure up to 16 atmospheres or pressure decreased to one-fifth atmosphere fell into 2 categories; a) effects of pressure per se, and b) phenomena associated with gaseous equilibrium. That the primary pressure effects were observed if the auditoty tubes and paranasal sinuses were obstructe Pain was elicited as a diagnostic symptom and was associated with congestion and hemorrhage of the mucosal lining of the occluded spaces. Of the gases absorbed under pressure (a) atmospheric nitrogen exerted a narcotic effect, minimized or abolished by

substitution of helium and (b) pure oxygen at atmospheric pressure elicited symptoms of bronchial irritation or vasomotor spasm if breath for six or seven hours, and at a pressure of four atmospheres induced convulsive seizures followed by apparently complete recovery. The air emboli formed following decompression from high pressure atmospheres affected those parts of the body having poor circulation.

In reviewing the works on hyperpressure condition hitherto Bean (18, 1945) enumerated the main systems adversely affected by and the possible mechanisms of oxygen action at increased pressure as follows: pulmonary, central nervous system, increase carbon dioxide accumulation, increased tissue due to increased tissue tension of carbon dioxide, decreased tissue metabolism, enzymatic inhibition, 'hyperoxic anoxia' and finally formation of toxic substances.

3. Pathological Features.

The pathologic changes associated with oxygen toxicity was pointed out by Karsner (189, 1916) that in laboratory animals and man subjected to increased partial oxygen pressure for protracted length of time from 24 to 72 hours, the pulmonary changes such as congestion, edema, epithelial degeneration and desquamation, fibrin formation and finally pneumonia probably of irritative origin were to be described as fibrinous bronchopneumonia. The gross alteration in the right side of the heart he thought was secondary to the lung changes. This physiologic disturbance led to degeneration of the distal parenchymatous organs due to

chronic passive congestion.

4. Biochemical Studies.

A more precise biochemical study at the enzyme level in tissue homogenates or slices under oxygen pressure was reported by Libbrecht et Massart (211, 1937). These investigators demonstrated that hyperbaric oxygen decreased the activity of the dehydrogenase system. Bohr and Bean (49, 1940) observed reduction of dehydrogenase activity from 9% to 50% after two and one-half hours of exposure at 7.5 atmospheres of oxygen. Similar results were obtained when anoxia condition was introduced instead of high pressure oxygen (16, 1940). It was opined that the common pathologic defect was tissue anoxia. The same group in 1944 (17) showed that the more rapidly acting cyanide-sensitive oxyidase reductase system as well as the slowly activated dehydrogenase system were affected by the toxic action of hyperbaric oxygen.

Stadie and Haugaard (304, 1945) concluded that the mechanism of inactivation of dehydrogenase system in rat liver, kidney or brain was that of oxidation of the active sulfhydryl group to the inactive thiol form when subjected at seven atmospheres. Such inactivation was slow and prevented by the addition of malonate and succinate and reversed by reduced glutathione or cysteine. Cytochrome oxidase and cytochrome c were unaffected at this pressure.

Haugaard (149, 1955) postulated that the toxic action of oxygen at high tension may be related to an oxidation of essential metabolites such as coenzymes containing glutathione and free sulfhydryl radicals such as coenzyme A and lipoic acid rather than

due to inactivation of enzymes in general.

Two years later Haugaard and associates (150, 1957) reported further that the enzyme system in heart muscle oxidizing glucose and pyruvate was gradually inhibited at 1 atmosphere of oxygen which was greatly accentuated by trace amounts of cupric ions. Ethylenediaminetetraacetic acid, a chelating agent protected the inactivation.

Wood and Watson (337, 1962) showed that rats injected intraperitoneally with gama-aminobutyric acid (GABA) prior to exposure to 5 atmospheres of oxygen were protected from the ill effects of oxygen toxicity with reference to the frequency and severity of convulsion. GABA is present in detectable amounts only in central nervous system. The levels in the brain (338, 1963) was low in those rats that were exposed for a long period of time and had severe convulsions and normal levels were noted in those rats exposed for short periods of time and in the un-exposed. The degree and speed of recovery was related to the duration of exposure to the hyperbaric environment. They suggested that since the citric acid cycle was reported to be inhibited by oxygen at elevated pressures at the alpha-ketoglutaric dehydrogenase step (Thomas and Neptune, 1962), then the shunt GABA pathway may become the major route in the cycle necessitating a greater requirement of GABA. Under normal conditions this pathway accounted 40% of the total metabolic activity.

A suggestion was put forward by Thomas and Neptune (318, 1962) that the chemical mechanisms of oxygen toxicity were intimately concerned with the inactivation of enzyme systems. It seemed

to them that there were two types of inhibition by oxygen:
(1) slow and irreversible inactivation of certain enzymes by the effects of oxygen activity directly upon the protein apo-enzymes themselves. This inactivation they claimed may be related to the slowly fatal and irreversible effects of oxygen in vivo,
(2) the rapid and reversible inhibition of specific enzyme systems by the action of hyperbaric oxygen on co-factors such as alpha-lipoic acid in 2 key enzymes; pyruvic oxidase and alpha-oxo-glutarate dehydrogenase.

In attempting to unravel the instability of cytochrome reductase at room temperature, Dixon and co-workers (113, 1960) and Armstrong and others (8, 1960) found that if was inactivated by oxygen and suggested that it may have played a role in connection with oxygen poisoning.

Brosemer and colleague (56) noted strong inhibition under high oxygen tensions of strain c mouse fibroblast which ordinarily multiply even under absolute nitrogen environment with virtual absence of oxygen. The inhibition would be reversed by lowering the oxygen tension within 48 hours. Longer periods of incubation in high oxygen, resulted in irreversible changes including cell degeneration.

5. Recent and Current Clinical Applications.

The revival of recent interest on the clinical application of hyperbaric oxygen was ushered in, a decade ago by the work of Boerema (43, 1956) although a year earlier Churchill-Davidson (87, 1955) has been using hyperbaric oxygen in conjunction with x-ray irradiation in certain tumours. The employment of this rediscovered

experimental therapeutic tool remained largely in the institutions that initiated it until some startling results were published by Smith (292, 1958) and by Boerema (44, 1960). The latter demonstrated rather convincingly that hemoglobin was indeed 'superfluous' as stated by Haldane (143, 1895) around three scores before.

The enthusiasm of the scientific community in the resurgence of the modern usage of hyperbaric oxygen was on the basis of sound principle of gas physics (Henry's Law).

Valuable lessons were gleaned from the efforts of Haldane (143, 1895) and Bohr (48, 1905). It was stated that in an animal breathing 100% oxygen at ambient conditions, the amount of molecular oxygen physically dissolved in the plasma was increased fivefold from the solubility constant of 0.3 vols% to 1.5 vols% and this increment was linearly related to each atmospheres of added pressure. Since the basal metabolic tissue need as reflected by the mean arterial and venous oxygen difference was around 5 vols%, 3 atmospheres of oxygen pressure was sufficient to provide the necessary oxygen requirement. Thus at 3 atmospheres hemoglobin can be dispensed with entirely.

Interesting fundamental work which gave the clinician a better insight into the dynamics of blood transport, cerebral circulation and cerebral metabolism was provided by Lambertsen and co-workers (202, 1953; 203, 1959). It was shown that administration of oxygen at 2 atmospheres to human subject during leg exercise lowered ventilation, restored arterial pH and pCO_2 towards resting levels and caused pCO_2 to rise above the resting

level; the cerebral venous pH remained elevated despite reduction of blood fixed acid. These data suggested to these workers that either a slight elevation of cerebral blood flow or a reduction in the rate of cerebral oxygen consumption occurred during exercise breathing oxygen at 2 atmospheres without gross elevation of cerebral venous pO_2.

Nairn and others reported progressive reduction of pulmonary capillary blood flow and diffusing capacity with rising intracapillary pO_2. Similar studies were performed by Bain and others (1964) confirming the association of vasoconstriction with elevation of pO_2 of blood perfusing the pulmonary capillary bed.

The problem of man in an oxygen millieu (151, 1963) and the role of CO_2 tension in the blood or plasma in effecting an early onset of oxygen toxicity was clearly demonstrated by Bean (19,1961), Patel and Bowdey (256, 1964), and Bergofsky et al (26, 1964). Apparently the vasoconstriction if any occurred during oxygen hyperbaria was neither sufficient to curtail oxygen delivery to the tissue nor prevented the onset of toxicity. On the contrary the initiation of convulsion and lethal irreversible pulmonary changes was hastened.

Subsequent reports of Boerema (45, 1961), Smith et al (294, 1961) and Illingworth and associates (177, 1961) further explored the everwidening spectrum of clinical states that benefited the use of oxygen up to 3 atmospheres; e.g., the improvement of ventricular fibrillation threshold of hearts to hypothermia and prolonged circulatory arrest, the prompt emergence from carbon mono-

xide poisoning and barbiturate coma, the relief of pain in cases of peripheral vascular ischemia, the dramatic healing of chronic bed sores, the maintenance of cortical activity as monitored by EEG during carotid artery occlusion, the prevention of likely distal gangrene due to injury of the main artery to the affected limb and finally the prevention and alleviation of the ravaging effects of gas gangrene infections.

Tremendous progress have been achieved in the recent past in delineating the possible extent of the useful application of hyperbaric oxygen under experimental laboratory and clinical settings.

1. Cardiac Surgery on Cyanotic Infants

One of the major obstacle during cardiac surgery in blue babies is the unavoidable hypoxia during the correction of the anomaly. Performance of either palliative or corrective surgery is best carried out at 3 atmospheres of oxygen pressure. The additional safety is provided by the fact that the priming of small disc oxygenator could be done without blood and at a low perfusion rate (46, 1962; 233, 1963; 29,1963; 30, 1963).

2. Coronary Artery Occlusion

The sudden excruciating pain descriptive of acute coronary episode was found to be relieved in patients breathing 100% oxygen under pressure (13,1940; 50, 1963). Subjective and objective benefits have been elicited in post-cardiac arrest patients as well (196, 1962).

The two major acute complications of acute coronary occlusion, e.g., ventricular fibrillation and cardiogenic shock were mitigated

and/or prevented when the victim was exposed to high pressure oxygen reasonably early (238, 1962; 297, 1964). The overall efficacy of oxygen on these conditions was reflected in the significant reduction of the mortality rates in experimental laboratory and clinical trials (297, 1962; 233, 1963; 180, 1964; 86, 1964). However a note of caution was sounded by the unimpressive results of the work of Cameron et al (1965) on a well controlled clinical studies.

3. Gas Gangrene Infection

Oxygen therapy have resulted in a rapid control and regression of toxemia within 24 hours in gas gangrene myositis and septsis if exposed to 3 atmospheres. This mode of treatment has proved to be life saving and permitted conservative regimens in the face of manifest clostridial necrosis (Brummelkamp et al - 1961, Smith et al - 297, 1962; Brummelkamp and others - 59, 1963; 60, 61, 1963; Glad and associates - 136, 1965; and Brummelkamp - 297, 1965).

Clostridial infection of the liver with the associated anaerobic septicemia following ligation of the hepatic artery was controlled if the animal was pressurized at 3 atmospheres of oxygen within 6 hours. Similar protection was afforded by antibiotics. Both treatments however failed to prevent necrosis of the liver (193, 1963; 188, 1963).

Jacobsen reported (179, 1965) protection of the liver against periods of transitory total inflow occlusion up to 120 minutes at 4 atmospheres but not at 3 atmospheres of oxygen.

Inspite of the extreme sensitivity of Clostridium welchii,

the rest of the strains of chlostridia organisms like Clostridium tetanus and butolinum have not consistently responded well to high oxygen tension (60, 1963; 132, 1965; 254, 1963).

4. Tumour Radiotherapy

Following publication of Churchill-Davidson's work (87) valuable lessons have been gained in the use of hyperoxygenation combined with radiotherapy.

Kluft (194, 1963) was able to slow down the development of tumour growth in the primary and metastatic foci although there was no significant prolongation in the life expectancy. Obviously extensive experience has to be accumulated before a definite statement could be made concerning the usefulness of high pressure oxygen combined with radiotherapy (Chuechill-Davidson, 88, 1963; 53, 1963) and/or chemotherapy (1, 1963).

The mechanism of action of oxygen on tumour suppression has been the subject of sharp debate.

Carter and Watkinson (82, 1963) attributed the ameliorating effect of hyperbaric together with deep x-ray to the increment of the attendant hypoxic state.

Measurements of tissue oxygen during compression has been notoriously difficult.

Evans (125, 1963) found the oxygen saturation of tumours to be slow compared to the normal skin at 4 atmospheres. An increase in oxygen tensions in tumours was noted following irradiation.

Quantitation of the oxygen saturation patterns in different parts of the tumour and the relation of the tumour tissue oxygen

tension to the rest of the body at ambient and at hyperbaric conditions were beautifully described by Jamieson and Brenk (1965). These investigators recorded wide scatter of pO_2 values in tumors than in normal tissues under ambient conditions. A higher proportion of electrodes gave low readings in tumors than in normal tissues but a proportion of tumors showed regions of high pO_2. With pressurization of patients to 4 atmospheres absolute, the mean tumor pO_2 rose rapidly and reached mean values more than twice those in the normal tissues. While the pressure was maintained for a period of 30 minutes, the mean oxygen tensions continue to rise to double the value at the completion of compression. Decompression was followed by a considerable lag in the decline of oxygen tension recorded.

5. Carbon Monoxide and Cyanide Poisoning

It is generally held that the physiopathologic end-result of carbon monoxide intoxication is tissue anoxia due to the avid oxidation or fixation of hemoglobin to a more stable non-oxygen carrying carboxyhemoglobin. Thus the body is totally deprived of the oxygen carrying capacity of free or unbound hemoglobin.

Haldane (143, 1895) needed to increase the lethal dose of carbon monoxide to laboratory animals in the presence of oxygen at elevated pressures.

In the presence of oxygen at 2 atmospheres the normal half-time elimination of carbon monoxide of 4 hours was shortened to 45 minutes (252, 1950) and left no doubt that high pressure oxy-

gen was the most efficient means available whereby the blood may be cleared of carboxyhemoglobin and revived victims early in carbon monoxide and coal gas poisoning (Campbell - 66, 1929; Smith et al - 117, 1961; 118, 1963; 209, 1961; 293, 1960; 295, 1962.

6. Hemorrhagic and Bacteremic Shock

Tissue anoxia is a prominent physiologic feature of hemorrhagic shock on account of curtailment of oxygen delivery to the tissues. The arrest and reversibility of this condition necessitates a prompt and vigorous rectification of the existing oxygen deficit. Should restoration of effective circulating blood volume fail to improve perfusion and or circulation at the tissue level, hyperbaric oxygen exposure has been advocated specifically to provide readily available molecular oxygen in physical solution.

Provision of adequate arterial oxygen supply improved the mortality rates of dogs in hemorrhagic shock from 90% in the control to 27% in those receiving 100% oxygen (96, 1963; 121, 1965; 123, 1962). The maximum benefit was elicited only in the early phase in the less severe stages of shock (40, 1965) and seemed necessary for the inhibition and subsequent survival of animals in bacteremic shock. It has been proved useful in the treatment of endotoxin shock (124, 1964), although Frank (131, 1943) failed to demonstrate any value whatsoever.

7. Ashyxia Neonatorum

The first few hours after birth is generally known to be

the most critical period in an infant's life. Most of those who develop asphyxia respond to non-surgical intervention and a proportion of others is amenable to tracheal intubation. However a small minority of marginal cases fail to be helped by either method. In this group benefit has been derived from exposure to oxygen at high pressure during this critical time. (Hutchison et al - 172, 1962; 173, 1963; 174, 1965).

8. Tensile Strength of Healing Wounds

Conflicting reports of the influence of hyperbaric oxygen on healing wound are being made known of late.

Lundgren and Sandberg (216, 1964) noticed impaired wound healing in rats exposed to 2 atmospheres of oxygen pressure. This impairment displayed a dose response pattern. Beckham and Hitchcock (21) on the other hand found significant omprovement in tensile strength of wounds in dogs exposed to two and one-half atmospheres given eight 2-hour daily treatments.

9. Miscellaneous Conditions

Isolated encouraging reports were described on the use of hyperbaric environment on vascular injuries of the limb (231, 1963) and ergot poisoning of the foot which prevented likely amputation.

Attar (10, 1964) and others (122, 1963) subjected dogs to 3 atmospheres after inducing massive pulmonary embolism by autologous blood clots. Survival was improved from 50% in the untreated to 69% in the oxygen treated group. The survival rate was correlated with the improvement in the hemodynamic, biochemical and electrocardiographic changes.

Schwartz and associates (282, 1965) administered 0.2 ml of kerosene intratracheally in rats to induce kerosene pneumonitis and followed by exposure to 3 atmospheres of oxygen. Although the duration of survival was altered the survival statistics were not affected.

The irreversible hypoxic damage to the spinal cord which often results from temporary occlusion of the descending thoracic aorta was largely prevented by high oxygen tension. The occlusion time could be prolonged significantly as well (54, 1963).

Direct application of oxygen to bedsores has promoted rapid healing in otherwise indolent ulcer (Gorecki - 139, 1964).

Gool and Jong (138, 1964) showed that various afflictions of the eye in which vascular insufficiency of the retinal artery and/or optic nerve was the common pathologic disturbance were improved under a regulated oxygen pressurization. This improvement was explained on the basis of development of collateral circulation and other reparative process during the period of oxygen exposure. This finding is at variance with the work of Behnke (23, 1936) on healthy volunteers.

III. Experimental Design and Methods

A. Experimental Design

A preliminary investigation of 42 kidney autotransplants to either the neck or pelvis strongly suggested that the pelvic transplant was superior for long-term studies. The principal causes of failure when the kidney was placed in the cervical area were late pyelonephritis, infected hydronephrosis and wound complications. The groups of animals herein reported, all of which received an autotransplant to the pelvis are summarized in Table I.

Accordingly, the project was divided into the following order of investigations:

1) Development and perfection of nephrectomy and pelvic autotransplantation, as a standard technique and establishment of a control group of animals,

2) Exploration of the protective influence of hypothermia and hyperbaric oxygen on isolated autologous canine kidneys for 24 hours,

3) Determination of the circulatory behaviour of recently removed and 24-hour preserved kidneys,

4) Determination of the best combination of hypothermia and hyperbaric oxygen and the optium range if there are any and,

5) Definition of the value of the operating hyperbaric chamber on such kidneys stored by hypothermia-hyperbaric method of preservation for 48 hours.

B. Methods

1. Kennel and Standard Care

The room is air-conditioned and well ventilated and each

GROUPS	TEMPERATURE	PERIO
I CONTROLS	27°C	25 MI
II 5 ATA*HBO	27°C	24 HO
III HYPOTHERMIA	4°C	" "
IV 2 ATA-HBO	"	" "
V 3 ATA-HBO	"	" "
VI 5 ATA-HBO	"	" "
VII 10 ATA-HBO	"	" "
VIII 20 ATA-HBO	"	" "
IX 30 ATA-HBO	"	" "
X 10 ATA-HBO	"	48 HO
XI 10 ATA-HBO**	"	" "
XII 5, 20, 30 ATA-HBO	"	72 HO

TABLE I - EXPERIMENTAL DESIGN

* Atmospheres absolute of hyperbaric oxygen.

** Excision and replantation of kidney were done in operating hyperbaric ch
dog breathing 100% oxygen.

	TEMPERATURE	PERIOD OF ISCHEMIA
	27°C	25 MINUTES
	27°C	24 HOURS
	4°C	" "
	"	" "
	"	" "
	"	" "
	"	" "
	"	" "
	"	" "
	"	48 HOURS
	"	" "
)	"	72 HOURS

TABLE I - EXPERIMENTAL DESIGN

lute of hyperbaric oxygen.

lantation of kidney were done in operating hyperbaric chamber at 3 ATA with 0% oxygen.

dog is provided with a separate numbered cage corresponding to each number on the collar. Larger cages are available as temporary recovery spaces for the newly operated animals. Following their recovery from anesthesia, they are replaced into their original cages. Animals on medium and long term experiments are housed in the interim at the farm and at the spacious kennel at the McIntyre Building. Sick animals are either secluded or sacrificed, dictated by the general condition of the animals and the availability of spaces. The cages are washed with detergent and luke-warm water daily delivered through a manually operated spray gun.

2. Diet

Dogs are fed once daily with a mixture of equal parts of commercial dog biscuits; Burgerbits of Ballard's* and Purina Dog Chops**. Water is provided ad libitum. One pound of good quality lean meat is given twice weekly and sick animals are provided with milk and vitamin supplements as indicated. Animals scheduled for operation the following day are fasted at least 12 hours starting the evening before.

3. Selection of Animals

Adult mongrel dogs of either sex weighing between 15 to 25 kilograms were used in these studies. Upon arrival at the

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* -Dr. Ballard's Animal Foods Limited, Montreal, P.Q., Canada.

** -Mon Dou Grain Limited, Montreal, P.Q., Canada.

Kennel the healthy appearing well-fed dogs, free of gross evidences of disease were selected and assigned metallic numbers secured around their neck a chain collar, weighed and injected with a poly-valent canine vaccine*** at a recommended dose of one milliliter per kilogram body weight. They were matured for one week prior to the initial operation. Particular attention was focused to the presence of any possible acute respiratory ailment, characterized by the presence of either a dry or an unduly moist nostrils, nasal discharge and grunting respiration. The state of appetite and the general behaviour during the period of observation were also noted.

4. Pre-operative Care

On the morning of the procedure, the dog was anesthetized with intravenous Pentobarbital Sodium**** at an initial dose of 25 to 30 milligrams per kilogram body weight and if prolonged anesthesia was necessary, subsequent supplementary dose of 30 to 60 milligrams was given in addition. After a satisfactory level of anesthesia had been induced, the abdomen was shaved and the dog was transferred to an operating table and tied securely. The operative site was prepped with 1% Cetavlon+ and 2.5% Tincture of Iodine following insertion of an endotracheal tube.

*** Anti-Canine Distempter-Hepatitis Leptopsira Canicola Serum. Supplied by the American Cyanamid Company, Princeton, New Jersey, U.S.A.

**** Pentobarbital Sodium 6% - supplied by Siegfried S.A., Zofingue, Switzerland.

\+ 1% Cetrimide Solution 1:1000 - supplied by Ayerst Laboratories, Montreal, P.Q., Canada.

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5. Operative Care

During the procedure, 500 milliliter of 5% Dextrose in Saline containing 500 milligrams of Tetracycline* was infused at a slow drip intravenously, for hydration and general prophylactic purposes. At the onset of recirculation, 10% Low Viscosity Dextran** was rapidly given one gram per kilogram body weight as an anti-sludging agent. Strict aseptic technique was maintained at all times. After the approximation of the anterior rectus sheath, the wound bed was dusted with Sulfathiazole Powder.+ The line of coaptation was sealed with Aeroplast Spray++ at the conclusion of skin closure. Every attempt was made to arouse the animal at the termination of the operation. If need be, one milliliter of Coramine was administered intramuscularly to stimulate prompt recovery and was generally followed by an active response.

6. Post-Operative Care and Follow-up

Two milliliters of Fortymycin+++ was injected intramuscularly once daily for the first five days to minimize respiratory and wound complications.

Urinalysis, weekly blood urea nitrogen and serum creatinine determinations were made for 10 weeks and once a month thereafter Renal clearances were performed under general anesthesia 3 to 4

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* Tetracyn 500 - supplied by Pfizer Co. Ltd., Montreal, P.Q.
** Rheomacrodex - supplied by Pharmacia, Uppsala, Sweden.
+ Sulfathiazole, N.F.X.- Microcrystalline Powder - supplied by Ingram and Bell Ltd., Montreal, P.Q., Canada.
++ Supplied by Aeroplast Corporation, Dayton, Ohio, U.S.A.
+++ Fortimycin Suspension 1/2 - Procaine Penicillin 400,000 U and 0.5 Gm. of Streptomycin. Supplied by Ayerst Laboratories, Montreal, P.Q., Canada.

weeks after contra-lateral nephrectomy and at the end of one year.

7. Biochemical Determinations

a) Blood Urea Nitrogen - It was determined by the Hyland UN-Test Method* (84, 175) (Phenate-Hypochlorite Method). It was based on the principle that ammonia produced by the action of urease on urea, preformed ammonia or ammonia derived from Kjeldahl digestion reacts with phenol in the presence of hypochlorite and nitroprussdite (Berthelot Reaction) to form a blue color, the intensity being directly proportional to the amount of ammonia present in the sample in question. The color reaction is highly sensitive and requires only small specimen volume, thus eliminating any deproteinization step.

After a heparinized peripheral blood sample was withdrawn from the dog, it was immediately spun down for 10 minutes at 1800 revolutions per minute (rpm) in an International Centrifuge, Universal Model UV**. A measured amount of the plasma (0.02 ml.) or 1:100 diluted urine was added to 0.2 ml. of urease to release the ammonia and made to react with 1 ml. of Phenol Color Reagent in the presence of 1 ml. of Alkaline Hypochlorite. The resulting blue solution was diluted with 8 ml. of distilled water and the optical density (o.d.) was read spectrophotometrically together with 2 batches of simultaneously determined 15 mg. nitrogen standard and Labtrol** at 640 millimicrons of the Coleman Uni-

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* Hyland Laboratories, Los Angeles, California, U.S.A.
** Manufactured by International Equipment Company, Needham, Heights, Massachusetts.

versal Model Spectrophotometer*.

b) Creatinine Determination in the Serum and Urine

They were determined by an adsorption technique using Lloyd's reagent (302). This is based on the higher recovery of creatinine chromogen in procedures that yield acid filtrate from plasma, serum or diluted urine and it has proved itself as the most satisfactory method of determination of creatinine in these fluids. The clearance is derived from the formula; UV/P = ml/min U = creatinine concentration in milligrams per 100 ml in the urine; V = rate of urine flow in ml per minute and P = serum or plasma creatinine concentration in milligrams per 100 milliliters.

Six ml of 10% Trichloracetic acid was added to 2 ml of plasma to deproteinate it and centrifuged for 10 minutes at 2800 rpm. Five ml of the filtrate was added with 0.5 ml of Saturated Oxalic Acid and adsorbed with 98 to 102 mg. of Lloyd's Reagent. The resulting precipitate was treated with alkaline Picric Acid after centrifugation, shaken for 10 minutes and centrifuged for another 10 minutes at 2800 rpm. The clear yellow supernatant was read at 520 millimicrons in the Beckman B spectrophotometer together with 5 standards at 22.5°C (room temperature).

In urine containing more than 30 mg% of protein, the latter was thrown out of solution after 1:100 or 1:200 dilution with distilled water, by the addition of 10% Sodium Tungstate and 1/12 N Sulfuric Acid. The filtrate was subsequently treated like the protein-free plasma filtrate.

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* Manufactured by Coleman Instrument Inc., Maywood, Illinois.

c) Determination of Sodium Para-Amino Hippurate

It was determined by the N-(1-naphthyl) ethylenediamine Dihydrochloride Method of Bratton and Marshall as modified by Smith et al (302).

The method depends in diazotizing the para-amino group of the para-aminohippurate with nitrous acid, destruction of the excess acid with sulfamate and coupling it with n-(1-naphthyl) ethylene-diamine dihydrochloride. The clearance = UV/P as in the creatinine clearance.

Protein-free filtrate was made by adding Cadmium Sulfate and Sodium Hydroxide to plasma or 1:1000 diluted urine. The white precipitate was allowed to stand for 10 minutes and centrifuged for another 10 minutes at 2800 rpm. To 5 ml of the filtrate or 1:1000 diluted urine was added 1 ml of 1.2 N Hydrochloric Acid and 0.5 ml of Sodium Nitrite. After 3 to 5 minutes, 0.5 ml Ammonium Sulfamate was added. In another 3 to 5 minutes 0.5 ml of n-(1-naphthy) ethylenediamine Dihydrochloride was added. The optical density of the resulting lilac colored solution was read at 540 millimicrons of the Beckman B spectrophotometer. The value obtained is adjusted by multiplying it with the reciprocal of the simultaneously determined plasma fraction of the blood sample and expressed in 100 milliliters %.

d) Ancillary Procedures

a) Visualization of the Renal Vascular Tree by Means of Schlesinger Radio-opaque Mass (Gelatin-Potassium Io-

dide Barium Sulfate 280)

The mass consists of a gelatin base which is kept liquid at room temperature by the addition of Potassium Iodide (KI) and a contrast medium, Barium Sulfate. It is mixed by means of a Waring Blendor. Formalin, acting upon gelatin much like an activator on plastic, is added prior to the injection in concentration which can be varied so as to assure solidification of the mass with a pre-determined period of time (30 to 60 minutes) at pH 6.2 and 21°C room temperature. Once solidified, the mass yields a flexible casts which handles easily and which greatly facilitates dissection of the vessels. The mass reaches the terminal arterioles with diameters between 40 to 50 millimicrons and does not cross the capillary bed.

The kidney to be injected was suspended in a metal stand and connected to a system consisting of a pressure source from a compressed air with a constant pressure reservoir, a needle valve, a manometer and feeding bottles joined to each other by flexible tubing. The needle valve and the manometer were inserted on a T-tube in the line between the source of pressure and the feeding bottles. The needle valve acted as bleeder to reduce the pressure of the source to that desired in the vessels.

The artery was injected first followed by the vein at 150 and 20 mm.Hg. respectively. Injection time lasted from 10 to 20 minutes. Roentgenographic pictures were taken.

b) Neoprene Injection

Commercial Neoprene was injected in a similar manner as the Schlesinger Mass with the same set-up. Red was used for the

arterial system and blue for the venous system for convenient identification. After the latex had set, the specimen was corroded in concentrated Hydrochloric Acid. In some specimens the casts were subsequently digested with 10% Methyl Salicylate and the outline of the cortical vessels were beautifully revealed(211a).

c) Intravascular India Ink (Higgins) Staining

Thirty milliliters of India ink was injected intraarterially just before disconnecting the recirculated kidney to outline the extend of intra-renal circulation. Fixed paraffin sections were cut and stained with Hematoxylin and Eosin Stain Method.

8. Technique of the Study on the Circulatory Behaviour of Stored (Hypothermia - 4°C and Hyperbaric-hypothermic at 3 ATA-HBO) and Immediately Removed Kidneys

Immediately following nephrectomy or at the end of the preservation period, the kidney was placed on a scale and connected to a femoral vessel of the original host with polyethylene tubings measuring from 1 to 1-1/2 feet in length.

Flow was measured by disconnecting the proximal end of the venous tubing and catching the venous return from the kidney for a measured period of 100seconds at one half, one, two, five, fifteen, thirty, forty-five and sixty minutes from the start of recirculation, with a becker containing heparin and re-infused immediately. The arterial and venous oxygen saturation was determined by means of a Spectrophotometric oximeter*. The blood pressure

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* AO Oximeter, model 10800, American Optical Co.

was monitored by a carotid arterial canula connected to a mercury manometer. The weight was measured with a scale and net weight gain was calculated. After recirculation the kidney was examined grossly, histologically and by injection using India Ink, radio-opaque mass and Latex.

9. Technique of Nephrectomy and Pelvic Autotransplantation

A) Control Group - In a group of 25 dogs, the abdomen was opened through a long vertical midline incision after a satisfactory general anesthesia and aseptic preparation of the operative site. Hemostasis was effected by both electro-cautery and non-absorbable ligatures. The edges of the wound were covered with water repellant towels to prevent contagion and to isolate the viscera. Exposure was aided by means of a self-retaining retractor. The intestines covered with wet large abdominal pads, were retracted to the right side by an able assistant. Then, the left kidney was mobilised by opening the posterior parietal peritonium between hemostats laterally. Exploration of the renal pedicle was carried out posteriorly to ascertain that there were no anomalous blood supply. Only kidneys that were not scarred or congested and having good brownish pink color and soft doughy consistency with good-sized single artery and vein were selected. If the left kidney was otherwise not suitable for transplantation, it was reperitonealized and a similar critical and rigid evaluation of the opposite kidney was undertaken. First preference was given to the left kidney because of the lower location and the ease of removal due to the absence of the multilobar liver

as in the case of the right kidney eventhough the incidence of anomalous blood supply was more frequent on the left side. After the selection was decided, the isolation of the kidney was continued very carefully and gently. Hemostasis had to be nearly if not wholly perfect to visualize the peri-ureteral vasculature. All transections were made between clamps not vice-versa. Painstaking care was exercised to leave the adrenal gland in situ. With the kidney isolated, the ipsilateral iliac vessels were similarly dissected, isolated and tied at the level of the termination of the internal iliac vein after Bulldog clamps were applied proximally. The stumps were irrigated with 10% Low Viscosity Dextran (38a, 135a, 156a, 157a, 183a). The ureter was then sectioned at the junction of the middle and lower third followed by ligatures of the artery and vein in that order flush with the aorta and inferior vena cava respectively, with a heavy non-absorbable material (1-0 silk). The severed kidney was manually irrigated with 10% LVD through the artery, at room temperature (27°C) by means of a plastic syringe with a perfusion pressure approximately 140 mm. Hg. until the venous effluent became clear. The pale and bloodless kidney was promptly connected end to end to the iliac vessels by joining the renal vein to the common iliac vein and the renal artery to the external iliac artery with an over and over technique using waxed 6-0 cardiovascular silk. At the completion of the anastomoses, the renal circulation was re-established. The period of ischemia was around 25 minutes. Ten percent Low Viscosity Dextran was rapidly infused at a dose of one gram per kilogram body

weight at the onset of recirculation, as an anti-sludging agent instead of heparin. The recirculated kidney at this initial period was actively congested and salmon-pink (Figs. 1 and 2).

Fig. 1. - Vascular anastomoses immediately after re-establishment of circulation. Note the absence of constriction. The larger lower vessel is the renal vein joined to the common iliac vein and the smaller upper vessel is the renal artery connected to the external iliac artery.

Fig. 2. - Appearance of kidney shortly after replantation exhibiting salmon-pink hue due to active congestion.

The lowest level of the ureter was selected which was more or less similar in gross appearance as the kidney, indicating a good vascular supply. This segment was transected and split longitudinally along its anterior surface avoiding the delicate ureteral vessels for a distance of 5 to 8 millimeter and was spread out in a fan-like shape. The distal end was telescoped through a transmural opening in an avascular area of the trigone in the immediate vicinity of the natural uretero-vesical ostium. A mucosa to mucosa approximation between the ureter and the bladder was carried out through a counter-incision on the supero-anterior surface of the bladder with a 4-0 plain catgut with a swedge-on needle. Patency of the ureteral opening was ascertained by gently introducing a closed straight mosquito clamp and most often by the spurting of a stream of urine (Fig. 3). The uretero-neocystostomy was further secured by suturing the ureter to the serosal surface of the bladder emploing 6-0 cardiovascular silk. The counter-incision was closed in a three-layered conventional fashion. The kidney was anchored snugly in the pelvis making sure that the vessels were not kinked or compromised in any way. The rent in the peritoneum was repaired after the opposite kidney was removed. The operation was terminated by closing the abdomen in the usual manner.

10. Technique of Hypothermic Preservation

The left kidney of 10 animals was removed as described above and cooled immediately by combined surface and core-cooling method accomplished by immersion of the ischemic kidney in a kidney basin

Fig. 3. - Uretero-vesical Anastomosis.

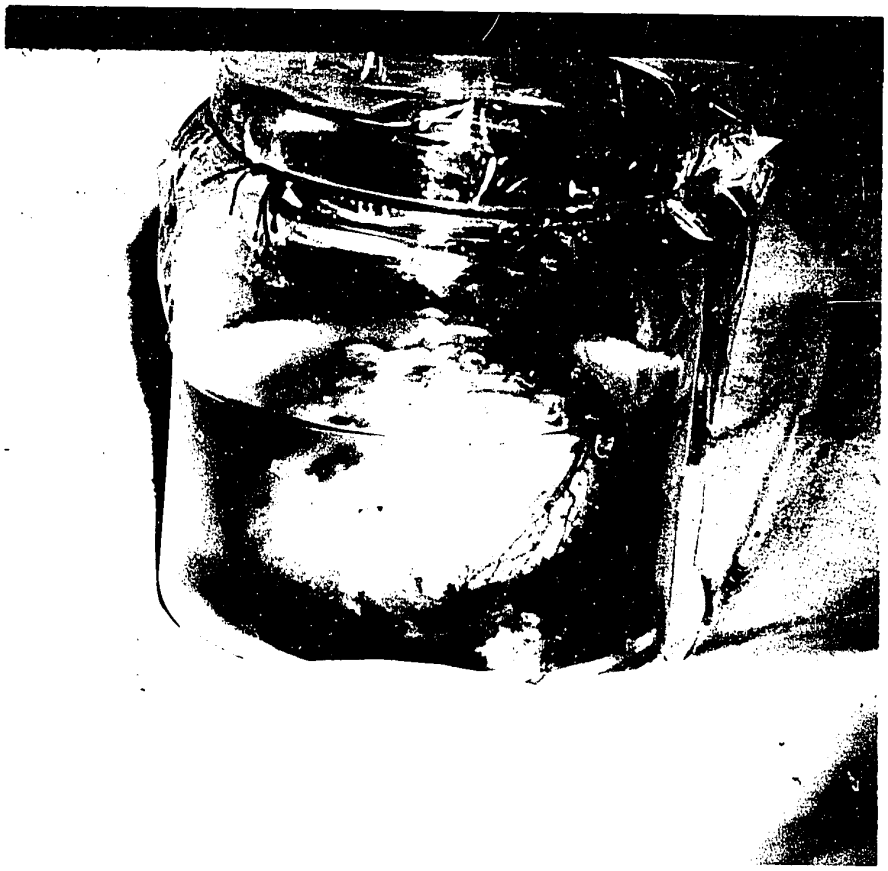

Fig. 4. - Cooled Kidney Immersed in Tis-U-Sol Inside a Sterile Glass Container.

filled with cooled (4°C) Tis-U-Sol* and irrigation with cooled (4°C) 10% LVD in Water in the standard fashion until the venous outflow became clear. The super-cooled kidney was transferred into a suitable glass jar half-filled with refrigerated Tis-U-Sol and covered with a fenestrated plastic material (Vi-Drape)**

The fenestration was done to prevent possible accumulation of gaseous metabolic by-products that might be deleterious to the stored kidney (Fig. 4).

This manoeuver was carried out within 10 minutes. The glass jar was promptly kept in the storage room whose thermostat was set at 4°C with less than one degree of fluctuation. The following day, 24 hours later, the preserved kidney was autotransplanted into the pelvis of the original host in the usual manner.

During the replantation, the kidney was covered with a constantly cooled wet abdominal pad to effect a gradual rewarming process. In the first 30 minutes of recirculation the flow was restricted by partial occlusion of the artery with a bulldog clamp applied over a circumferentially wrapped gauze pad, the flow was regulated to prevent undue turgidity.

Stored kidneys have lost some amount of their vascular tone and are not able to maintain their auto-regulatory function and usually lead to a markedly increased consistency of the kidney eventuating in various degrees of venous outflow block which is considered harmful to the ultimate fate of the kidney. Thus, it

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* Physiological Irrigating Solution - Supplied by the Baxter Laboratories of Canada, Ltd., Alliston, Ontario, Canada.
** Supplied by the Aeroplast Corporation, Dayton, Ohio, U.S.A.

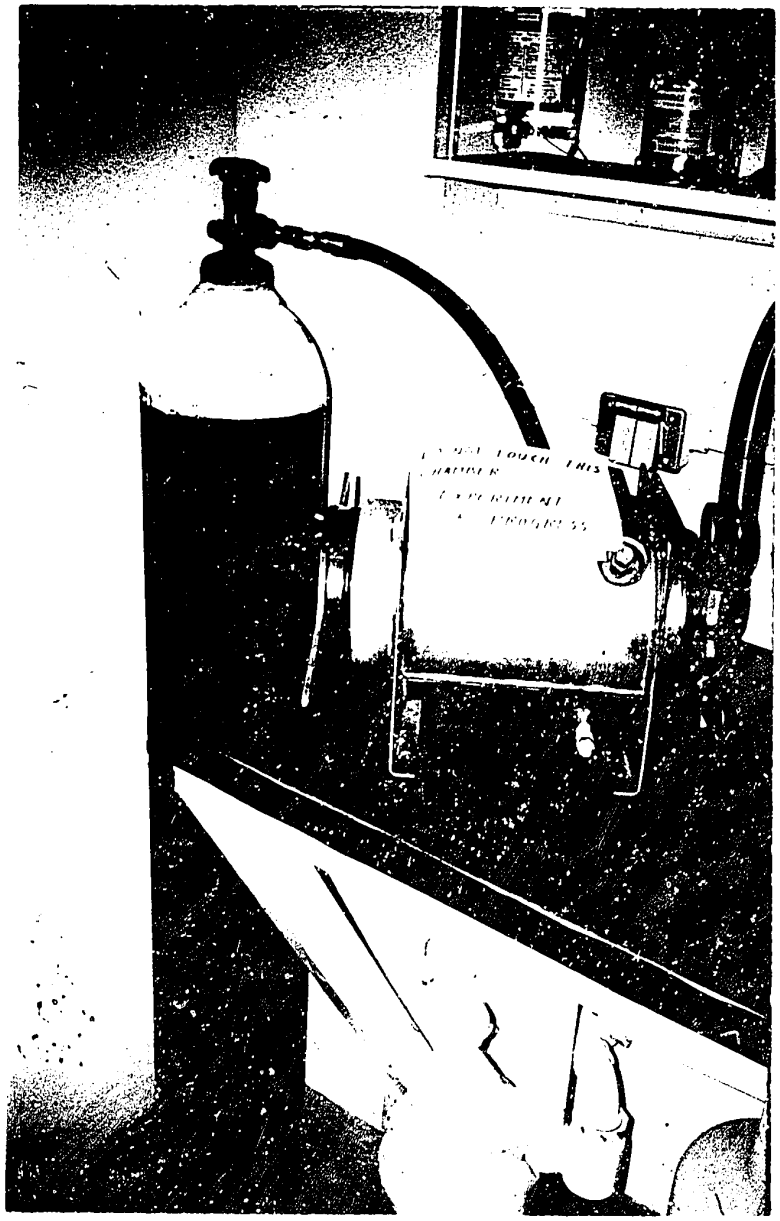

Fig. 5. - Hyperbaric Chamber suitable for small animal and individual organ work.

was felt that artificial restriction of the volume flow initially, would prevent the occurrence of this vicious cycle in the meantime that the renal vasculature is regaining its inherent auto-regulatory mechanism.

Corollary studies along this concept have demonstrated a gradual increase of weight of isolated kidney associated with an inverse decline of the volume flow and oxygen extraction as measured by arterial and venous oxygen saturation difference and most often with a diminution or cessation of visible urine flow.

Hyperbaric Chamber

It is shown in Fig. 5*. It is horizontally propped double-walled stainless steel cylinder designed to withstand pressures up to 500 pounds per square inch - approximately 35 atmospheres, absolute. There is an inlet at the top and an outlet at the bottom of the outer jacket to permit circulation of fluid at an appropriate temperature to control the inner chamber temperature. Mounted through one end of the cylinder are: a pressure gauge and a thermometer from which inner chamber pressure and temperature are monitored and 2 one-way needle valves permitting ingress and exit of oxygen through a flexible connector from a portable tank. The opposite end is closed by an O-ring type of rubber seal.

11. Technique of Hyperbaric Preservation

The kidney of 10 dogs was promptly pressurized at 5 ATA-HBO** after it was similarly irrigated with 10% LVD at 27°C. The hyperbaric tank was kept at room temperature (27°C). Twenty-four

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* - Designed and constructed by the Dominion Welding and Engineering Company of Canada, Ltd., Montreal, P.Q., Canada.

** - Atmospheres, absolute, Hyperbaric Oxygen.

hours later, the kidney was replanted into the pelvis of the host.

Operating Hyperbaric Chamber*

It was designed and built by the Dominion Welding Engineering Company Limited (Fig. 6, 118a) in accordance with the American Society of Mechanical Engineers Pressure Vessel Code Section VIII and Inter-provincial Regulations. The maximum pressure is 100 pounds per square inch gauge (p.s.i.g.) or 6.8 atmospheres, absolute.

It has a main chamber and a man-lock. The former has two bunk seats of which one can be swung out to form a suitable bed for patient care while the latter serves the purpose of initial compression and decompression room to allow switching of personnel during diving.

A medical lock is provided in the wall of the main chamber with an internal and external locks to permit passing of equipment and drugs while the main chamber is pressurized. The rise and fall of temperature during compression and decompression are controlled by an air-conditioning system which automatically adjusts the chamber temperature to a pre-set level.

Compressed air comes from two sources:

1) A low-pressure system consisting of a water-sealed 20 H.P. compressor with a bypass system feeding directly to the chamber console manifold.

2) A high-pressure system of a non-lubricated water-cooled 10 H.P. reciprocating compressor which discharges air into a large

* - Designed and Constructed by the Dominion Welding Engineering Company of Canada, Ltd., Montreal, P.Q., Canada.

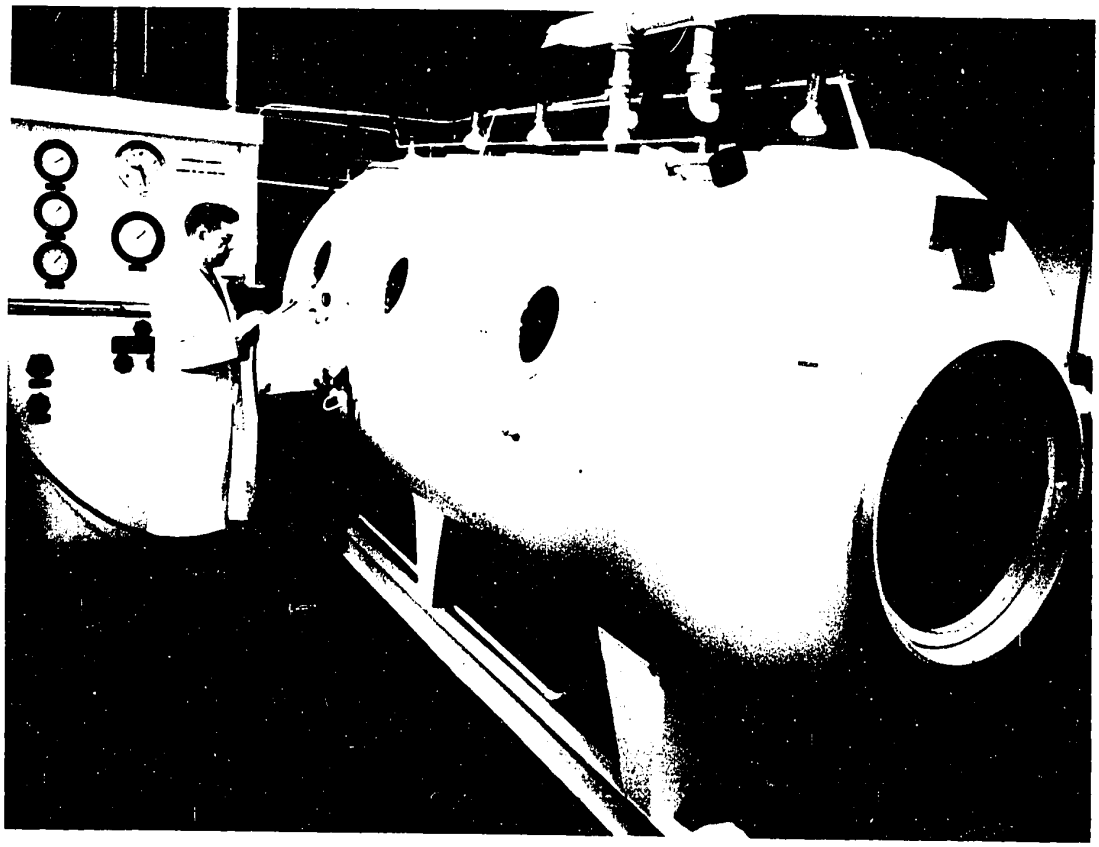


Fig. 6. - Operating Hyperbaric Chamber at the Surgical Laboratories of the Royal Victoria Hospital, Montreal, Province of Quebec, Canada

volume receiver which is also used for rapid pressurization.

The chamber is never pressurized with pure oxygen. Lights and other electrical gadgets are located outside to minimize fire and explosion hazards. Flammable material in the chamber is reduced to a minimun. Lighting is provided through ports in the ceiling.

Pyrene fire extinguishers (Type "K" air repellant) are provided in each compartment.

A two-way station intercommunicating system is used so that the chamber operator is able to hear conversation of the chamber occupants at all times, in addition to two explosion-proof telephone receivers, one in each chamber. Five glass ports are present in the side walls of the chamber to allow observation of those inside.

12. Technique of Hyperbaric-Hypothermic Preservation

a) Twenty-Four Hour Preservation

Supercooling and irrigation of the isolated kidney of 56 dogs were carried out as described. The glass jar containing the kidney was deposited with dispatch in the hyperbaric chamber which was kept permanently in the storage room at 4°C and pressurized to 2, 3, 5, 10, 20 or 30 ATA-HPO. The speed of pressurization was determined solely by the elevation of the recorded inner chamber temperature as shown by the gauge due to the heat of compression. Extreme care was exercise to avoid exceeding 10°C reading. Although no precise measurements were made to correlate the ambient temperature rise with the core tempera-

ture of the stored kidney, nonetheless, it was thought that the temperature elevation should be minimized as much as possible. The time of compression was in the order of 5 to 10 minutes. If the kidney was not compressed until after 30 minutes of ischemia, it did not seem to get the full benefit of the increased oxygen pressure and the kidney invariably failed or behaved like a hypothermic one.

Twenty-four hours later, the storage tank was slowly decompressed at the rate of 10 ATA per hour for compression pressures above 5 ATA and replanted to the pelvis in the standard manner. Failure to observe this decompression precaution by too rapid a pressure release, resulted in a precipitous temperature drop to sub-zero points and in massive air embolism which were grossly observed especially in the peri-renal fatty tissue. It was congested and abundantly studded with air emboli. This was thought to occur in the kidney substance itself. Both factors were proved to be deleterious to the immediate and ultimate fates of the transplant. In earlier preliminary studies, kidneys that were rapidly decompressed became engorged and cyanotic with no venous outflow (Fig. 7-9). These organs did not improve with time and were doomed to failure..

b) Forty-Eight Hour Preservation

A similar procedure was followed in the kidney of 18 dogs stored for a period of 48 hours.

In the kidney of another 11 animals, both nephrectomy and replantation were done in the operating hyperbaric tank at 3 ATA

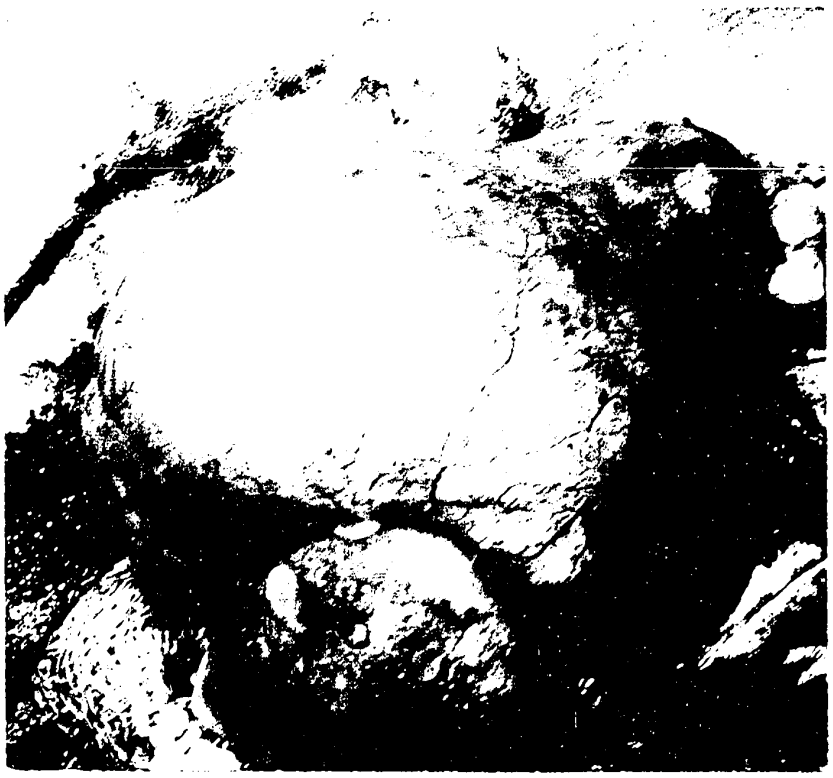


Fig. 7. - Gross appearance of a kidney preserved at 30 ATA-HBO for 24 hours with rapid decompression. Congestion, cyanosis and gas bubbles in the perinephric tissues are evident. There was no venous return in this kidney on re-circulation.

Fig. 8. - Cross-section of a kidney which was rapidly decompressed following 24 hours storage at 4 C and 30 ATA-HBO. Congestion of the medulla and cortico-medullary junction was present.

Fig. 9. - Cut-Section of the vascular anastomoses of the kidney in Figs. 7 & 8. No clot was found in either vessel.

room air with the dog breathing 100% oxygen.

c) Seventy-Two Hour Preservation

The preservation time in 7 kidneys was extended to 72 hours at 5, 20 and 30 ATA-HBO. Nephrectomy and replantation were performed at ambient air condition.

13. Technique of Biopsy and Contra-lateral Nephrectomy

Two to three weeks after replantation of the stored kidneys, under general anesthesia, the replanted organ was exposed through the original incision and a wedge of renal tissue was procured, fixed in 10% Formalin and stained with Hematoxylin and Eosin (H & E) and Periodic Acid Schiff (PAS) Techniques.

The renal defect was sutured with 3-0 chromic catgut in a swedge-on needle. The opposite kidney was removed and a cortico-medullary section (5-6 micra) was taken for fixed paraffin sections. The abdomen was then closed in layers.

14. Technique of Urinary Clearances of Creatinine and Para-Aminohippurate Sodium

a) Endogenous Creatinine Clearance

Within one month after contra-lateral nephrectomy in the experimental groups, and after a month following replantation in the control group, the 12-hour water restricted dog was tested under general anesthesia. Eight milliliter of heparinized peripheral venous blood was drawn to serve as a blank and baseline for blood urea nitrogen and serum creatinine determinations. Filliform or No. 10 to 14 milliliter-bag Foley catheter was introduced into the bladder for the male and female respectively,

under aseptic conditions and fasting specimen was collected and designated as such. The catheter was precisely positioned at the most dependent portion of the bladder by pulling it out gently from the bladder after it had started draining and then pushed in again just far enough to start draining again. After the bladder was emptied and washed with normal saline solution, 500 ml. of 5% Dextrose in Water was infused to induce diuresis. After a period of stabilization was achieved (30 to 45 minutes), four 20-minute period blood and urine samples were analyzed for creatinine concentrations.

b) Para-Amino-Hippurate Clearance (PAH)

A priming dose of 20% Sodium Para-Amino-Hippurate 0.04 mg. per kilogram body weight was infused and immediately followed by a sustaining dose of 0.015 x 600 im milliliters (estimated renal plasma flow) diluted to 250 ml of Ringers Solution and given at a constant drip of 2 ml per minute. After an equilibriation period of 30 to 45 minutes, 4 20-minute blood and urine samples were collected and determined for PAH concentrations.

c) Extraction Ratio Studies of PAH

At the conclusion of the urinary clearance studies of creatinine and PAH, the dog underwent an abdominal laparotomy through a midline abdominal incision. Three to four renal arterial and venous blood samples were taken simultaneously at 15-minute intervals and measured for PAH concentrations in mg. per 100 ml.

III. Results

A. Survival Study

Animals which survived contra-lateral nephrectomy are con-

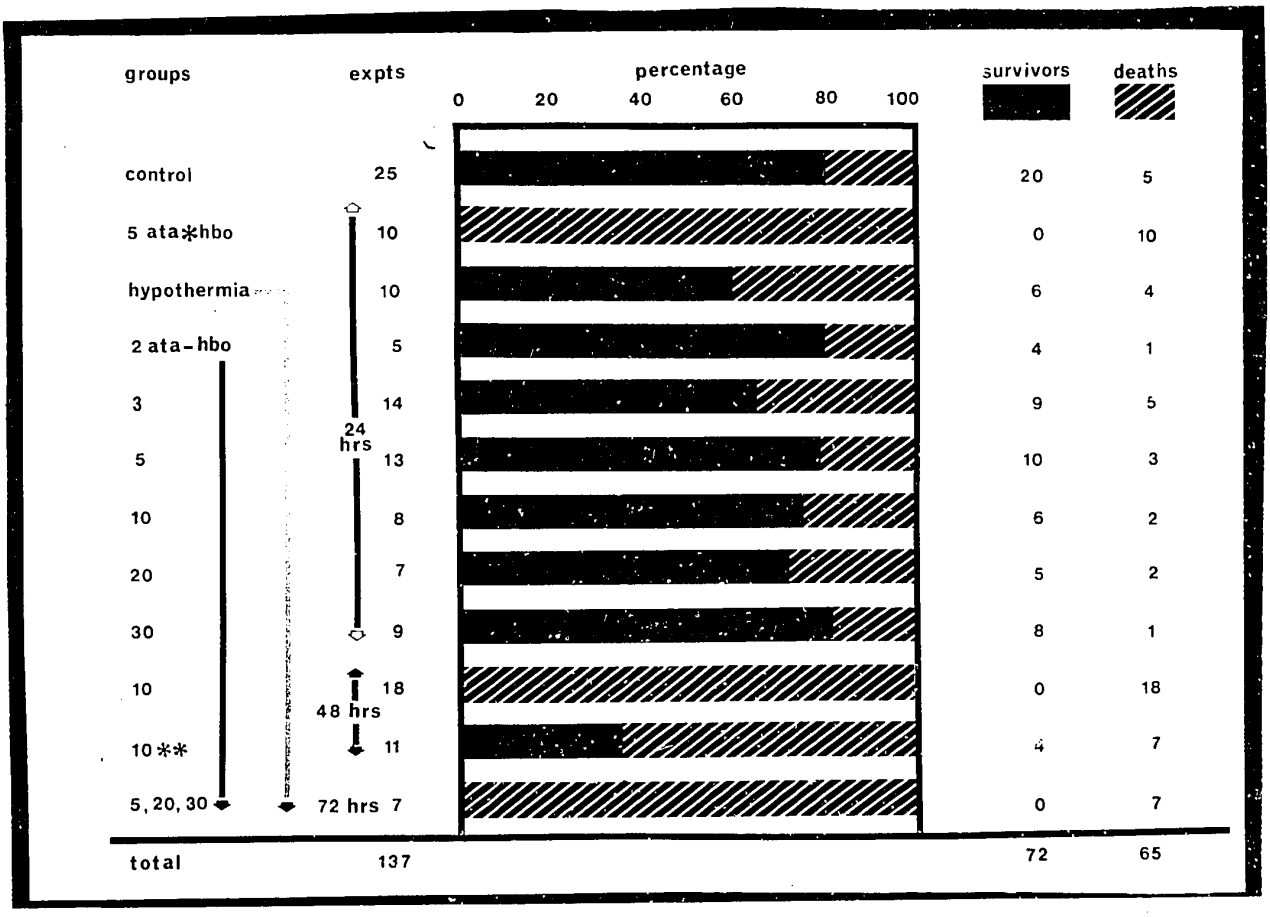


Fig. 10. - Survivors and Mortalities of all experimental groups.

* - Hyperbaric oxygen

** - Excision and replantation were done in the operating hyperbaric chamber.

sidered and all deaths listed in Table II and showb in Fig. 10, occurred after the opposite kidney had been removed. Deaths not directly related to renal failure but due to difficulty with multiple renal vessels, infections and failure of the hyperbaric chamber are listed as deaths due to technical faults. Those animals which had a prompt rise in blood urea nitrogen and other evidences of renal failure after removal of the opposite kidney are termed deaths from uremia. In a few animals, the cause of death was not determined.

There were 72 original survivors from a group of 137 animals autografted. Long-term survivors were sacrificed at 8 to 36 months. A survival rate of 80% was achieved for the control, 60% for the hypothermia group and from 64% to 89% for all hyperbaric-hypothermic preservation groups when the period of storage did not exceed 24 hours.

These results indicate that the kidney can be removed and replanted immediately without danger of late uremia. Causes of failure were technical in nature.

Hypothermia offered considerable protection for 24 hours of storage but late uremia, 4 of 10 was the major cause of death.

Hyperbaric oxygen alone did not confer any protection to the kidney stored for 24 hours at room temperature.

Hyperbaric oxygen combined with hypothermia as a method of storage was followed by late death due to renal failure in 8 of 56 transplants. Two of these animals which succumbed had immediate contra-lateral nephrectomy. Technical factors including anesthesia

GROUPS	EXPERIMENTS	SURVIVORS AND %	CAUSE OF DEATH UREMIA	TECHNICAL	U
I CONTROLS	25	20, 80%		5	
II 5 ATA-HBO+27°C+24 HOURS	10	0, 0%	10		
III HYPOTHERMIA(4°C) " "	10	6, 60%	3		
IV 2 ATA-HBO " " "	5	4, 80%		1	
V 3 ATA-HBO " " "	14	9, 64%	5		
VI 5 ATA-HBO " " "	13	10, 77%		2	
VII 10 ATA-HBO " " "	8	6, 75%	1	1	
VIII 20 ATA-HBO " " "	7	5, 71%	1	1	
IX 30 ATA-HBO " " "	9	8, 89%	1		
X 10 ATA-HBO " 48 "	18	0, 0%	18		
XI 10 ATA-HBO* " " "	11	4, 36%	7		
XII 5, 20, 30 ATA-HBO (4°C) 72 HOURS	7	0, 0%	7		
TOTAL	137	72	53	10	

TABLE II - SURVIVORS AND CAUSE OF DEATH OF VARIOUS GROUPS

* Excision and replantation performed in operating hyperbaric chamber at 3 A

	EXPERIMENTS	SURVIVORS AND %	CAUSE OF DEATH UREMIA	TECHNICAL	UNDETERMINED
	25	20, 80%		5	
+27°C+24 HOURS	10	0, 0%	10		
IA(4°C) " "	10	6, 60%	3		1
" " "	5	4, 80%		1	
" " "	14	9, 64%	5		
" " "	13	10, 77%		2	1
0 " " "	8	6, 75%	1	1	
0 " " "	7	5, 71%	1	1	
0 " " "	9	8, 89%	1		
0 " 48 "	18	0, 0%	18		
0* " " "	11	4, 36%	7		
ATA-HBO (4°C)	7	0, 0%	7		
	137	72	53	10	2

TABLE II - SURVIVORS AND CAUSE OF DEATH OF VARIOUS GROUPS

and replantation performed in operating hyperbaric chamber at 3 ATA.

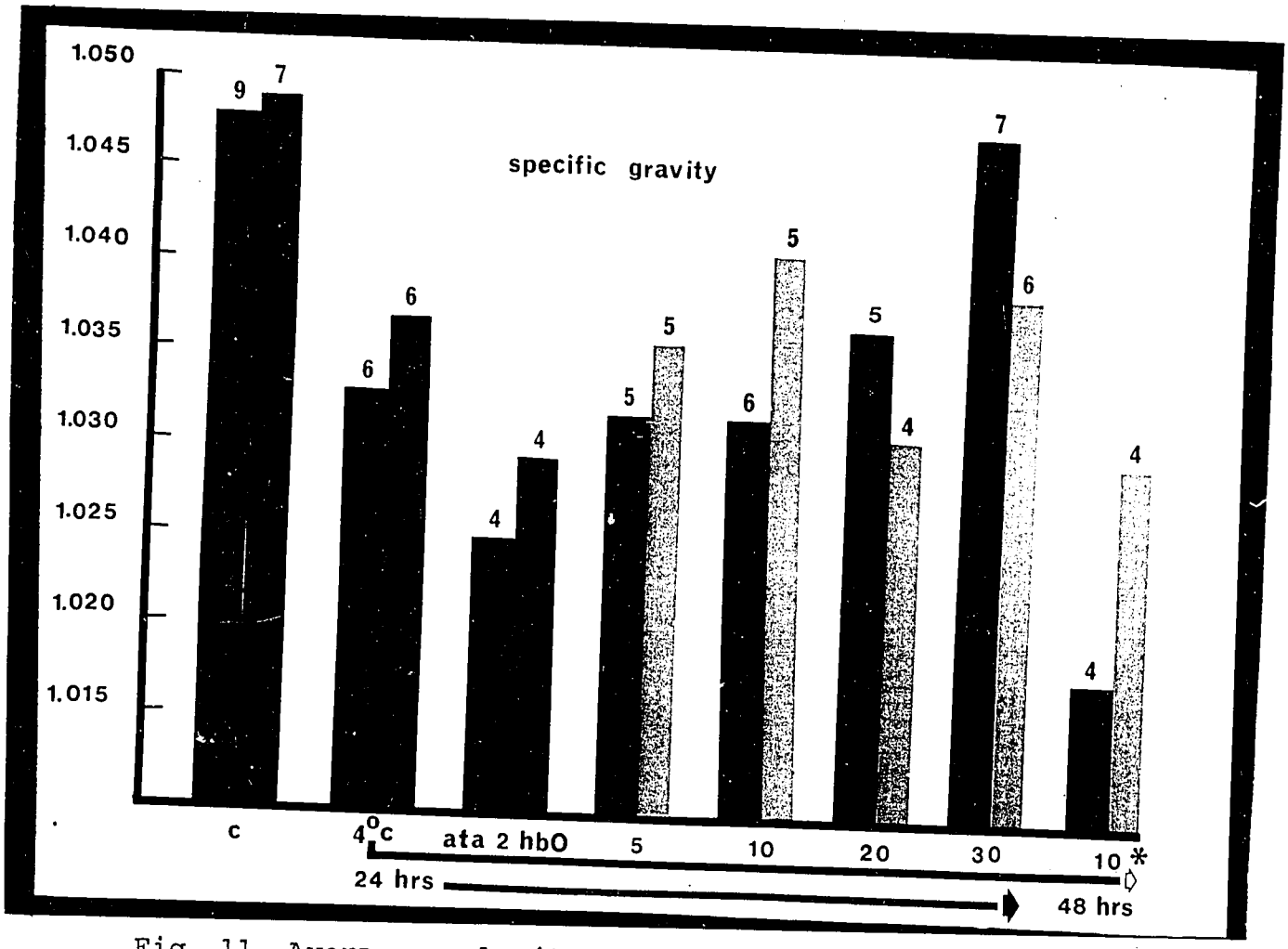


Fig. 11. Average early (1st months) and late (prior to sacrifice) specific gravity of 12-hour fasting urine of long-term survivors.

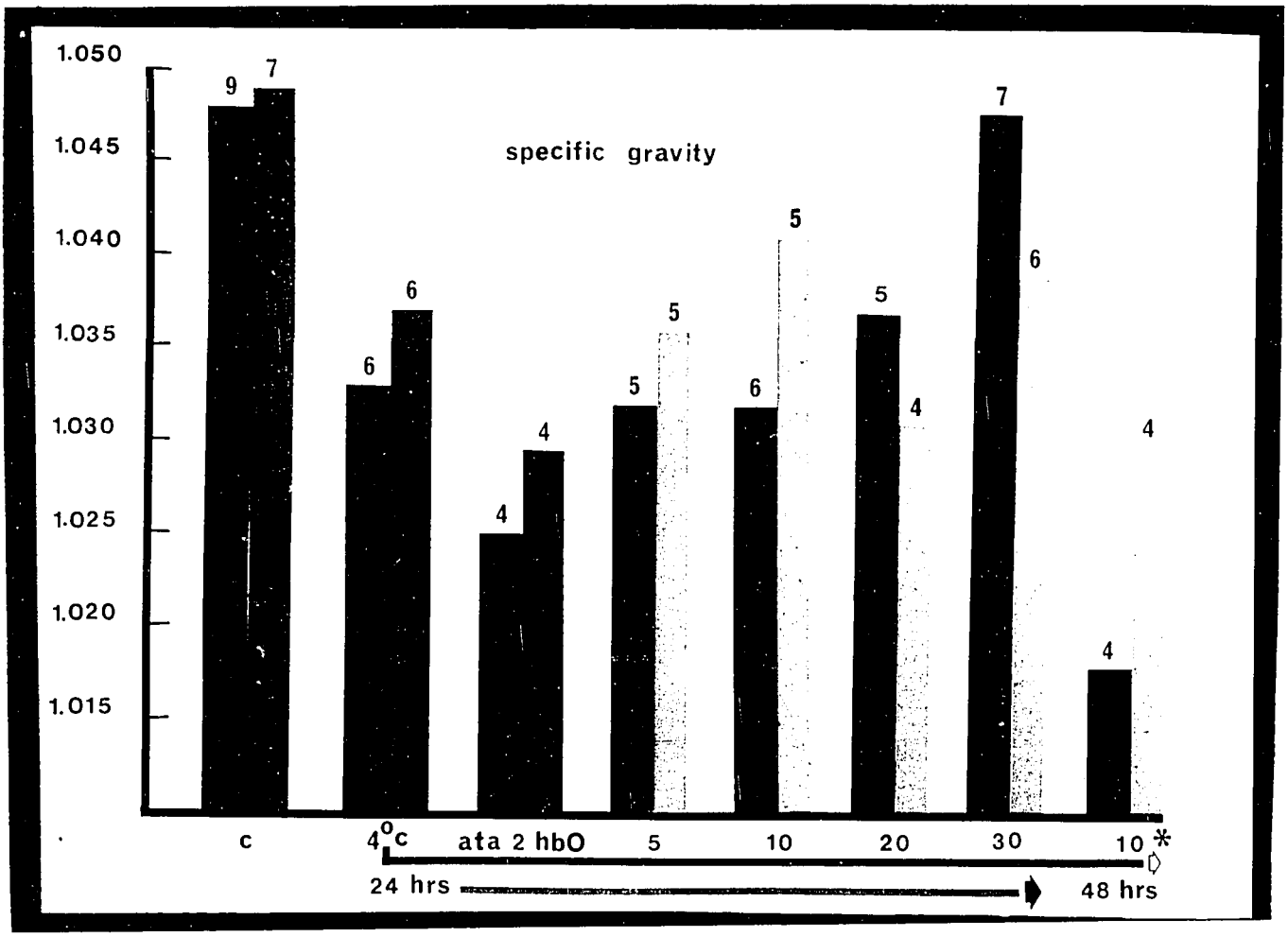


Fig. 11. Average early (1st months) and late (prior to sacrifice) specific gravity of 12-hour fasting urine of long-term survivors.

TABLE III - COMPARISON OF EARLY AND LATE SPECIFIC GRAVITY OF 12-HOUR FASTING URINES

GROUPS	No.	SPECIFIC GRAVITY EARLY (1st 6 months)	OSMOLARITY LATE (at sacrifice)	
I CONTROL	1	1.035		
	2	1.035		
	3	1.052	1.053	
	4	1.060	1.052	
	5	1.051	1.053	
	6	1.050	1.045	1735
	7	1.054	1.052	2060
	8	1.055	1.046	1785
	9	1.039	1.041	
MEAN	9	1.048	1.049	1860
II HYPOTHERMIA (4°C)	162	1.029	1.037	1500
	164	1.024	1.039	1392
	193	1.030	1.024	
	201	1.039	1.044	1591
	219	1.046	1.042	1781
MEAN	5	1.034	1.037	1566
III 2 ATA-HBO+4°C 24 HOURS	206	1.031	1.032	1288
	232	1.027	1.027	1072
	236	1.014	1.020	695
	238	1.029	1.036	1362
MEAN	4	1.025	1.029	1004
IV 5 ATA-HBO+4°C 24 HOURS	13	1.052	1.043	
	14	1.042	1.032	1311
	16	1.030	1.045	1754
	38	1.025	1.013	
	50	1.012	1.044	1722
MEAN	5	1.032	1.035	1596

TABLE III - CONTINUED

GROUPS	No.	SPECIFIC GRAVITY EARLY (1st 6 months)	OSMOLARITY LATE (at sacrifice)	
	87	1.024	1.054	
IV	100	1.034	1.033	1163
10 ATA-HBO+4°C	106	1.037	1.034	
24 HOURS	109	1.024		
	112	1.044	1.031	1011
	127	1.029	1.052	1810
MEAN	6	1.032	1.041	1328
	117	1.017		
V	119	1.027	1.015	650
20 ATA-HBO+4°C	128	1.039	1.026	1039
24 HOURS	138	1.039	1.031	1011
	161	1.064	1.053	1960
MEAN	5	1.037	1.031	1165
	54	1.049	1.030	1212
VI	141	1.054	1.045	1820
30 ATA-HBO+4°C	142	1.046	1.037	1591
24 HOURS	148	1.059	1.033	1260
	155	1.044	1.045	1290
	160	1.038	1.045	1658
MEAN	6	1.048	1.039	1472
VII	169	1.013	1.015	765
10 ATA-HBO+4°C	212	1.016	1.018	
48 HOURS	220	1.015	1.025	1062
	270	1.028	1.032	1274
MEAN	4	1.018	1.023	1034

and pneumonia as well as uremia contributed to the 10 deaths from animals which received kidneys stored for 24 hours using hyperbaric oxygen in combination with hypothermia.

When hyperbaric oxygen and hypothermia was used to preserve kidneys for 48 or 72 hours, uremia was the cause of death in all animals. Four of 11 animals survived when their kidney had been excised and replanted in the hyperbaric chamber even when their kidney had been preserved for 48 hours, with the dog breathing 100% oxygen through a cuffed endotracheal tube.

B. Quality of Survival

1. Concentration Test

Renal function was assessed in all survivors which were followed at least 6 months and up to 3 years after auto-transplantation and contra-lateral nephrectomy. The results of the 12-hour water restriction test are summarized in Table III and depicted in Fig. 11. Kidneys in all groups were able to achieve urine concentrations above 1.030 and to maintain their concentrating abilities during the period of study except the kidneys which had been previously stored for 48 hours which sustained severe impairment and sustained only modestly with time and those kidneys preserved for 24 hours at 2 ATA-HBO.

2. Blood urea nitrogen (BUN) and serum creatinine (Cr)

The alterations of BUN and Cr with time is illustrated in Figs. 12-16. Sudden elevations in BUN and Cr are noted immediately after removal of the opposite kidney which subsequently dec-

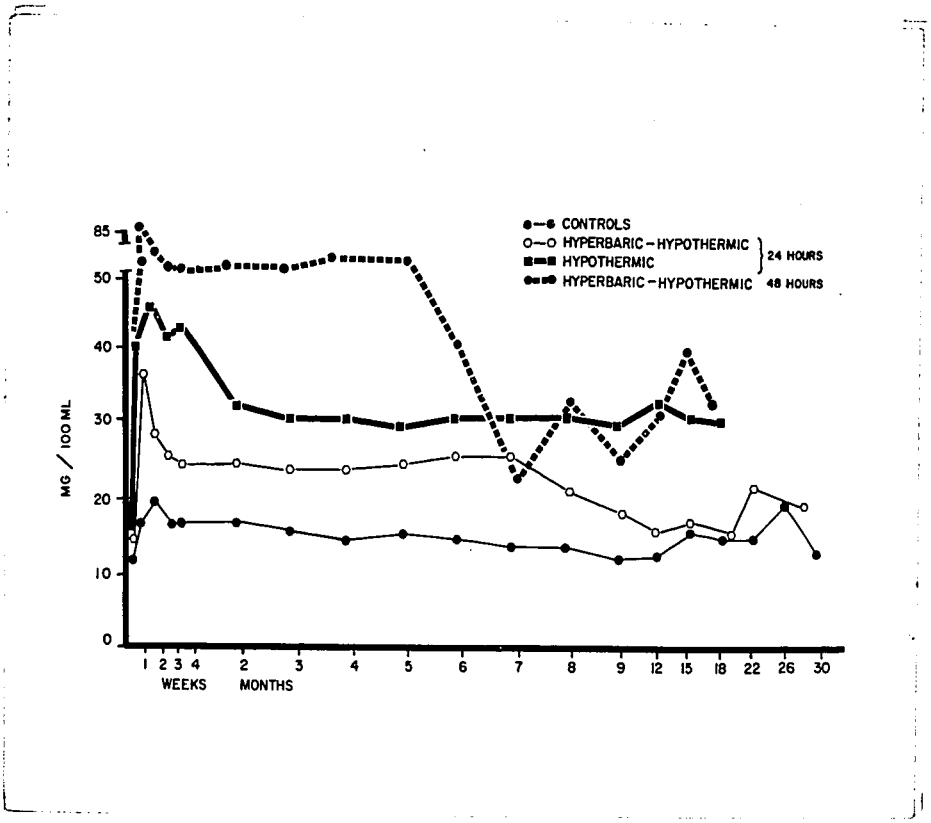
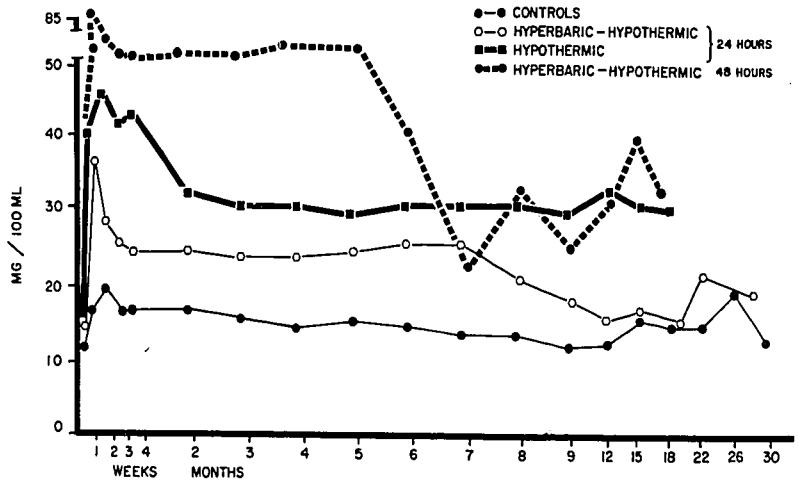


Fig. 12. - Blood urea nitrogen. There is a rise in BUN levels after the removal of the contralateral kidney which subsequently decline to a stable level in 3 to 8 weeks. This is least and shortest for the controls and greatest and longest for those animals which received kidneys stored for 48 hours.



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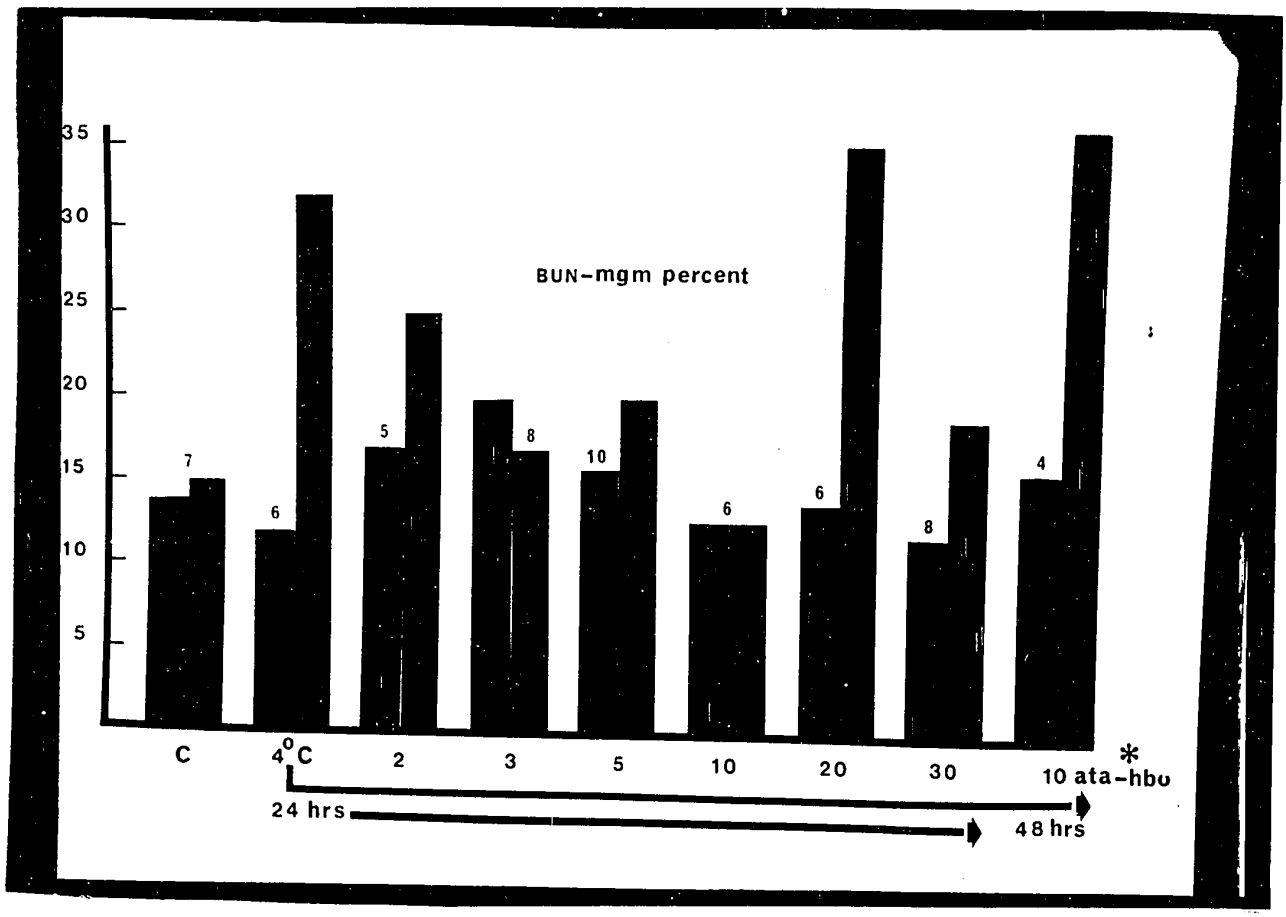

Fig. 13. Average pre-operative and latest BUN of all groups.

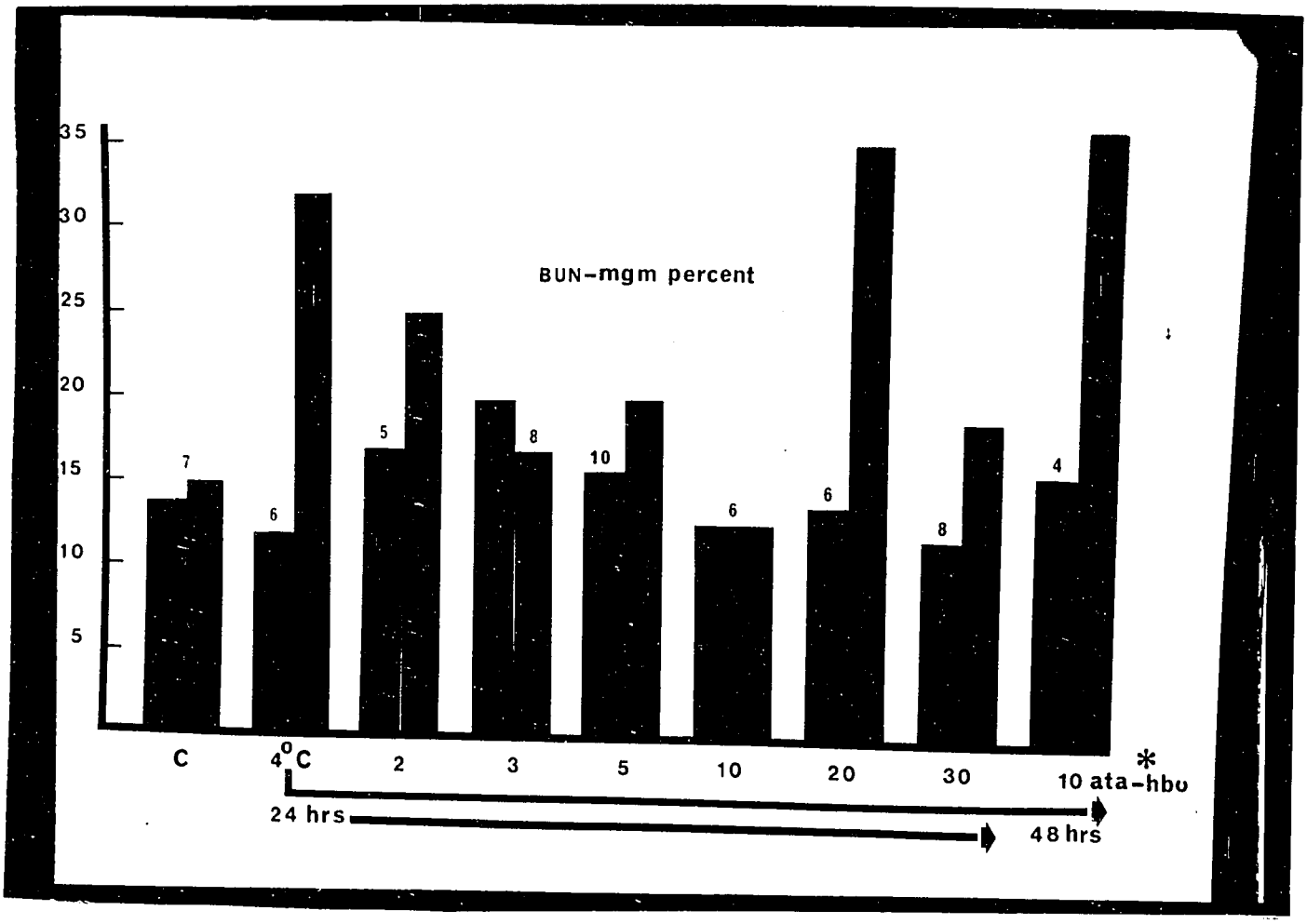

Fig. 13. Average pre-operative and latest BUN of all groups.

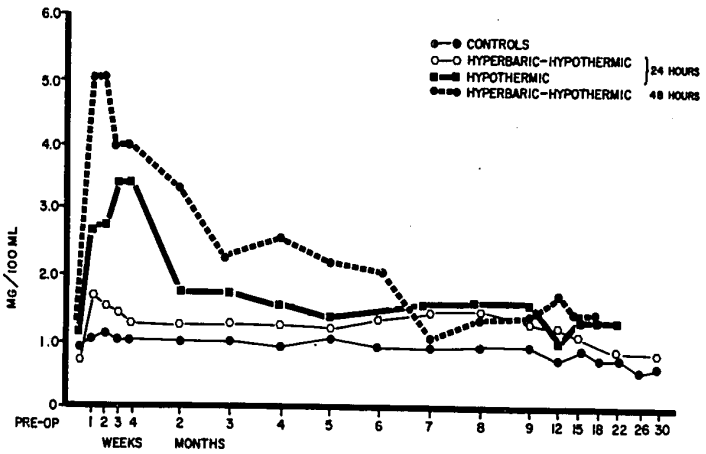

Fig. 14. - Serum creatinine levels of the main experimental groups depict curves parallel to the BUN.

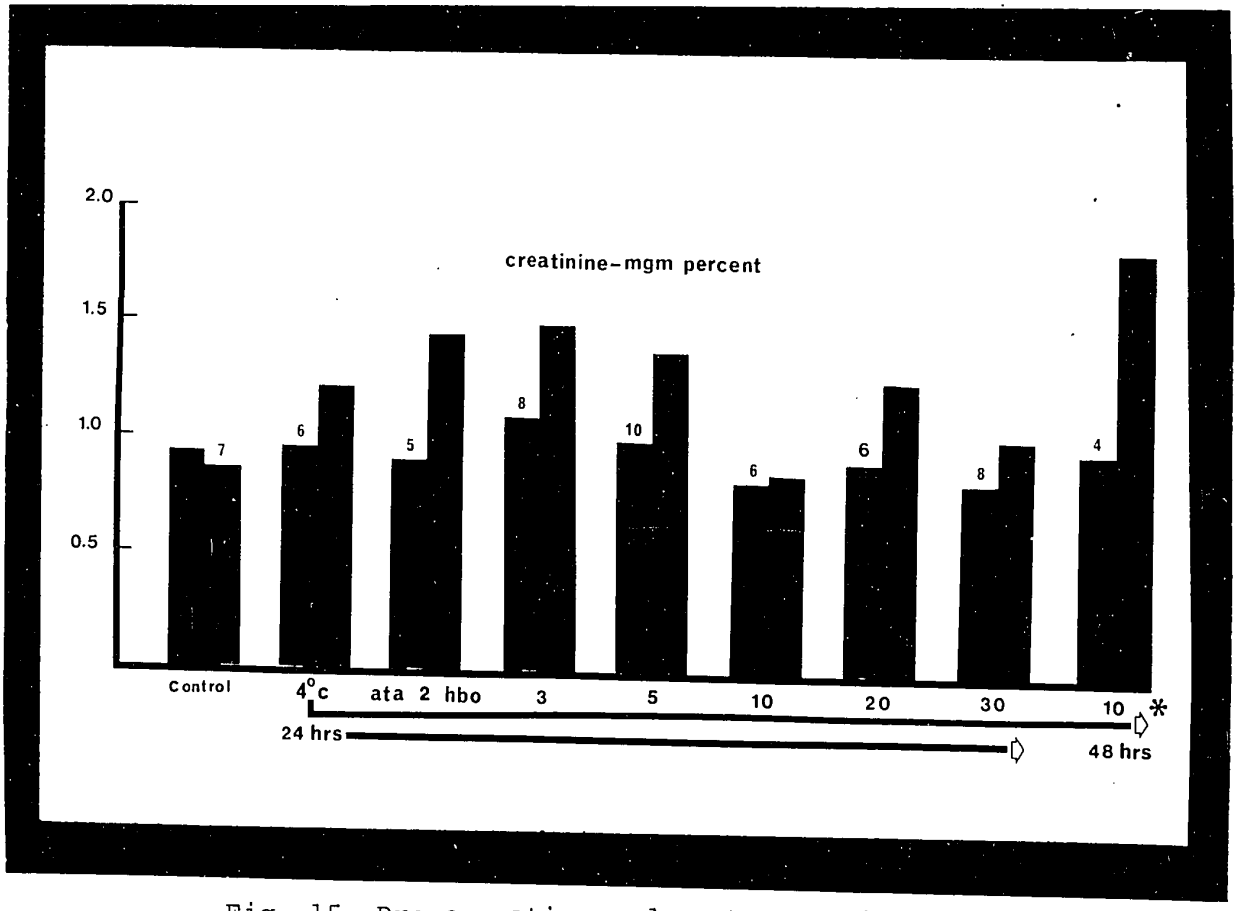

Fig. 15. Pre-operative and post-operative serum creatinine levels of all groups.

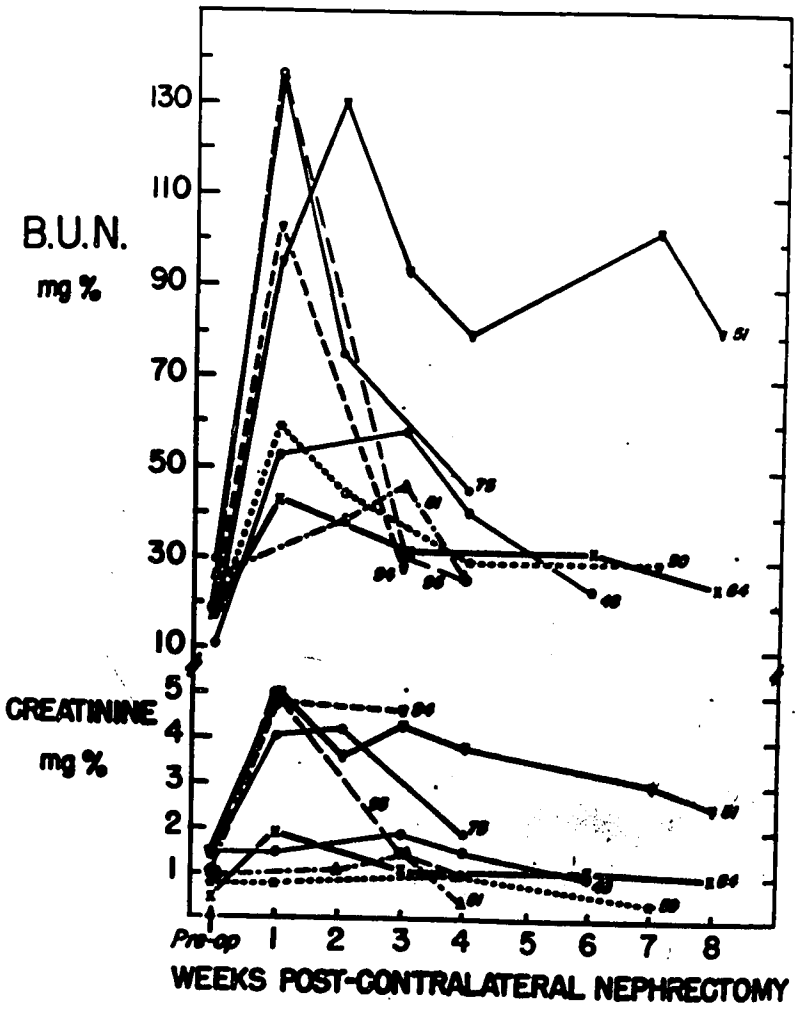

Fig. 16. - Levels of BUN and serum creatinine during the early weeks of kidneys preserved at 3 ATA-HBO for 24 hours.

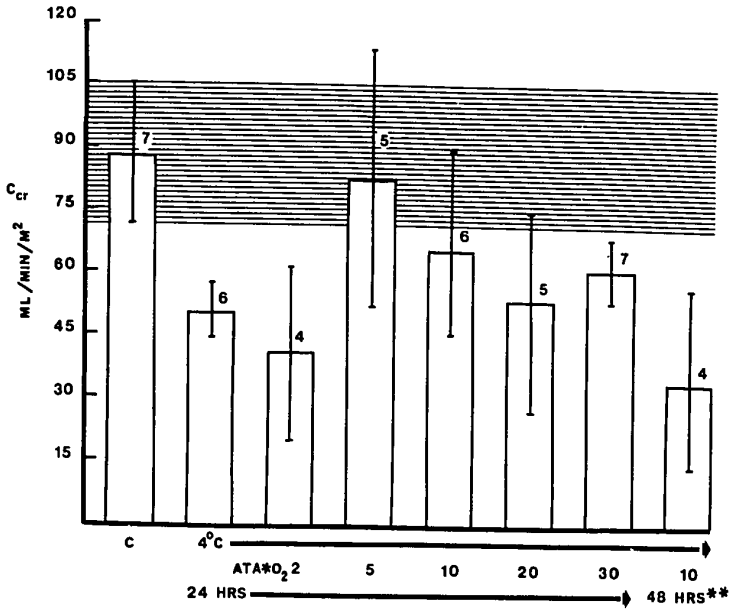

Fig. 17. - Histogram showing the average creatinine clearance with standard deviations of long-term survivors. The GFRs of kidneys stored at 5 and 10 ATA-HBO for 24 hours compare well with the controls.

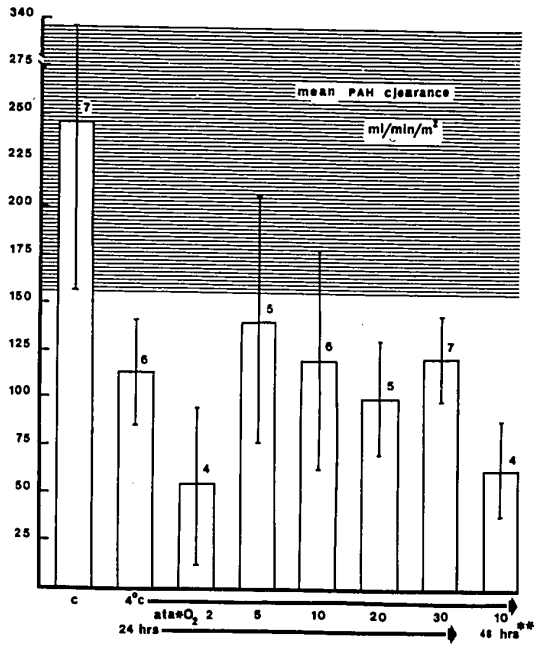

Fig. 18. - Histogram illustrating average PAH clearance with standard deviations of long-term survivors. The tubular excretions of PAH by kidneys stored at 5 ATA-HBO can be considered comparable to the control group.

line to stable levels in 3 to 8 weeks. Controls and hyperbaric-hypothermic groups have lesser rises of BUN and Cr and return to normal levels sooner. When hypothermia alone was used or if the period of preservation exceeded 24 hours, much higher levels were found and the return towards normal was very prolonged. Persistent elevations of BUN and Cr beyond 8 weeks usually reflected severe damage to the kidney which would not be able to sustain life of the animal for an extended period of time. The precipitous drops in BUN and Cr in animals carrying kidneys stored for 48 hours after the 6th month were due to the demise of a dog having such severely damaged kidney.

Very few dogs survived auto-transplantation of a kidney stored for 48 hours and the fact that these few regained some of the lost excretory and homeostatic functions as here assessed must indicate only that it is possible for a kidney to survive this prolonged period of storage.

3. Average clearances of creatinine(C_{cr}; 251, 254) and PAH (302) in milliliters per minute per square meter of body surface ($ml/min/m^2$; 95, 106).

The average glomerular filtration rates (GFR) as measured by C_{cr} and the average renal plasma flows (RPF) as assessed by C_{pah} of the various groups together with the corresponding levels of significance are shown in Table IV and Figs. 17 and 18, while the individual GFR and RPF are depicted in Table I, Appendix A.

Normal GFR was demonstrated by the controls and the hyperbaric-hypothermic series at 5 and 10 ATA-HBO (Fig. 17). Mild

Table IV. - Comparison of the clearances of creatinine and PAH of the different groups during the survival period. The GFR of kidneys preserved for 24 hours at 5 and 10 ATA-HBO are the same as the control group at the 95% confidence limits. The other groups differ with the controls. Hypothermia alone, on the other hand differs with the kidneys stored for 24 hours at 5 and 30 ATA-HBO, otherwise the rest of the groups are similar to the hypothermia group. The RPF of controls is significantly different from the others except the kidneys stored at 5 ATA-HBO for 24 hours.

GROUPS	No.	MEAN Ccr (ml/min/m^2)	CONTROL vs. OTHERS	HYPOTHERMIA vs. OTHERS	MEAN Cpah (ml/min/m^2)	FILTRATIO ml/min/ FRACTIO
I CONTROL	3	82			182	0.45
	4	69			126	0.55
	5	98			232	0.42
	6	104			280	0.37
	7	65			205	0.32
	8	110			296	0.37
	9	85			403	0.21
AVERAGE ± S.D.	7	88±17		< .01	246±90	0.38
RANGE		69-110			126-403	21-55
II HYPOTHERMIA 4°C 24 HOURS	162	45			81	0.56
	164	43			95	0.45
	187	48			155	0.31
	193	55			107	0.51
	201	61			139	0.44
	219	55			98	0.56
AVERAGE ± S.D.	6	51±7	< .01		113±28.4	0.45
RANGE		43±61		.01	81-155	0.56
III 2 ATA-HBO & HYPOTHERMIA 24 HOURS	206	66			114	0.58
	232	50			45	1.1
	236	24			25	0.96
	238	24			30	0.80
AVERAGE ± S.D.	4	41±21	< .01	> .10	54± 41	0.76
RANGE		24-66			25-114	0.58-1.1

No.	MEAN Ccr (ml/min/m^2)	CONTROL vs. OTHERS	HYPOTHERMIA vs. OTHERS	MEAN Cpah (ml/min/m^2)	FILTRATION FRACTION	CONTROL vs. others	HYPOTHERMIA vs. others
3	82			182	0.45		
4	69			126	0.55		
5	98			232	0.42		
6	104			280	0.37		
7	65			205	0.32		
8	110			296	0.37		
9	85			403	0.21		
7	88±17		< .01	246±90	0.38	0.01	< 0.01
	69-110			126-403	21-55		
162	45			81	0.56		
164	43			95	0.45		
187	48			155	0.31		
193	55			107	0.51		
201	61			139	0.44		
219	55			98	0.56		
6	51±7	< .01		113±28.4	0.45	< 0.01	
	43±61		.01	81-155	0.56		
206	66			114	0.58		
232	50			45	1.1		
236	24			25	0.96		
238	24			30	0.80		
4	41±21	< .01	> .10	54± 41	0.76	< .01	< .05
	24-66			25-114	0.58-1.1		

GROUPS	No.	MEAN Ccr (ml/min/m^2)	CONTROL vs. OTHERS	HYPOTHERMIA vs. OTHERS	MEAN Cpah (ml/min/m^2)	FI ml FF
IV 5 ATA-HPO & HYPOTHERMIA 24 HOURS	13	107			166	0
	14	86			236	0
	16	81			106	0
	38	30			62	0
	50	111			132	0
AVERAGE ± S.D.	5	83±31	> .50	< .05	140±66	0
RANGE		30-111			62-236	0
V 10 ATA-HBO & HYPOTHERMIA 24 HOURS	87	34			40	0
	100	85			120	0
	106	80			135	0
	109	38			72	0
	112	78			149	0
	127	82			203	0
AVERAGE ± S.D.	6	66±24	> .05	> .10	120±58	0
RANGE		34-85			40-203	0.
						0.
VI 20 ATA-HBO& HYPOTHERMIA 24 HOURS	117	17			43	0.
	119	49			102	0.
	128	68			133	0.
	138	79			124	0.
	161	44			96	0.
AVERAGE ± S.D.	5	51± 24	< .02	> .50	100± 30	0.

No.	MEAN Ccr (ml/min/m^2)	CONTROL vs. OTHERS	HYPOTHERMIA vs. OTHERS	MEAN Cpah (ml/min/m^2)	FILTRATION FRACTION	CONTROL FRACTION vs. OTHERS	HYPOTHERMIA FRACTION vs. OTHERS
13	107			166	0.64		
14	86			236	0.36		
16	81			106	0.76		
38	30			62	0.48		
50	111			132	0.84		
5	83±31	> .50	< .05	140±66	0.59	< .05	> .10
	30-111			62-236	0.84		
87	34			40	0.85		
100	85			120	0.71		
106	80			135	0.59		
109	38			72	0.53		
112	78			149	0.52		
127	82			203	0.40		
6	66±24	> .05	> .10	120±58	0.55	< .02	> .50
	34-85			40-203	0.40-0.85		
117	17			43	0.40		
119	49			102	0.48		
128	68			133	0.51		
138	79			124	0.64		
161	44			96	0.46		
5	51± 24	< .02	> .50	100± 30	0.51	< 0.01	< 0.10

GROUPS	No.	MEAN Ccr (ml/min/m^2)	CONTROL v.s. OTHERS	HYPOTHERMIA v.s. OTHERS	MEAN Cpah (ml/min/m^2)
RANGE		17-79			43-133
VII 30 ATA-HBO & HYPOTHERMIA 24 HOURS	156	63			126
	54	54			79
	141	70			131
	142	54			143
	148	64			145
	155	52			121
	160	70			105
AVERAGE ± S.D.	7	61±10	$<.01$	$<.05$	121±23.1
RANGE		52-70			79-145
VIII 10 ATA-HBO & HYPOTHERMIA 48 HOURS	169	26			51
	212	11			44
	220	44			51
	270	56			100
AVERAGE ± S.D.	4	34±20	$<.01$	$>.05$	62±26
RANGE		11-56			44-100

	No.	MEAN Ccr (ml/min/m^2)	CONTROL v.s. OTHERS	HYPOTHERMIA v.s. OTHERS	MEAN Cpah (ml/min/m^2)	FILTRATION FRACTION	CONTROL v.s. OTHERS	HYPOTHERMIA
		17-79			43-133	0.40-0.64		
HBO & RMIA	156	63			126	0.50		
	54	54			79	0.68		
	141	70			131	0.53		
	142	54			143	0.38		
	148	64			145	0.44		
	155	52			121	0.43		
	160	70			105	0.67		
	7	61±10	$<.01$	$<.05$	121±23.1	0.50	<0.01	$>.50$
		52-70			79-145	0.38-0.68		
HBO & RMIA S	169	26			51	0.51		
	212	11			44	0.25		
	220	44			51	0.86		
	270	56			100	0.56		
	4	34±20	$<.01$	$>.05$	62±26	0.55	$<.01$	$<.05$
		11-56			44-100	0.25-0.86		

impairment of filtration rates were observed in kidneys stored in hypothermia alone, and in those groups of kidneys kept at 20 and 30 ATA-HBO. The kidneys preserved at 2 ATA-HBO for 24 hours and those at 48 hours have deranged functions ranging from severe to moderate.

Statistical analysis of the different GFR by means of Student's t-test disclosed significant differences between the controls and the others except those kidneys stored at 5 ATA-HBO for 24 hours. When one compared the kidneys in which hypothermia alone was employed as a method of preservation, with the other groups, significant differences existed between hypothermia and the ones preserved at 5 and 30 ATA-HBO for 24 hours.

Except the kidneys preserved at 5 ATA-HBO for 24 hours, there was substantial losses of renal tubular excretory ability in all experimental preservation groups as revealed by C_{pah} when compared with the controls (Fig. 18). These impaired tubular functions as determined by PAH clearance was reflected also in the increments of the filtration fractions, confirmed by statistical analysis and supported by extraction ratio studies performed on randomly selected representative animals (Table V).

Table V and V-a* summarize the different clinical and biochemical parameters gleaned immediately prior to sacrifice of long-term survivors. By and large there appears a not-too-significant nonetheless consistent elevation of BUN and serum creatinine

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* Clearance studies of some animals which received kidneys stored for 24 hours at 3 and 5 ATA-HBO were not available and assessment of kidney function was made by comparing pre-operative BUN and creatinine levels with values prior to sacrifice.

Table V. Summary of biochemical and other clinical data of long-term survivors together with extraction ratios on randomly selected animals immediately prior to sacrifice.

* - Calculated using extraction ratio.

GROUP	DOG No.	OSMOLARITY 12-HOUR FASTING URINE (milliosmols)	BUN - mg% Pre-Operative	BUN - mg% Post-Operative	SER Pre-
I CONTROL	6	1735	16	14	
	7	2060	14	16	
	8	1785	10	17	
MEAN		1860	13	16	
II HYPOTHERMIA 4°C 24 HOURS	162	1500	12	26	
	164	1392	14	76	
	201	1591	12	19	
	219	1781	13	16	
MEAN		1566	13	34	
III 2 ATA-HBO & HYPOTHERMIA 24 HOURS	206	1288	18	14	
	232	1072	16	21	
	236	695	19	39	
	238	1362	16	22	
MEAN		1104	17	24	
IV 5 ATA-HBO & HYPOTHERMIA 24 HOURS	14	1311	7	18	
	16	1754	11	19	
	50	1722	16	22	
MEAN		1596	13	20	

DOG No.	OSMOLARITY 12-HOUR FASTING URINE (milliosmols)	BUN - mg% Pre-Operative	BUN - mg% Post-Operative	SERUM CREATININE - mg% Pre-Operative	SERUM CREATININE - mg% Post-Operative
6	1735	16	14	1.0	0.74
7	2060	14	16	0.7	0.86
8	1785	10	17	1.0	0.77
	1860	13	16	0.9	0.79
162	1500	12	26	1.0	1.4
164	1392	14	76	1.0	1.8
201	1591	12	19	1.0	1.0
219	1781	13	16	1.1	1.1
	1566	13	34	1.0	1.3
206	1288	18	14	0.9	0.61
232	1072	16	21	0.8	1.98
236	695	19	39	1.1	1.9
238	1362	16	22	0.8	1.45
	1104	17	24	0.9	1.49
14	1311	7	18	0.8	1.0
16	1754	11	19	0.5	1.2
50	1722	16	22	1.0	1.0
	1596	13	20	0.8	1.06

FINAL URINARY CLEARANCES (ml/min/m^2) Creatinine (C_{cr})	PAH (C_{pah})	FILTRATION FRACTION (C_{cr}/C_{pah})	RENAL PLASMA* FLOW - RPF ml/min/m^2	RENAL BLOOD FLOW RBF* ml/min/m^2	EXTRACTION RATIO OF $\frac{(A-V)}{A}$
105	404	0.26			
71	145	0.49	230	443	0.63
97	350	0.28			
91	299	0.34	230	443	0.63
38	64	0.59	121	220	0.53
35	97	0.37			
43	111	0.39	171	294	0.65
62	92	0.57			
45	91	0.48	146	257	0.59
53	92	0.58	170	316	0.54
35	52	0.67			
23	33	0.69	60	103	0.55
15	31	0.45			
32	52	0.60	115	210	0.55
131	344	0.41			
42	87	0.48			
74	148	0.50			
82	193	0.46			

FILTRATION FRACTION (C_{cr}/C_{pah})	RENAL PLASMA* FLOW - RPF ml/min/m^2	RENAL BLOOD FLOW RBF* ml/min/m^2	EXTRACTION RATIO OF PAH $\frac{(A-V)}{A}$	BLOOD PRESSURE	SURVIVAL (MONTHS)
0.26				120/90	30
0.49	230	443	0.63	140/95	30
0.28				140/95	30
0.34	230	443	0.63	133/93	30
0.59	121	220	0.53	145/85	22
0.37					21
0.39	171	294	0.65	120/70	20
0.57				135/90	20
0.48	146	257	0.59	133/82	21
0.58	170	316	0.54	100/60	20
0.67				135/65	19
0.69	60	103	0.55	125/80	18
0.45				140/100	18
0.60	115	210	0.55	125/76	19
0.41				125/95	29
0.48				170/130	28
0.50				125/100	28
0.46				140/108	28

GROUP	DOG No.	OSMOLARITY 12-HOUR FASTING URINE (miiliosmols)	BUN - mg% Pre-Operative	BUN - mg% Post-Operative	SERUM Pre-Op
V.10 ATA-HBO & HYPOTHERMIA 24 HOURS	100	1163	14	11	
	112	1011	9	12	
	127	1810	18	13	
MEAN		1328	13	12	
VI. 20 ATA-HBO & HYPOTHERMIA 24 HOURS	119	650	9	38	
	128	1039	20	19	
	138	1011	16	16	
	161	1960	16	16	
MEAN		1165	15	22	
VII. 30 ATA-HBO & HYPOTHERMIA 24 HOURS	54	1212	19	20	1
	141	1820	14	22	0
	142	1591	9	26	0
	148	1260	12	22	1
	155	1290	10	15	0
	160	1658	8	25	0
MEAN		1472	12	22	0
VIII. 10 ATA-HBO & HYPOTHERMIA 48 HOURS	169	765	9	40	0
	220	1062	8	27	0
	270	1274	30	22	1
MEAN		1034	16	30	0

DOG No.	OSMOLARITY 12-HOUR FASTING URINE (miiliosmols)	BUN - mg% Pre-Operative	BUN - mg% Post-Operative	SERUM CREATININE - mg% Pre-Operative	SERUM CREATININE - mg% Post-Operative
100	1163	14	11	0.7	1.0
112	1011	9	12	1.1	1.05
127	1810	18	13	0.7	0.7
	1328	13	12	0.8	0.92
119	650	9	38	0.9	1.76
128	1039	20	19	1.0	0.9
138	1011	16	16	0.9	1.2
161	1960	16	16	1.0	1.2
	1165	15	22	0.9	1.25
54	1212	19	20	1.0	1.0
141	1820	14	22	0.9	1.0
142	1591	9	26	0.9	1.0
148	1260	12	22	1.1	1.4
155	1290	10	15	0.8	0.8
160	1658	8	25	0.7	1.1
	1472	12	22	0.9	1.05
169	765	9	40	0.6	1.73
220	1062	8	27	0.8	1.46
270	1274	30	22	1.3	0.79
	1034	16	30	0.9	1.33

FINAL URINARY CLEARANCES (ml/min/m^2) Creatinine (C_{cr})	PAH (C_{pah})	FILTRATION FRACTION (C_{cr}/C_{pah})	RENAL PLASMA FLOW - RPF* ml/min/m^2	RENAL BLOOD FLOW RBF* ml/min/m^2	EXTRACTION RATIO OF PAH $\frac{(A-V)}{A}$
50	155	0.32			
57	128	0.42			
71	139	0.51			
58	141	0.42			
33	57	0.58			
46	103	0.45	240	393	0.43
45	73	0.62			
46	114	0.44			
43	87	0.52	240	393	0.43
48	70	0.69			
52	145	0.36			
66	97	0.67	156	319	0.62
59	176	0.34			
53	116	0.46			
64	197	0.32			
57	134	0.48	156	319	0.62
31	52	0.68			
40	76	0.53			
79	100	0.79	286	461	0.35
50	76	0.64	286	461	0.35

FILTRATION FRACTION (C_{cr}/C_{pah})	RENAL PLASMA FLOW - RPF* ml/min/m^2	RENAL BLOOD FLOW RBF* ml/min/m^2	EXTRACTION RATIO OF PAH $\frac{(A-V)}{A}$	BLOOD PRESSURE	SURVIVAL (MONTHS)
0.32					25
0.42				155/110	24
0.51				140/100	24
0.42				148/105	24
0.58				130/110	24
0.45	240	393	0.43	145/105	24
0.62					23
0.44				140/90	22
0.52	240	393	0.43	138/90	23
0.69				145/100	27
0.36				120/80	23
0.67	156	319	0.62	120/85	23
0.34				120/80	23
0.46				145/115	22
0.32				140/90	22
0.48	156	319	0.62	132/90	23
0.68				110/75	21
0.53				145/100	20
0.79	286	461	0.35	150/100	9
0.64	286	461	0.35	135/92	17

Table V-a. Individual BUN and Creatinine Determinations (mg%) of Long-Term Survivors.

Group	Dog No.	Pre-Operative BUN	Pre-Operative Creatinine	Post-Operative BUN	Post-Operative Creatinine
3 ATA-HBO & HYPOTHERMIA 24 HOURS	48	10	1.5	17	1.5
	50	18	0.8	16	1.6
	51	24	1.6	18	1.8
	64	17	0.5	13	1.3
	75	29	1.4	20	1.4
	81	25	1.0	17	1.5
	94	17	1.1		
	95	18	1.1	18	1.8
Mean	8	20	1.1	17	1.6
5 ATA-HBO & HYPOTHERMIA 24 HOURS	103	29	1.4	17	1.4
	113	17		20	2.0
	114	20	1.4	12	1.6
	125			17	1.8
Mean	4	22	1.4	17	1.7

thresholds save the groups of kidneys stored in hypothermia alone, hyperbaric-hypothermic method of storage at 2 ATA-HBO and the 48-hour preservation group. This is indicative of the homeostatic adjustments that the remaining stored kidney undertakes in handling relatively increased excretory load in the face of diminished renal function due to the extended period of ischemia.

The results indicate that hyperbaric oxygen combined with hypothermia preserved renal function better than that provided by hypothermia alone. The additional safety is modest and requires clearance techniques to elucidate. Hyperbaric oxygen below 5 or above 10 atmospheres is of no additional demonstrable value.

C. Miscellaneous Parameters

The general condition of survivors as reflected by the hematocrit levels, blood pressures as well as the body and kidney weights is depicted in Table VI and the photographs of survivors are shown in Appendix B.

The blood pressures and the levels of hematocrit were within normal range except in a few animals which received kidneys preserved for 48 hours and 24 hours at 2 ATA-HBO. The body weights were not materially altered. The kidneys on the other hand, increased in weights in the order of 40% to 106% based on estimated kidney weights at the time of replantation. However this increase in weights indicative of the existing hypertrophy was not positively correlated with results of the function tests as determined by clearance techniques.

TABLE VI. - MISCELLANEOUS CLINICAL PARAMETERS AND COMPARISON OF BODY AND KIDNEY WEIGHTS OF LONG-TERM SURVIVORS

GROUPS	DOG No.	Hct	BLOOD pressure	BODY WEIGHTS (Kg) PRE-OP	BODY WEIGHTS (Kg) POST-OP	KIDNEY WEIGHT (grams) Nephrectomy*	KIDNEY WEIGHT (grams) Sacrifice	Pe we in
I CONTROLS	6	42	120/90	24	22	61	99	
	7	47	140/95	28	31	71	147	1(
	8	46	140/95	19	17	48	70	
MEAN	3	45	135/95	24	23	61	105	
II. HYPOTHERMIA (4°C) 24 HOURS	162	47	145/85	26	25	66	92	3
	164	47		19	20	48	87	8
	193	52		15	17	38		
	201	50	120/70	15	18	38	65	7
	219	54	135/90	22	25	56	92	6
MEAN	5	50	135/80	20	21	51	74	6
III. 2 ATA-HBO & HYPOTHERMIA 24 HOURS	206	43	100/60	15	14	38	73	9
	232	33	135/65	16	12	41	56	3
	236	34	125/80	19	20	48	56	1
	238	40	140/100	15	19	38	62	6
MEAN	4	38	125/80	16	16	41	62	5

E VI. - MISCELLANEOUS CLINICAL PARAMETERS AND COMPARISON OF BODY AND KIDNEY WEIGHTS OF LONG-TERM SURVIVORS

OG o.	Hct	BLOOD pressure	BODY WEIGHTS (Kg) PRE-OP	POST-OP	KIDNEY WEIGHT (grams) Nephrectomy*	Sacrifice	Per cent weight increase	SACRIFICE (MONTHS)
	42	120/90	24	22	61	99	62	30
	47	140/95	28	31	71	147	107	30
	46	140/95	19	17	48	70	46	30
	45	135/95	24	23	61	105	72	30
	47	145/85	26	25	66	92	39	22
	47		19	20	48	87	81	21
	52		15	17	38			12
	50	120/70	15	18	38	65	71	20
	54	135/90	22	25	56	92	64	20
	50	135/80	20	21	51	74	66	19
	43	100/60	15	14	38	73	92	20
	33	135/65	16	12	41	56	37	19
	34	125/80	19	20	48	56	17	18
	40	140/100	15	19	38	62	63	18
	38	125/80	16	16	41	62	51	19

TABLE VI CONTINUED

GROUPS	DOG No.	Hct	BLOOD Pressure	BODY WEIGHTS (Kg) PRE-OP	POST-OP	KIDNEY WEIGHT (grams) Nephrectomy*	Sacrifice	Per cent weight increase
IV. 5 ATA-HBO&& HYPOTHERMIA 24 HOURS	13	45		26	24	66		
	14	52	125/95	23	24	58	70	21
	16	45	170/130	24	27	61		
	38	29		20	19	51		
	50	37	125/100	18	17	46	73	58
MEAN	5	42	140/110	22	22	56	72	40
V. 10 ATA-HBO & HYPOTHERMIA 24 HOURS	87	41		23	23	58		
	100	46		23	23	58		
	106	49		25	25	64		
	112	53	155/110	27	27	69	109	58
	127	42	140/100	16	16	41	62	51
MEAN	5	46	150/105	23	23	58	86	55
VI. 20 ATA-HBO & HYPOTHERMIA 24 HOURS	119	27	130/110	26	24	66	82	24
	128	47	145/105	20	19	51	96	88
	138	45		27	29	69		
	161	46	140/90	22	26	56	95	70
MEAN	4	41	140/100	24	25	61	91	61

TABLE VI CONTINUED

DOG No.	Hct	BLOOD Pressure	BODY WEIGHTS (Kg) PRE-OP	POST-OP	KIDNEY WEIGHT (grams) Nephrectomy*	Sacrifice	Per cent weight increase	SACRIFICE (MONTHS)
13	45		26	24	66			21
14	52	125/95	23	24	58	70	21	29
16	45	170/130	24	27	61			28
38	29		20	19	51			19
50	37	125/100	18	17	46	73	58	28
5	42	140/110	22	22	56	72	40	28
87	41		23	23	58			17
00	46		23	23	58			25
06	49		25	25	64			25
12	53	155/110	27	27	69	109	58	24
27	42	140/100	16	16	41	62	51	24
5	46	150/105	23	23	58	86	55	24
19	27	130/110	26	24	66	82	24	24
28	47	145/105	20	19	51	96	88	24
38	45		27	29	69			23
61	46	140/90	22	26	56	95	70	22
4	41	140/100	24	25	61	91	61	23

TABLE VI CONTINUED

GROUPS	DOG No.	Hct	BLOOD Pressure	BODY WEIGHTS (Kg) PRE-OP	POST-OP	KIDNEY WEIGHT (grams) Nephrectomy*	Sacrifice	Per wei incr
VII. 30 ATA-HBO & HYPOTHERMIA 24 HOURS	54	40	145/100	18	17	46	88	91
	141	44	120/80	18	20	46	86	87
	142	35	120/85	15	27	38	147	287
	148	55	120/80	18	18	46	70	52
	155	46	145/115	23	23	58	90	55
	160	56	140/90	19	19	48	92	71
MEAN	6	46	130/90	19	20	48	99	106
VIII. 10 ATA-HBO & HYPOTHERMIA 24 HOURS	169	36	110/75	15	14	38	65	71
	220	38	145/100	20	20	51	73	43
	270	38	150/100	16	15	41	78	90
MEAN	3	37	135/90	17	16	43	72	67

* - Estimates of kidney weights at the time of replantation were derived from 41 kidneys from a separate group of 24 dogs which had similar weight distribution as the experimental groups. There was 2.52 grams of kidney tissue per kilogram body weight. The mean kidney weight was 44.55±9.4 grams and the means body weight was 17.52±3.9 kilograms.

TABLE VI CONTINUED

DOG No.	Hct	BLOOD Pressure	BODY WEIGHTS (Kg) PRE-OP	POST-OP	KIDNEY WEIGHT (grams) Nephrectomy*	Sacrifice	Per cent weight increase	SACRIFICE (MONTHS)
54	40	145/100	18	17	46	88	91	27
141	44	120/80	18	20	46	86	87	23
142	35	120/85	15	27	38	147	287	23
148	55	120/80	18	18	46	70	52	23
155	46	145/115	23	23	58	90	55	22
160	56	140/90	19	19	48	92	71	22
6	46	130/90	19	20	48	99	106	23
169	36	110/75	15	14	38	65	71	21
220	38	145/100	20	20	51	73	43	20
270	38	150/100	16	15	41	78	90	9
3	37	135/90	17	16	43	72	67	17

timates of kidney weights at the time of replantation were derived
om 41 kidneys from a separate group of 24 dogs which had similar
ight distribution as the experimental groups. There was 2.52 grams
kidney tissue per kilogram body weight. The mean kidney weight
s 44.55±9.4 grams and the means body weight was 17.52±3.9 kilograms.

D. Pathology

The size and degree of fibrosis observed during biopsy, sacrifice or death as well as the estimated weight increase and the histopathologic features of the stored kidneys are shown in Table VII. The detailed clinical accounts and pathology of survivors and deaths are summarized in the master Tables 2 and 3 respectively in Appendix A.

The kidney weights of the stored kidneys were not determined at the time of transplantation. An estimated weight was then computed based on the ratio of kidney tissue and body weight. Thus 41 kidneys with a mean weight of 44.55±9.4 grams from 24 dogs with a mean weight of 17.52±3.9 gave 2.52 grams of kidney tissue per kilogram body weight. The weight distribution of these test animals corresponded very closely to the weights of the autologous hosts of the preserved kidneys. So from the known body weights of the experimental series and the assumed constant (2.52 gm. of kidney tissue/kilogram body weight) estimated kidney weights at the time of transplantation were derived.

The gross appearance of autografts at biopsy with reference to size and degree of fibrosis were rated by visual inspection as follows: 1. size reduction; 0=no appreciable change, 1-=25%, 2-=33%, 3-=50%, 4-=67% reduction and 5-=only a fibrotic remnant was found or the kidney was autolyzed, 1+=60 - 80 grams, 2+= 81 - 100 grams, 3+= 101 - 120 grams, 4+= 121 grams and up at the time of sacrifice or death, 2. presence of scars - the ratings ranged from 1+ representing very minimal scar formation to 4+ in which

Table VII. Summary of Gross and Microscopic Feature
Autografts of Long-term Survivors

I. GROSS

A. SIZE
- 1- 25% Reduction
- 2- 33% "
- 3- 50% "
- 4- 67% "
- 5- autolysis or fibrous remnant
- 0 no appreciable change
- 1+ 60-80 grams(autopsy or sacrifice)
- 2+ 81-100 " "
- 3+ 101-120 " "
- 4+ 121 and up "

B. FIBROSIS (1-4+)
- 1+ minimal
- 2+ moderate
- 3+ fairly extensive
- 4+ very extensive

II. MICROSCOPIC

- 1+ Normal
- 2+ Significa
  rophied c
  brane thi
- 3+ Presence
  morphnucl
  lymphocyt
  tium in a
- 4+ Extensive
  cation of

* The kidn
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44.55±9.
= 17.52±
of kidne

Table VII. Summary of Gross and Microscopic Features of Autografts of Long-term Survivors

% Reduction
% "
% "
% "
tolysis or fibrous remnant
appreciable change
-80 grams(autopsy or sacrifice)
-100 " "
1-120 " "
1 and up "

SIS (1-4+)
nimal
derate
irly extensive
cy extensive

II. MICROSCOPIC

1+ Normal
2+ Significant proportions of glomeruli atrophied or hyalinized with basement membrane thickening and crescents
3+ Presence of inflammatory cells; poly morphnuclear leucocytes, plasma cells and lymphocytes in the glomeruli and interstitium in addition to 2+.
4+ Extensive necrosis, fibrosis and calcification of the nephron and interstitium.

* The kidney weights were not recorded and were estimated from the ratio of kidney tissue in grams and the corresponding body weights in kilograms (gms/kg) at the time of transplantation. It was derived on 41 kidneys (mean weights = 44.55±9.4 grams) on 24 dogs (mean weights = 17.52±3.9 kg) and equalled to 2.54 gms of kidney tissue per kilogram body weight.

GROUPS	DOG No.	SURVIVAL (MONTHS)	TRANSPLANT BIOPSY 3-4 wks. SIZE	TRANSPLANT BIOPSY 3-4 wks. FIBROSIS	AUTOGRAFTED KIDNEY AT DEATH OF SACRIFICE SIZE	AUTOGRAFTED KIDNEY AT DEATH OF SACRIFICE FIBROSIS	% WEIGHT* INCREASE	C O K
I. CONTROL	6	30			2+	0	62	
	7	30			4+	0	107	
	8	30			1+	0	46	
MEAN	3	30				0	72	
II. HYPOTHERMIA 4°C 24 HOURS	162	22	2-	1+	2+	0	39	
	164	21	2-	1+	2+	3+	81	
	201	20	1-	0	+	0	71	
	219	20	1-	+	2+	+	64	
MEAN	4	21					66	
III. 2 ATA-HBO & HYPOTHERMIA 24 HOURS	206	20	1-	0	2+	0	92	
	232	19	0	2+	0	3+	37	
	236	18	2-	2+	0	3+	17	
	238	18	2-	2+	+	+	63	
MEAN	4	19					51	
IV. 5 ATA-HBO & HYPOTHERMIA 24 HOURS	13	21	0	2+		2+		1
	14	29	0	+	+	0	21	
	16	28						
	38	18	2-	+				
	50	28	+	+	+	0	58	
MEAN	5	28					40	

OG lo.	SURVIVAL (MONTHS)	TRANSPLANT BIOPSY 3-4 wks. SIZE	TRANSPLANT BIOPSY 3-4 wks. FIBROSIS	AUTOGRAFTED KIDNEY AT DEATH OF SACRIFICE SIZE	AUTOGRAFTED KIDNEY AT DEATH OF SACRIFICE FIBROSIS	% WEIGHT* INCREASE	Control Opposite Kidney	Biopsy	Autopsy/ Sacrifice
6	30			2+	0	62	1	1	1
7	30			4+	0	107	1	1	1
8	30			1+	0	46	1	1	1
3	30				0	72	1	1	1
62	22	2-	1+	2+	0	39	1	1	2
64	21	2-	1+	2+	3+	81	1		
01	20	1-	0	+	0	71	1	2	2
19	20	1-	+	2+	+	64	1	1	1
4	21					66	1	1-2	2
06	20	1-	0	2+	0	92	1	1	2
32	19	0	2+	0	3+	37	1	1	3
36	18	2-	2+	0	3+	17	1		3
38	18	2-	2+	+	+	63	1	1	
1	19					51	1	1	3
.3	21	0	2+		2+		11	1	2
.4	29	0	+	+	0	21	1	1	2
.6	28						1	1	1
8	18	2-	+				1	1	1
0	28	+	+	+	0	58	1	1	1
	28					40	1	1	1

GROUPS	DOG No.	SURVIVAL (MONTHS)	TRANSPLANT BIOPSY 3-4 wks. SIZE	TRANSPLANT BIOPSY 3-4 wks. FIBROSIS	AUTOGRAFTED KIDNEY AT DEATH OR SACRIFICE SIZE	AUTOGRAFTED KIDNEY AT DEATH OR SACRIFICE FIBROSIS	% WEIGHT* INCREASE	HIST Control Opposit Kidney
V. 10 ATA-HBO & HYPOTHERMIA 24 HOURS	87	17	+	0				1
	100	25			+	2+		1
	106	16	1-	+				1
	112	24	1-	0	3+	0	58	1
	127	24	0	0	+	0	51	1
MEAN	5	24					55	1
VI. 20 ATA-HBO & HYPOTHERMIA 24 HOURS	119	24	0	0	2+	3+	24	1
	128	24	0	0	3+	+	88	2
	138	21	0	0				
	161	22	0	0	2+	0	70	1
MEAN	4	23					61	1
VII. 30 ATA-HBO & HYPOTHERMIA 24 HOURS	54	27	1-	2+	2+	3+	91	1
	141	23	1-	0	2+	0	87	1
	142	23	0	0	4+	0	287	2
	148	23	1-	0	+	0	52	1
	155	22	1-	2+	2+	2+	55	1
	160	22	1-	+	2+	+	71	1
MEAN	6	23					106	1
VIII. 10 ATA-HBO & HYPOTHERMIA 48 HOURS	169	21	3-	+	+	4+	71	1
	212	8	3-	2+				2
	220	20	2-	0	+	3+	43	1
	270	9	2-	0	2+	+	90	1
MEAN	4						67	1

	DOG No.	SURVIVAL (MONTHS)	TRANSPLANT BIOPSY 3-4 wks. SIZE	TRANSPLANT BIOPSY 3-4 wks. FIBROSIS	AUTOGRAFTED KIDNEY AT DEATH OR SACRIFICE SIZE	AUTOGRAFTED KIDNEY AT DEATH OR SACRIFICE FIBROSIS	% WEIGHT* INCREASE	HISTOPATHOLOGY Control Opposite Kidney	HISTOPATHOLOGY Biopsy	HISTOPATHOLOGY Autopsy/ Sacrifice
	87	17	+	0				1	1	1
x	100	25			+	2+		1	1	1
	106	16	1-	+				1	2	2
	112	24	1-	0	3+	0	58	1	4	1
	127	24	0	0	+	0	51	1	1	1
	5	24					55	1	1-2	1
	119	24	0	0	2+	3+	24	1	3	3
	128	24	0	0	3+	+	88	2		2
	138	21	0	0						
	161	22	0	0	2+	0	70	1	1	1
	4	23					61	1	2	2
	54	27	1-	2+	2+	3+	91	1	2	2
x	141	23	1-	0	2+	0	87	1	2	1
	142	23	0	0	4+	0	287	2		2
	148	23	1-	0	+	0	52	1	1	2
	155	22	1-	2+	2+	2+	55	1		2
	160	22	1-	+	2+	+	71	1	4	1
	6	23					106	1	2	2
&	169	21	3-	+	+	4+	71	1	2	3
	212	8	3-	2+				2	2	
	220	20	2-	0	+	3+	43	1	4	3
	270	9	2-	0	2+	+	90	1	2	2
	4						67	1	2	3

the whole surface of the kidney was scarred and covered with adhesions of either omentum and/or intestines. Representative photographs and photomicrographs of autotransplants from the various groups are shown in Appendix C.

1. Gross Appearance

Kidneys at sacrifice in the controls and in all experimental groups disclosed gross hypertrophy from 40% to 106%.

This weight increment does not correlate well with the parameters of kidney functions used in these studies.

a) Controls:- Varying degrees of hypertrophy was noted when the one remaining kidney was visualized one month or longer from the time of autografting (Figs. b & c, Appendix C).

b) Experimental Groups:-

1) 24-hour Preservation - These kidneys disclosed a wide range of size reduction; from a fibrotic remnant to those animals in which a technical factor and failure of preservation prevented survival, to one only slightly, but discernable smaller than the opposite kidney at the time of contra-lateral nephrectomy. Later sacrifice of long-term survivors (8 - 30 minutes) revealed compensatory hypertrophy (Figs. d-j, Appendiz C).

2) 48-hour Preservation - The transplants were all markedly reduced in size at the time of contra-lateral nephrectomy. Least tissue loss was present in the 4 animals which survived (Fig. k, Appendix C).

2. Histopathology

The histopathology of paraffin sections from the control

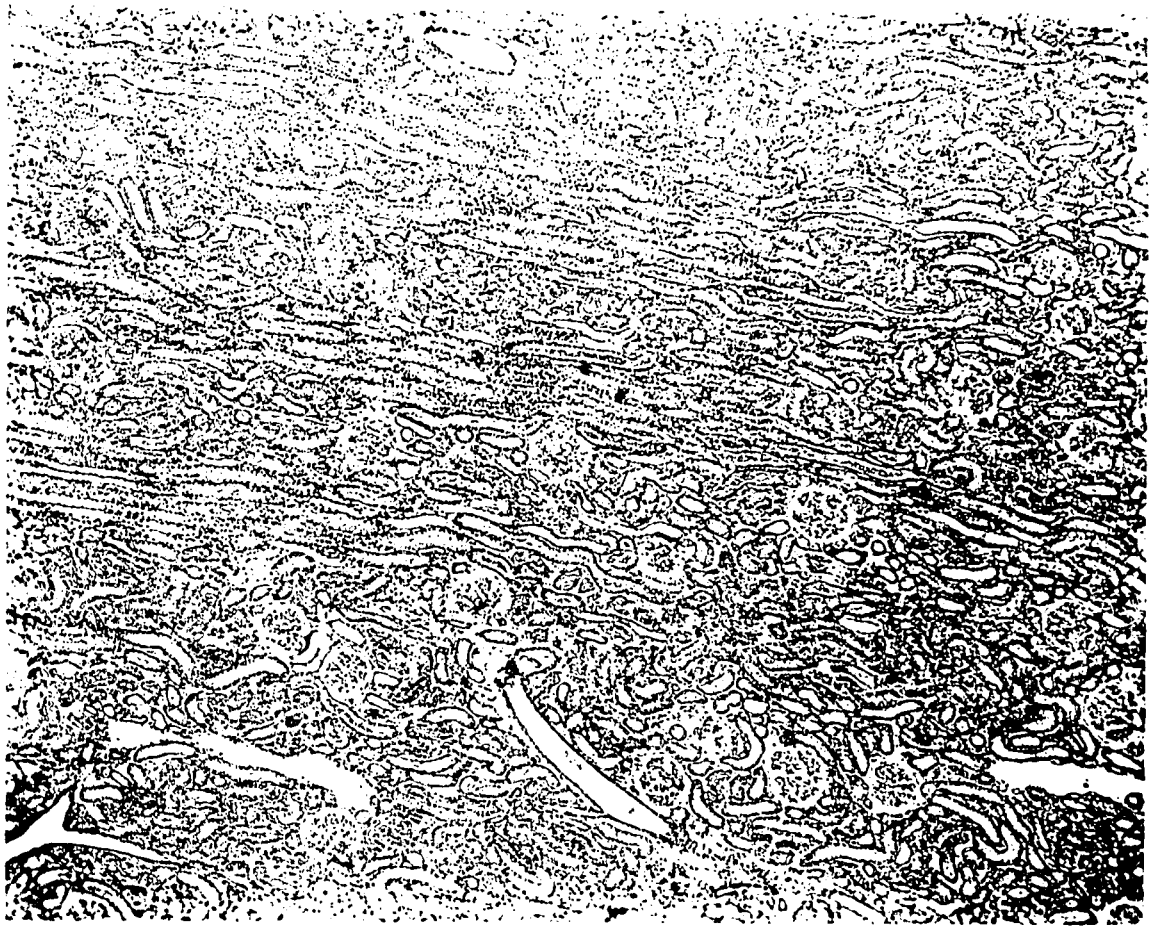


Fig. 19a-1. Grade 1+ - low magnification of a normal unpenetrated kidney section (D.C./1, H & E; x 50). This survey photomicrograph shows glomeruli, tubules, and blood vessels with approximately

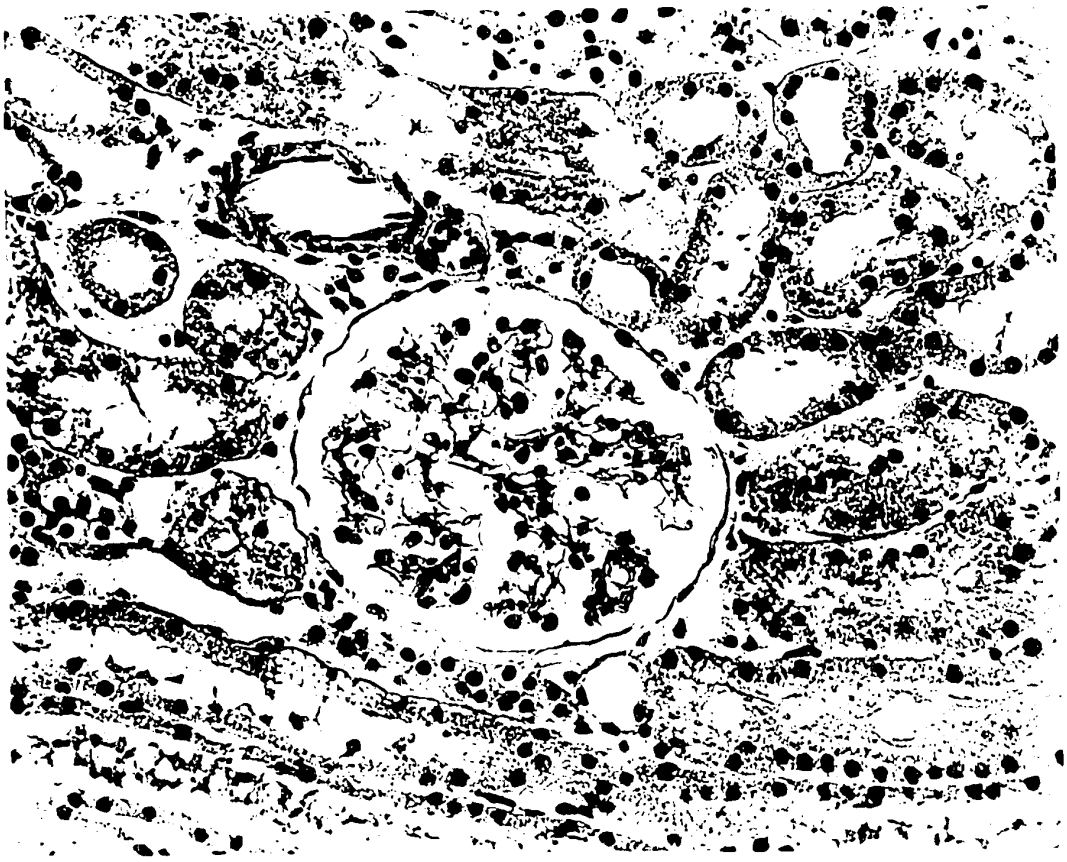
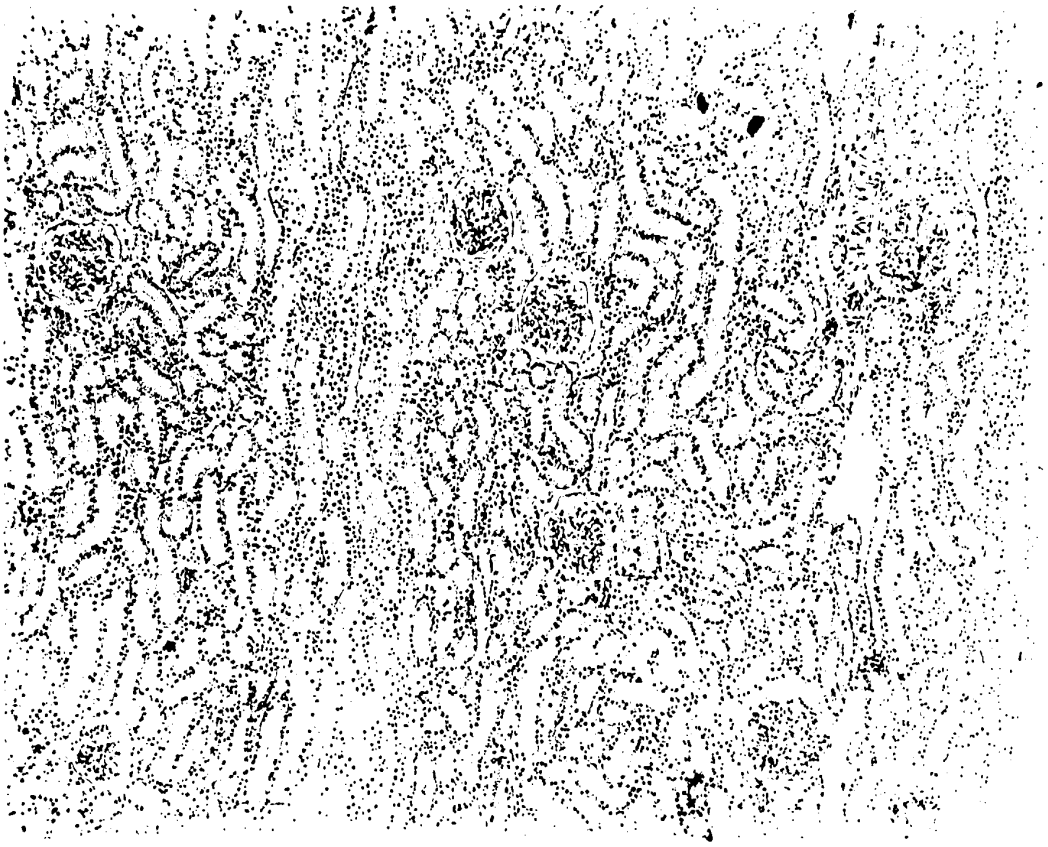


Fig. 19a-2. High magnification (H & E; x 400) of Fig. 19a-1. This glomerulus appears normal.



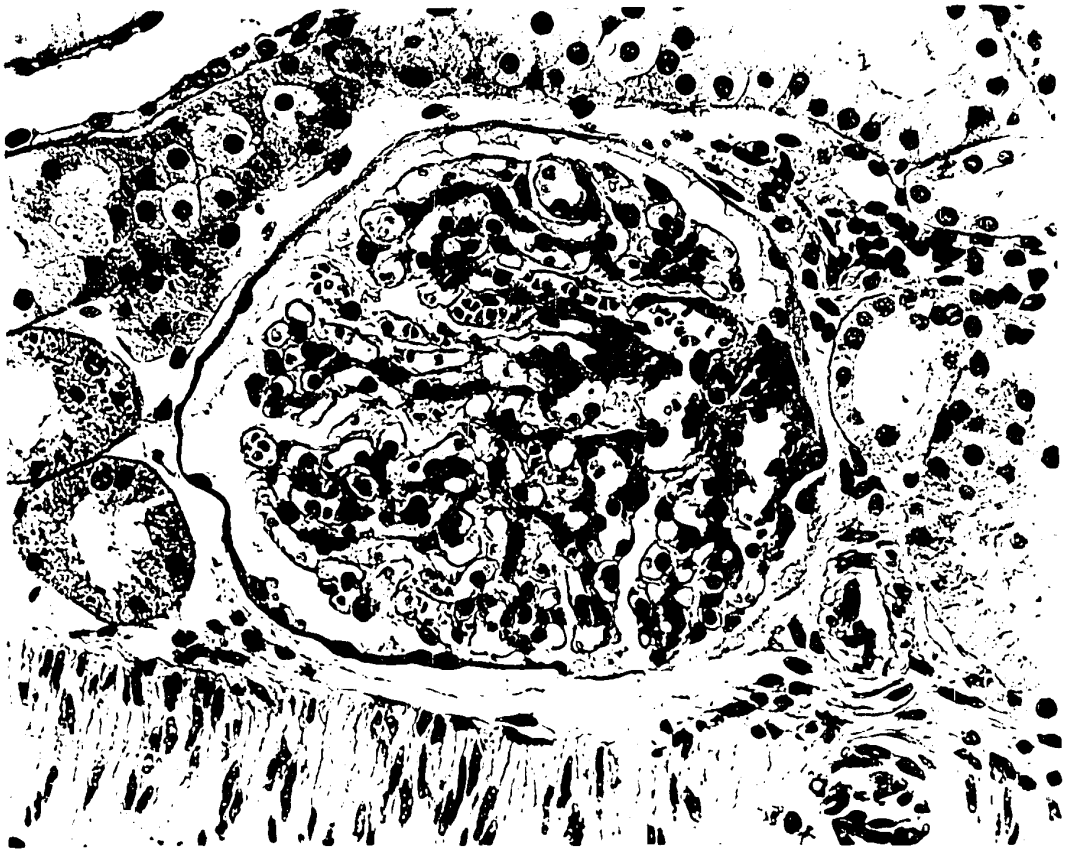
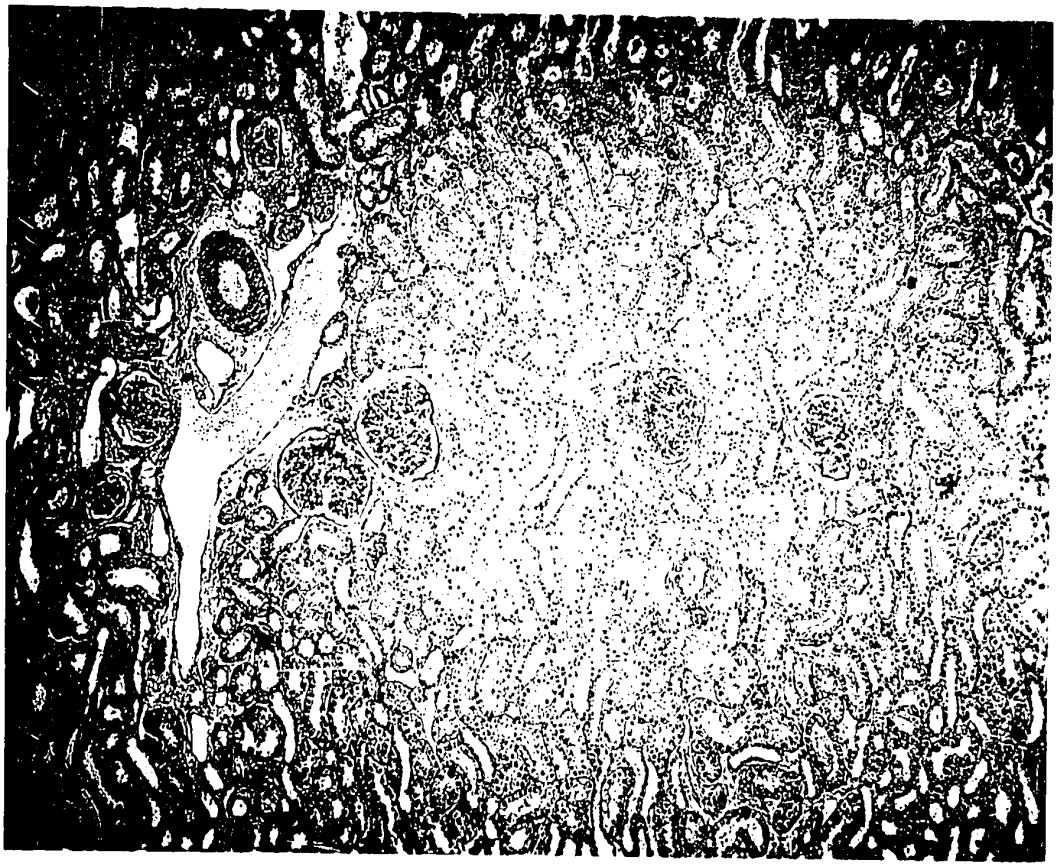


Fig. 19a-4. High magnification (H & E; x 400) of Fig. 19a-3. This glomerulus is larger than normal, more cellular, contains a few inflammatory cells and thickened basement membranes.



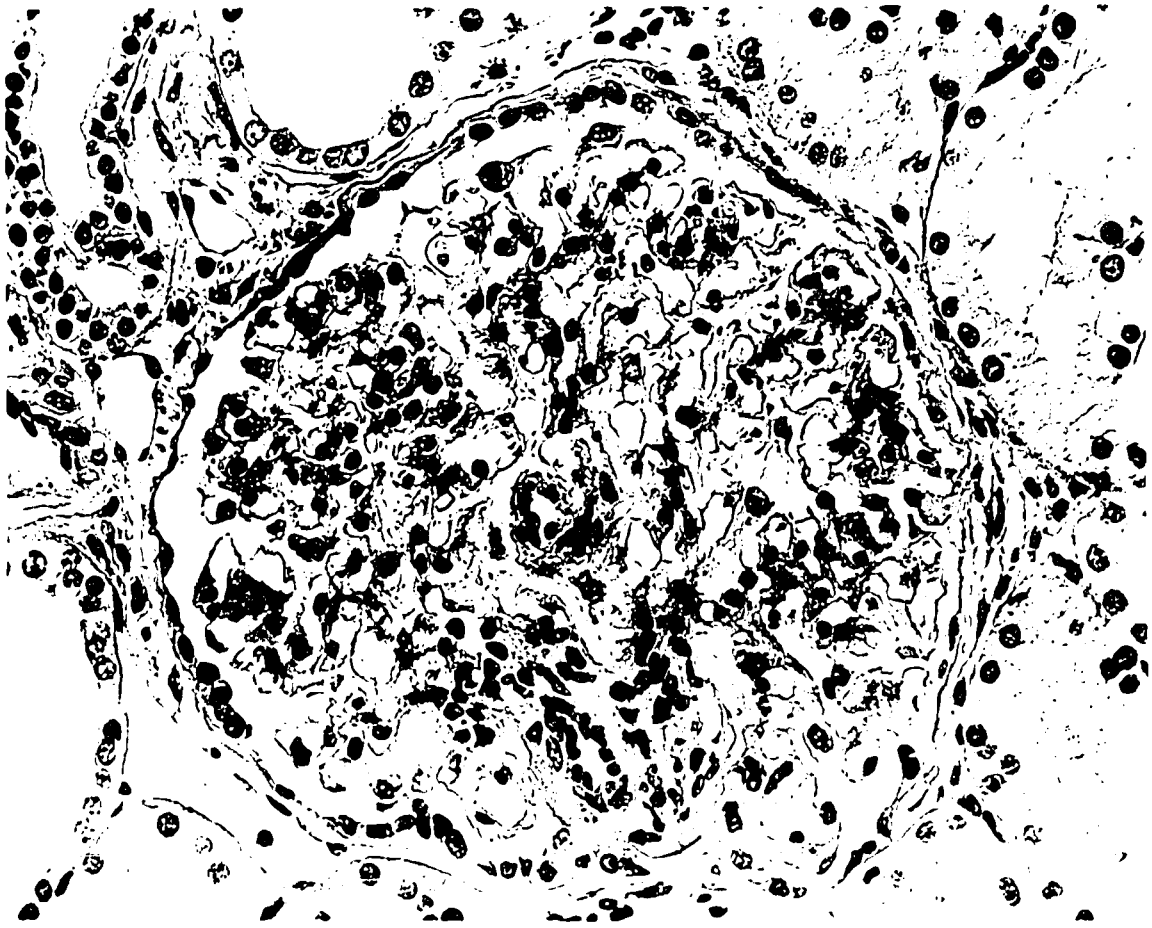
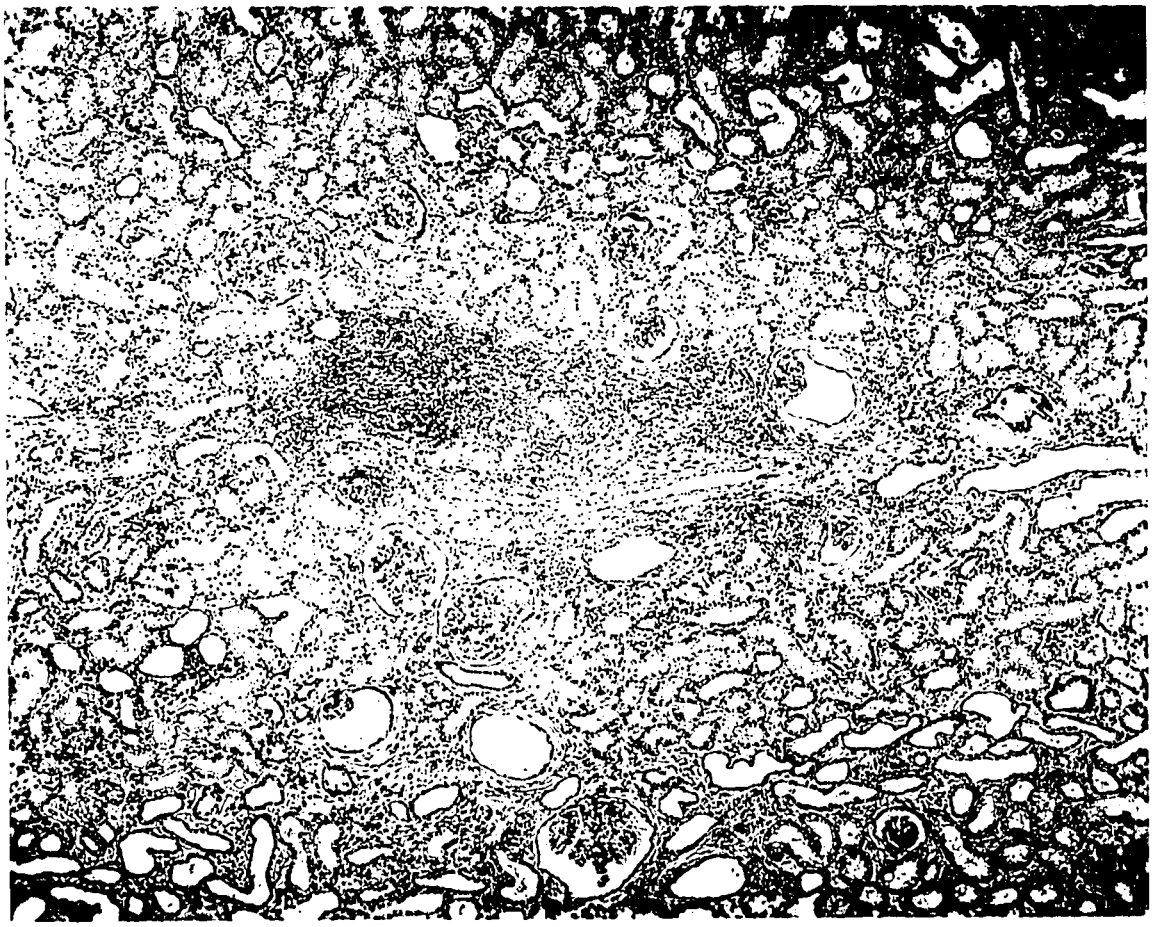
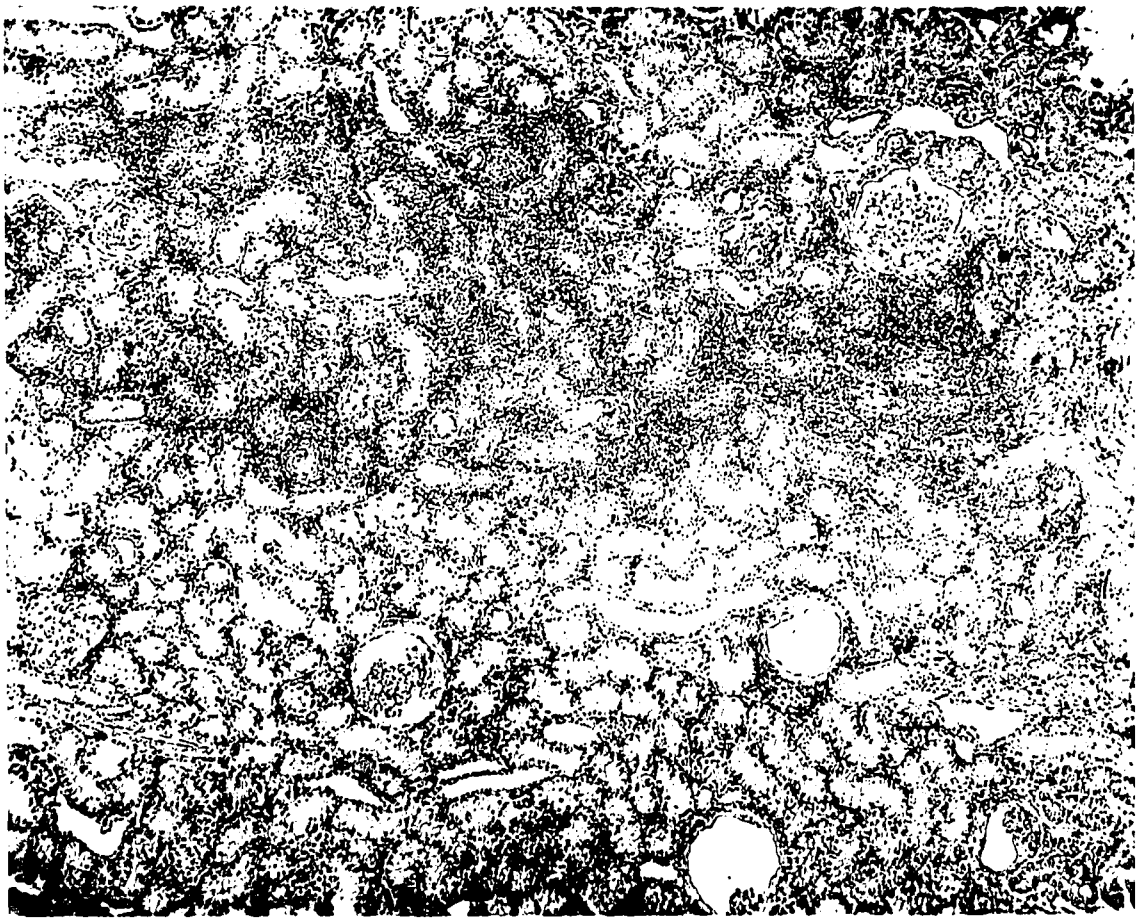
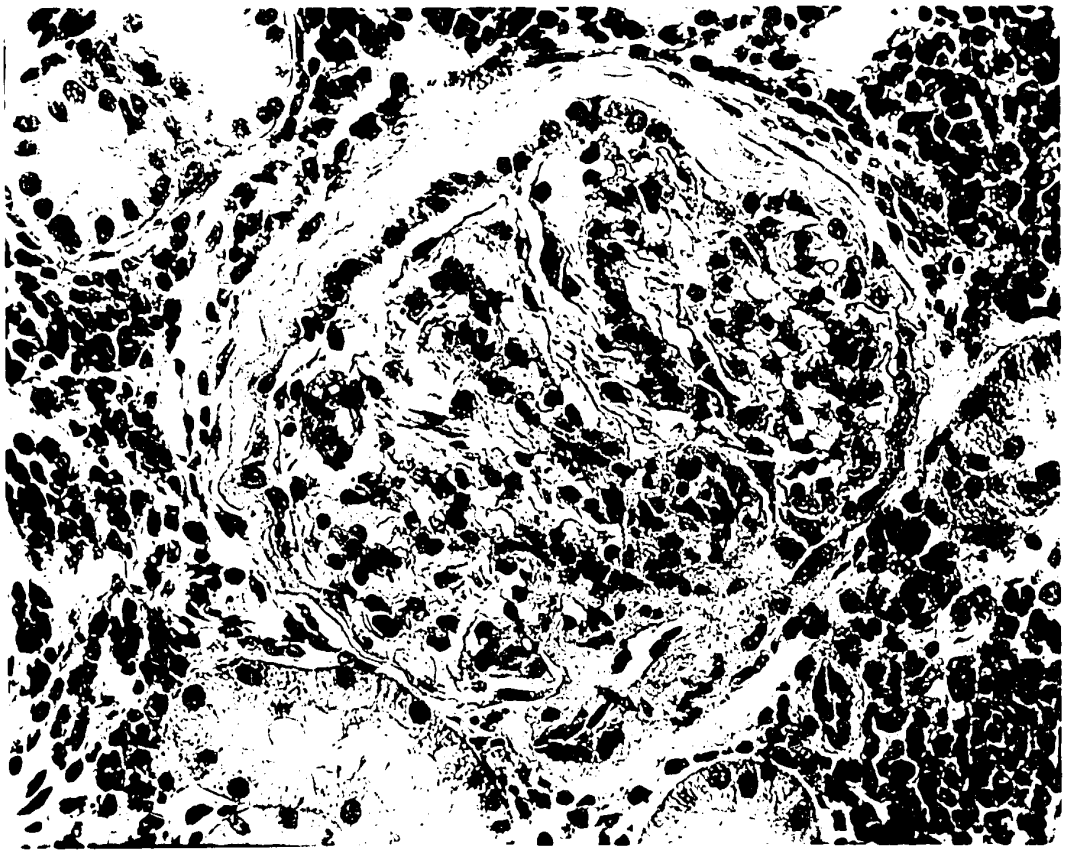


Fig. 195-2. High magnification of a section of a kidney preserved by hyperbaric-hypothermic method at 20 ATA-4°C for 24 hours (Dog /119, H & E; x 400). The marked cellularity of this glomeruli, the pronounced thickening of the basement membrane are frequently found in sections of kidneys which were placed in Grade 2 & /or 3.







(opposite) kidneys, as well as from the biopsy materials and tissues taken at sacrifice or death are illustrated in Figs. 19a-19-d & 1 to w in Appendix C. It is graded from 1 to 4; 1+=normal, 2+=significant numbers of glomeruli are abnormal; hyalinized, or show marked basement membrane thickening and presence of adhesions or cescent, 3+=presence of inflammatory cells including' polymorphonuclear leucocytes, and round cells in the nephron and interstitium in addition to 2+, and lastly 4+=extensive necrosis, and or calcification of the nephron and interstitium.

All sections from long-term survivors in the control and experimental groups revealed glomerular hypertrophy.

a. Controls - The microscopic studies pf paraffin sections from the survivors revealed normal kidney histology (Figs. 19a, n & o, Appendix C).

b. Experimental Groups - The small shrunken kidneys showed marked abnormalities with complete loss of glomeruli and extensive tubular necrosis and/or calcification. (Figs. 19d, 1 & m, Appendix C). The sections from kidneys preserved by hypothermia and hyperbaric oxygen at 3, 5 and 10 atmospheres were normal except in 2 animals (Figs. r - t, Appendix C).

Serious alterations nonetheless compatible with life, were observed in kidneys stored for 24 hours by hypothermic-hyperbaric oxygen method at 2 atmospheres and in the 48-hour preserved kidneys (Figs. q & w, Appendix C). Acute and/or subacute interstitial inflammations were noted in addition to the abnormal glome-

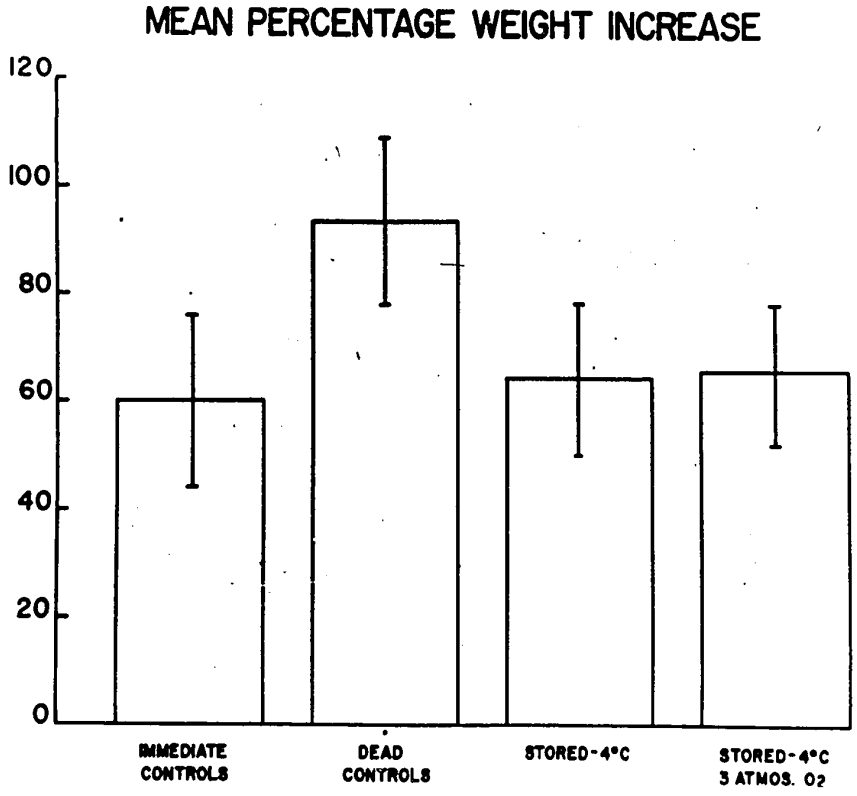


Fig. 20. Mean weight increase of various groups.

rular changes. The glomerular abnormalities which consisted of varying degrees of hypercellularity, hyalinization, adhesions, thickening of Bowman's membrane in a significant number of glomeruli characterized the kidneys preserved by hypothermia alone or in combination with hyperbaric oxygen technique at 20 and 30 atmospheres (Figs. p, u & v).

E. Circulatory Behaviour of Isolated and Preserved Canine Kidneys

The kidneys herein considered fall into 4 categories:

Group I - Hypothermia (4°C - 5 kidneys) for 24 hours;

Group 2 - 3 ATA-HBO and hypothermia for 24 hours (6 kidneys);

Group 3 - Immediate controls with ischemic time of 30 minutes or less (15 kidneys);

Group 4 - Dead controls kept at room temperature (27°C) for 24 hours.

1. Weight Changes: A comparison of the mean percentage weight increase for the four groups of kidneys revealed that the dead controls (Group 4) gained more weight than the other groups (significant at the 5% level by t-test) and there was no significant difference between the immediate control and preserved groups (Fig. 20).

2. Urine Output: Urine output of recirculated kidneys varied greatly between individual animals. Amounts between 1 and 47 ml were produced by immediate controls, less by preserved kidneys, and usually less than 1 ml by dead kidneys.

3. Blood Flow Rate: A comparison was made between the mean blood flow rates in ml/gm/min of each of the four groups,

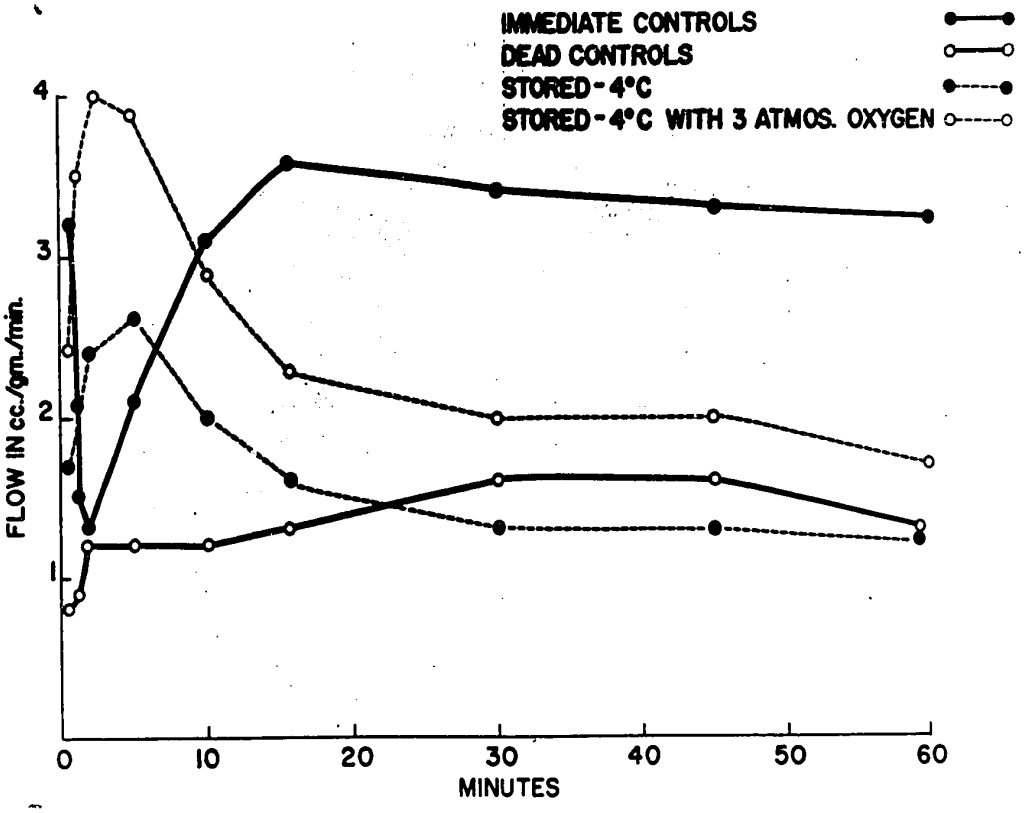


Fig. 21. - Mean blood flow rates for all groups during one hour or recirculation.

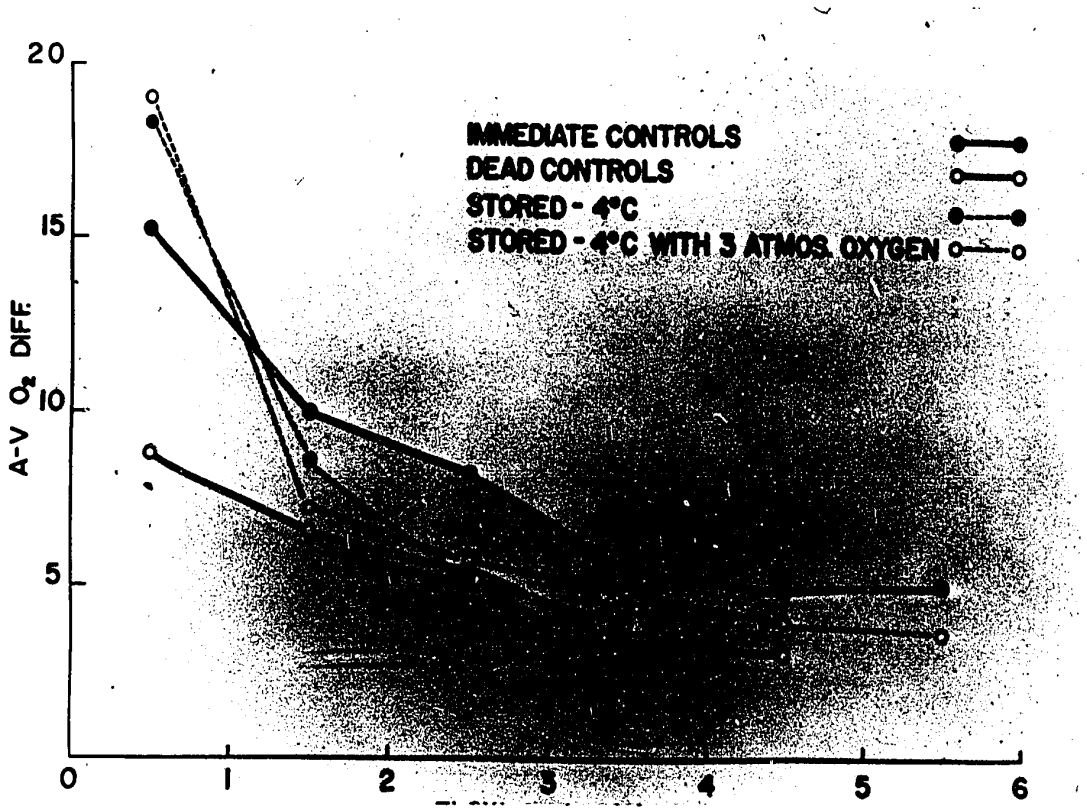


Fig. 22. - Relationship of A-V oxygen difference to blood flow rates for all groups.

plotted at intervals over one hour (Fig. 21). Applying tests of significance to these means, the following can be stated:

a. In the immediate control group (Group 3) there was a significant decrease in blood flow rate during the early minutes of recirculation (significant at the 2% level by t-test - mean at 1/2 minute against mean at 2 minutes) with return to initial levels by 10 minutes,

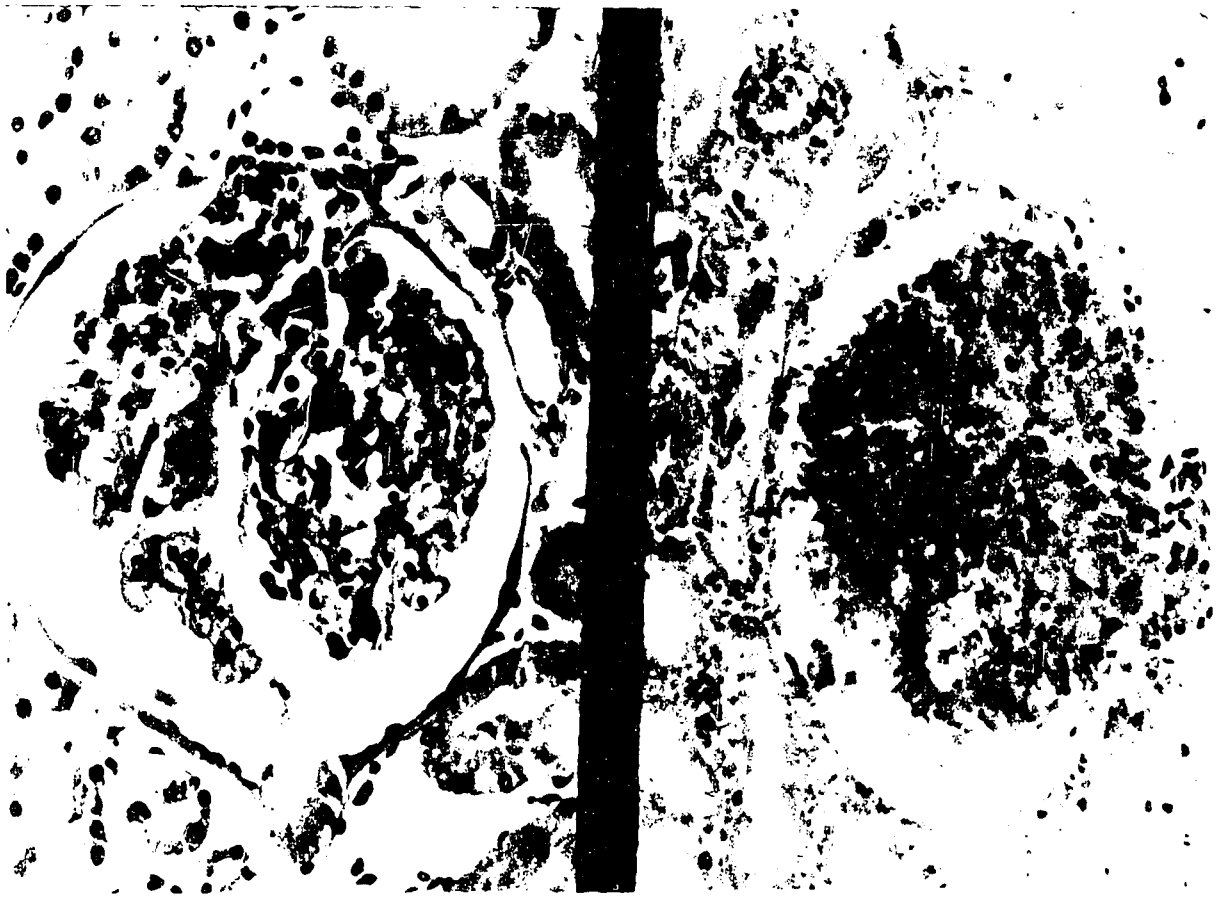
b. the flow rate of immediate control kidneys was significantly higher than that of all other groups after 10 minutes of recirculation (significant at 5% confidence limits by t-test),

c. the flow rate for hypothermic-hyperbaric kidneys (Group 2) was significantly higher than dead kidneys during the first 10 minutes (significant at the 1% level - the average of the means from 1/2 to minutes),

d. there is no statistically significant difference in flow rates between preserved and dead kidneys after 10 minutes.

4. Arterio-venous Oxygen Difference

The average arterio-venous oxygen difference for each group of kidneys was plotted against flow in ml/gm/min. (Fig. 22). The greatest A-V O_2 difference was seen at flow rates below 1 ml/gm/min and represented a significant difference, for all groups, from the A-V O_2 difference obtained with flow above 1 ml./gm/min. (significant at the 5% level by standard t-test). This difference was less marked in the dead kidney group than in the other three groups. The difference in oxygen consumption between the groups is proportional to the arterio-venous oxygen difference at any



one flow rate. The oxygen consumption of dead kidneys (Group 4) was statistically less than that of immediate controls (Group 3- significant at 5% level by t-test). However, it was not possible to demonstrate any statistical difference in oxygen consumption between the preserved and immediate kidneys or the preserved and dead kidneys. It is apparent that a considerable lowering of the blood flow rate produces a much more profound change in the A-V oxygen difference than change resulting from variations in the oxygen consumption capacity of the kidney.

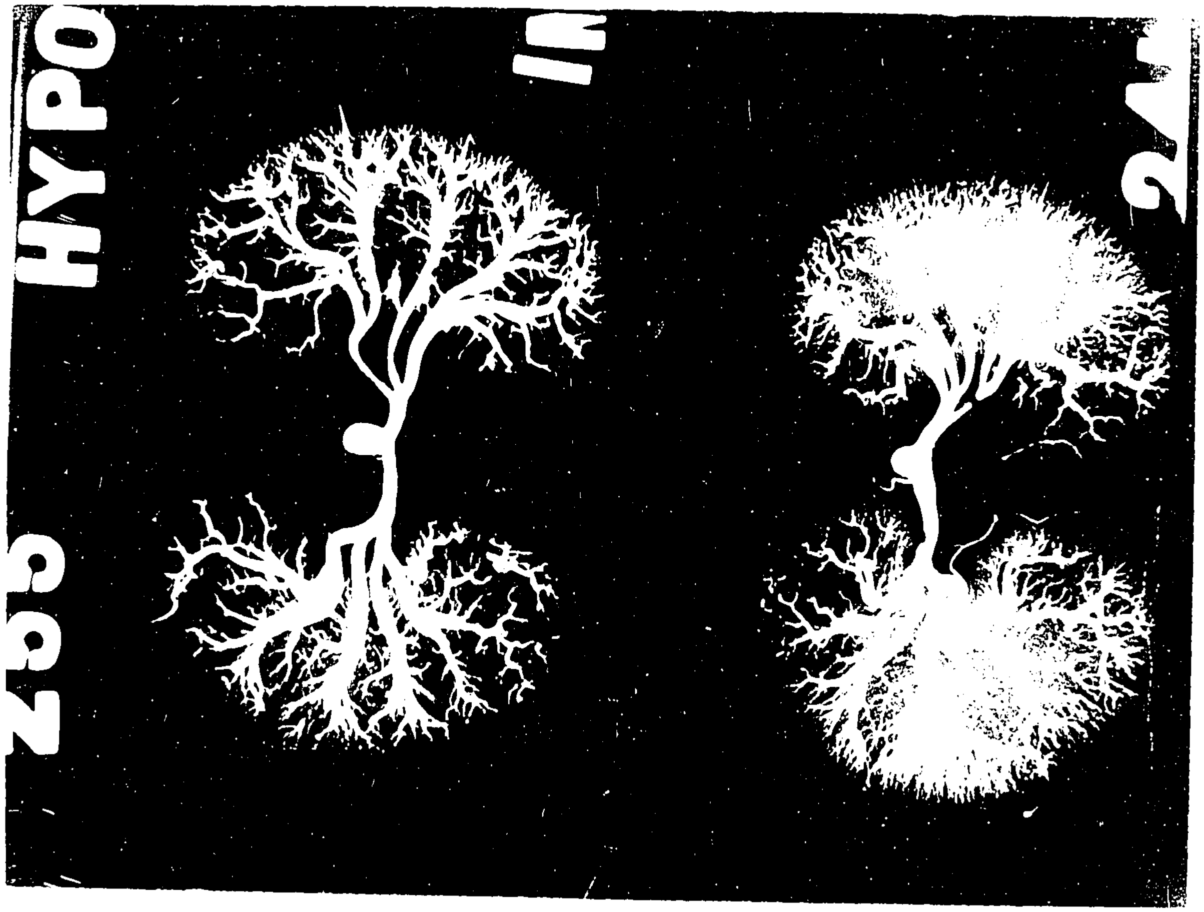
5. Gross Appearance

Dead control kidneys (Group 4) characteristically showed severe cortical and medullary congestion following recirculation, while preserved kidneys of both groups showed only a narrow band of congestion at the outer third of the medulla (Fig. 23). Considerable accumulation of edema fluid was commonly noted in the peri-renal connective tissue in dead and preserved kidneys, but was uncommon in immediate controls.

6. Microscopic Appearance

The microscopic appearance of dead kidneys was characterized by severe cortical hemorrhages, with rupture of glomeruli and hemorrhage into Bowman's space, together with severe tubular autolysis. By comparison, the architecture was well preserved in both groups of preserved kidneys with only minor tubular changes observed in the preserved kidneys (Fig. 24). The zone of congestion noted grossly was seen, microscopically, to be due to severe congestion of the vasa recta.

HYP
IN



[illegible] – R[illegible] after intra-arterial injection [illegible] control kidney on the left compared with [illegible] (C) [illegible] on the right. Note [illegible] kidney.

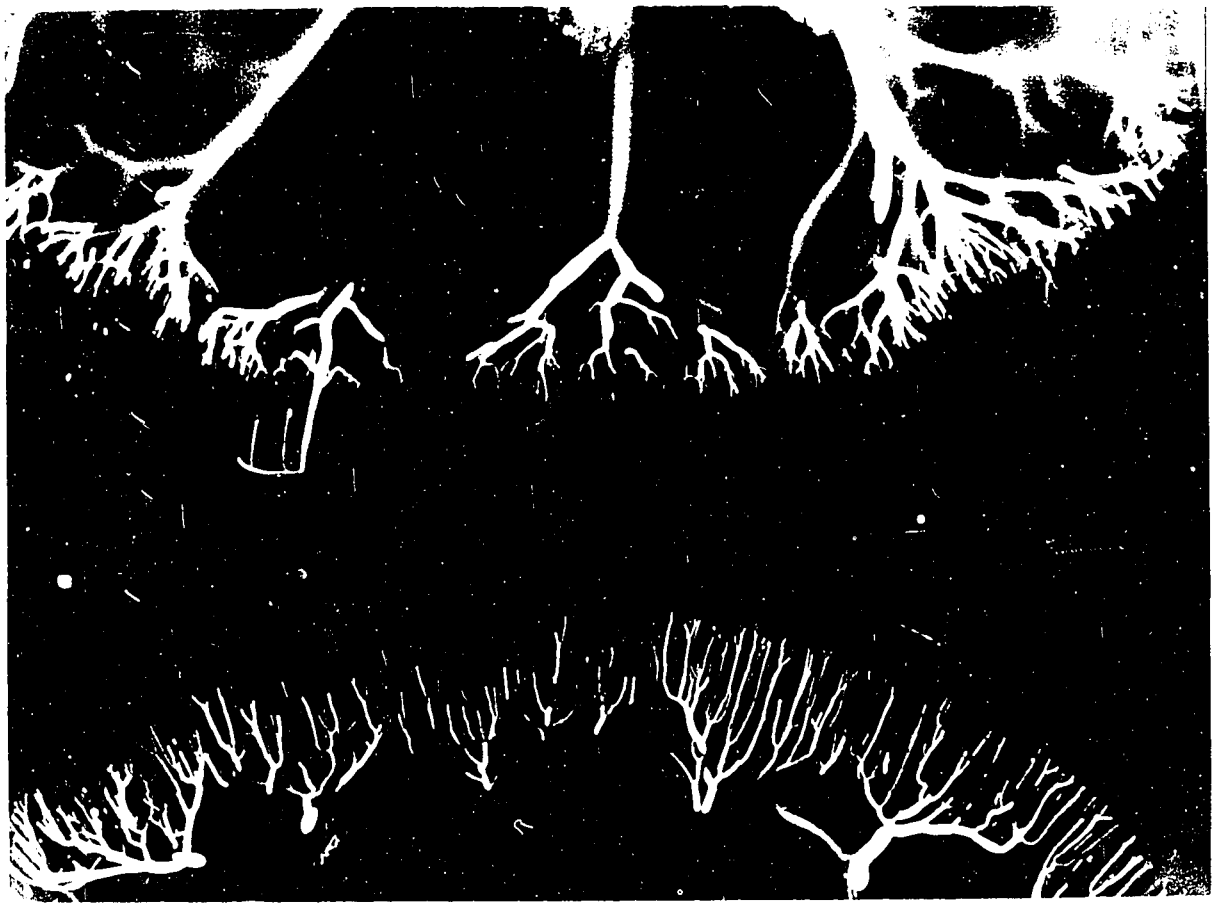


Fig. 27. - Schlesinger's mass injection with methyl salicylate clearing of an immediate control kidney (top) and a preserved kidney (bottom). Note dominance of dilated intertubular capillaries in the cortex of the preserved kidney.

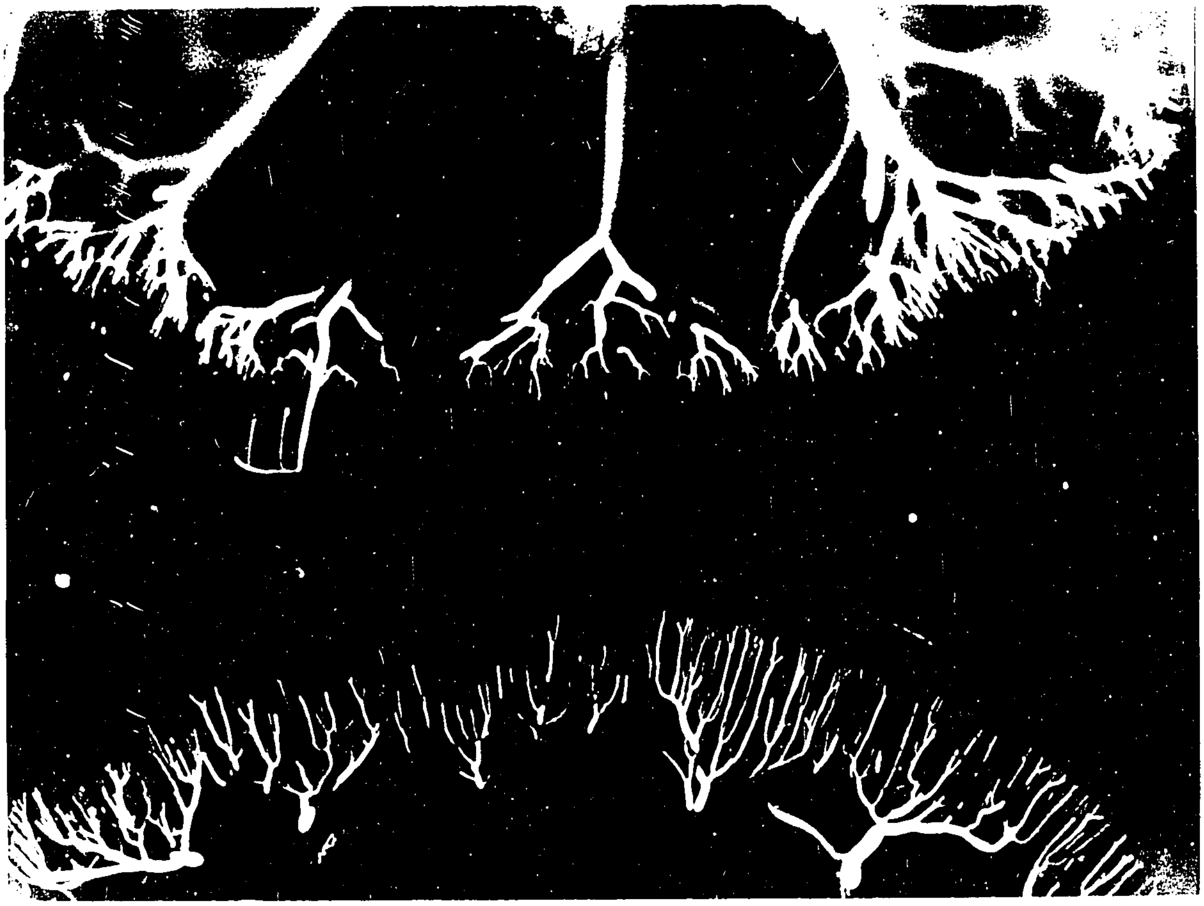


Fig. 27. - Schlesinger's mass injection with methyl salicylate clearing of an immediate control kidney (top) and a preserved kidney (bottom). Note abundance of dilated interlobular capil- lar[illegible] the [illegible] of the preserved kidney.

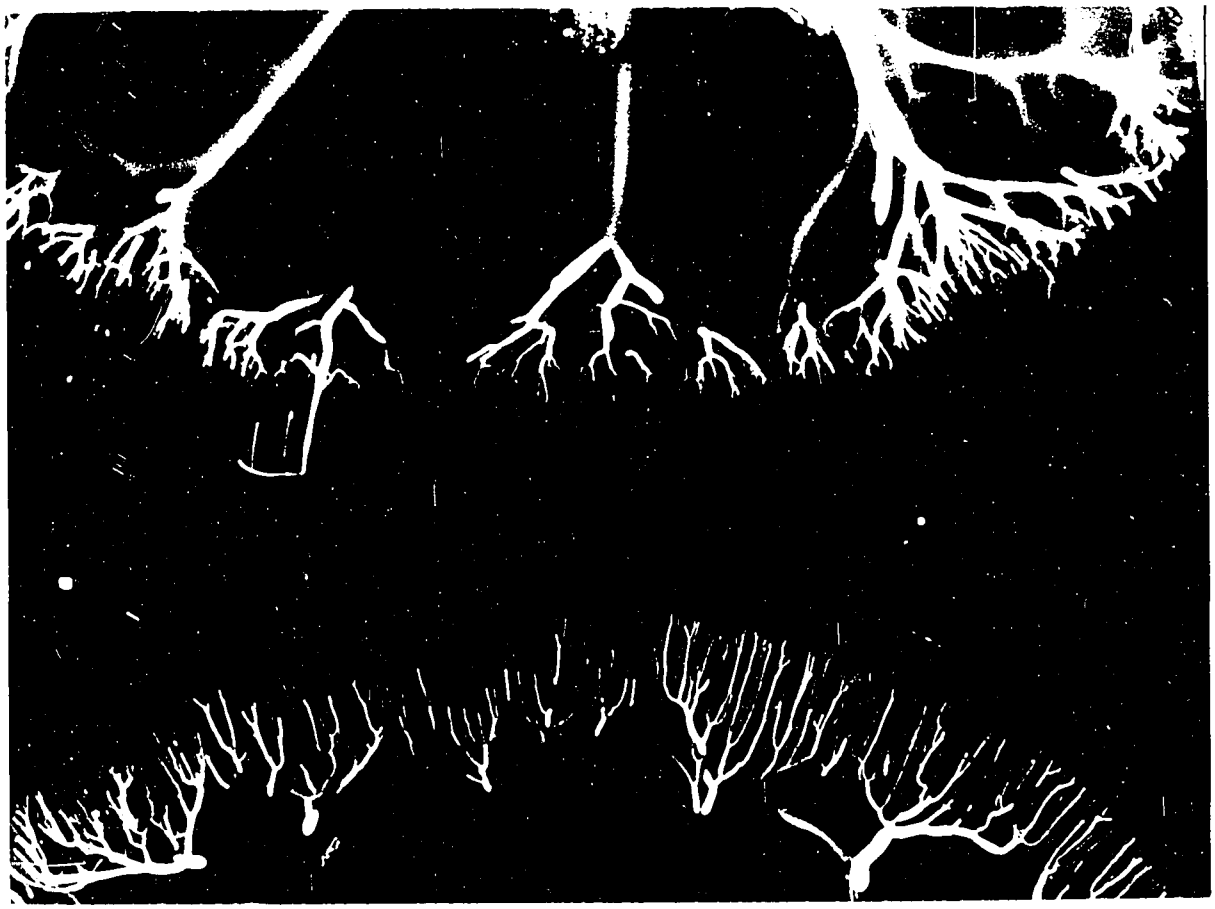


Fig. 27. - Schlesinger's mass injection with methyl salicylate clearing of an immediate control kidney (top) and a preserved kidney (bottom). Note abundance of dilated intertubular capillaries on the cortex of the preserved kidney.

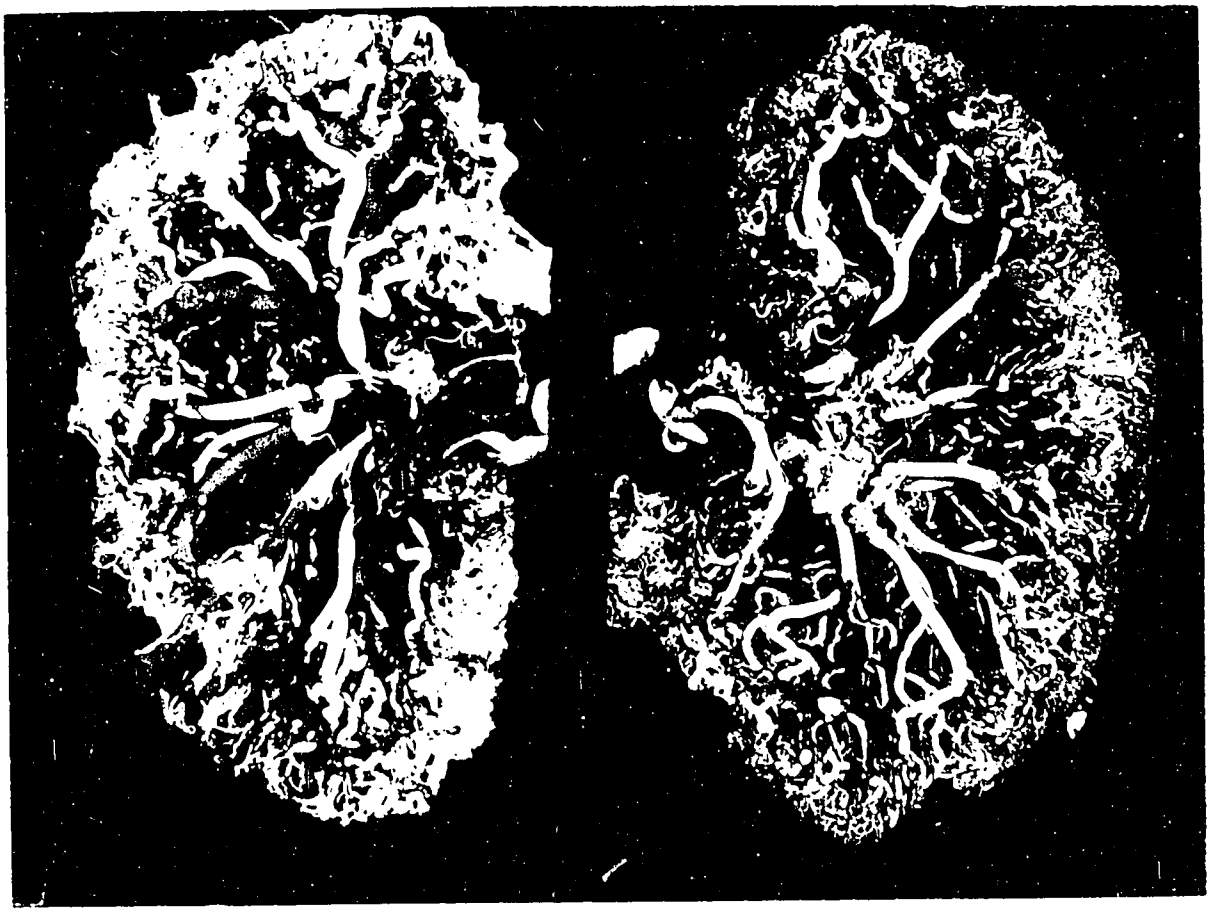


Fig. 28. - Latex casts of the arterial and venous system of immediate control kidney on the left and hypothermic preserved kidney on the right. (Arteries are represented by light pink latex and the veins with blue latex). The overfill of venous system is obvious in the stored kidney.

7. Appearance after India Ink Injection

The ink penetrated as far as the ruptured glomeruli in the dead group, but not into the intertubular capillaries, whereas in preserved kidneys and in immediate controls good filling of intertubular capillaries was observed (Fig.M25).

8. Radiography

Injection of Schlesinger's mass into the arterial tree consistently showed filling of interlobular arteries in the cortex of the preserved kidneys, which was not seen following injection of the immediate control kidneys (Fig. 26). This finding was confirmed by subsequent clearing of these kidneys with methyl salicylate (Fig. 27).

9. Latex Casts

The casts revealed marked overfilling of the venous tree in the preserved kidneys as compared to the immediate group (Fig. 28). The dead controls showed no filling of glomeruli, causing the arterial side to resemble a "dead tree appearance", as compared to preserved and immediate control kidneys in which the glomeruli hung in profusion from the arterial branches (Fig. 29).

DISCUSSION

Renal allografts from a cadaveric source have functioned and maintained life in a much larger percentage of instances than originally suspected (243a, 244a). Failure was predicted on the likelihood of great antigenic differences between donor and recipient. It has now become apparent that renal allografts

from living related donors do only slightly better in terms of absolute survival and renal function than do allografts from the cadaveric source (64a, 143a, 166a, 219b, 305a). Furthermore, it is apparent from paired transplants in which two recipients each received a kidney from the same donor that a very limited number of strong histocompatibility antigens are involved. In paired transplants the two recipients frequently behave similarly, either with prolonged allografts survival even without detectable evidence of rejection or by early, prompt, near simultaneous rejection (219b).

These observations support the use of cadaver donors, a policy which is laready recommended to gain experience for application when non-paired organs are to be transplanted.

The limiting feature of a cadaveric renal transplant programs is the paucity of donors. Permission from relatives prior to death of the donor must be obtained. The donor who succumbs as a result of generalized malignancy or infection, or is aged or has renal disease is not acceptable. In the experience of the Royal Victoria Hospital Transplantation Program, patients dying of central nervous system trauma, tumor or hemorrhage are the only suitable donors.

If patients salvageable by transplantation techniques are to be helped, organs presently wasted because of the lack of methods of preservation will have to become more generally available. The kidneys of suitable donors dying at a distance from a transplantation center could be used if a simple, effective technique of preservation was available. In addition, the poor

preparation of the patient attendant with the emergency nature of the present allografting procedures which contribute materially to morbidity and mortality could be largely be obviated. Transplantation could then be carried out under more convenient and ideal conditions.

The present method, an extension of many studies in the past, should meet immediate needs. In reviewing the works of Calne (64), Couch (93), Dempster (109), Kiser (191), Simso (289), and others (12, 110, 115, 116, 195, 259, 281, 315), hypothermia alone is generally unsuccessful for kidney preservation beyond 12 hours except for a few survivors (115, 116, 191, 259, 289). Longer periods of preservation have required either perfusion alone (93, 315) or with hypothermia (167 - 171, 205).

The use of a perfusion system, while most valuable for many other purposes, does not seem ideal for the present need, that is preservation of organs during transport to a transplantation center because of the involved nature of the technique.

Those who have advocated hyperbaric oxygen in the past have not reported survivors when hypothermia alone was used for 24-hour preservation. In contrast, in the present study the majority of animals autografted with a kidney preserved by cooling to 4°C for 24 hours, survived. Measurements of blood urea nitrogen and serum creatinine in the long-term survivors did not separate these animals from those which received a kidney preserved with hyperbaric oxygen and hypothermia. It required clearance tests to reveal the advantage of the latter technique.

That oxygen under increased pressure combined with hypothermia improves the quality of preservation achieved by hypothermia alone on ischemic kidneys is indicated by these experiments. It is also apparent that kidneys exposed to hyperbaric oxygen only, for 24-hour periods will not sustain the life of the host following contra-lateral nephrectomy. Further convincing evidence that hyperbaric oxygen does add something to hypothermia alone were the survivors which were recipients of kidneys stored for 48 hours, when the excision and replantation were done in the operating hyperbaric chamber. None survived 48 hours of storage when the procedures were conducted at ambient pressure. Attempts extend the period of preservation to 72 hours using combined hypothermia and hyperbaric oxygen were unsuccessful.

While hyperbaric oxygen does not appear from these studies to offer as much as was previously believed, it does suggest that it should be included for any preservation technique for times to exceed 8 to 12 hours.

The early results of the present study parallel that reported by the group of Manax (224-227) and others (208a, 273) although in the former study the preserved kidneys were replanted in the cervical region. The reports of Manax and associates does not include the long-term fate of animals receiving kidneys stored by hypothermic-hyperbaric method of preservation. In this investigation the majority of animals receiving renal autotransplants preserved for periods of 24 and 48 hours by means of a combina-

nation of hypothermia and hyperbaric oxygen were alive, normotensive, and possessing good renal function at least 36 months following removal of the opposite kidney.

Manax, Lillehei and others (41, 126, 212, 217, 218, 224-227) have suggested that the ischemic tolerance of isolated organs can be lengthened by increasing the pressure of oxygen during storage beyond 10 atmospheres. This is not borne out in the present investigation. Hyperbaric oxygen below 5 atmospheres or above 10 atmospheres is of no demonstrable value.

These results would lend support to the use of either systemic or regional cold perfusion immediately post-mortem in the procurement of organs to effect prompt cooling as suggested by Marchioro and colleagues (228) and the use of a hyperbaric theater which incorporates the functional features of an intensive care unit and operating suite wherein harvesting and replanting of organs could be carried out with safety as advocated by Hitchcock and collaborators(157,157a, 158).

The above concepts should be validated more extensively and should enjoy wider use clinically than practised at present.

The explanation for the preserving effect observed in these experiments is unclear. The metabolic reducing capacity of hypothermia has been extensively studied (27, 36, 39, 134, 135, 210, 335, 336). It can be postulated that the preserving effect of oxygen under increased pressure may be derived by supplying the markedly reduced oxygen requirements of cells under hypothermic conditions. On the other hand, it is also possible that

the inhibiting effect of hyperbaric oxygen on certain key metabolic enzyme systems (18, 23, 24, 11a, 149, 318, 337), may provide the tissue preserving mechanism.

It is claimed herein that the original contributions arising from this work consists in the determination of the comparative value of hypothermia alone, for renal preservation in dogs for 24-hour periods or when used together with hyperbaric oxygen to preserve isolated ischemic kidneys up to 24 hours with the consequent clearer definition of the optium range of oxygen pressure which confers maximum protection to isolated kidneys from ischemic damage. The delineation of the preserving influence of hypothermia alone, is thought crucial in the evaluation of the benefitial effects of hyperbaric oxygen in combination with hypothermia in organ preservation.

SUMMARY

An experiment was designed to evaluate the relative value of hypothermia alone with hyperbaric oxygen for renal preservation in the dog.

Kidneys were removed and either immediately reimplanted in the pelvis or stored for periods ranging from 24 to 72 hours. The opposite kidney was removed at the time of reimplantation in the control group but after 3 to 4 weeks in all other animals.

The results were assessed by mortality, renal function (specific gravity of fasting urine, blood urea nitrogen, serum creatinine, and clearances of creatinine and para-amino-Hippurate) and gross as well as microscopic pathology. The study was con-

ducted on 137 dogs of which 72 were long-term survivors (up to 3 years) which had renal function and pathological examinations.

Maximum survival and best long-term function was achieved in those animals which received a kidney stored at 4°C for 24 hours at between 5 and 10 atmospheres of oxygen. These did not vary significantly from controls (animals which had the kidney immediately reimplanted). The majority of these animals were normotensive and possessed good renal function and had a striking hypertrophy of the only remaining kidney. Levels of hyperbaric oxygen above 10 atmospheres or below 2 atmospheres decreased the quality of long-term survival.

Four of 10 animals survived on a kidney stored for 48 hours. In addition to storage under hyperbaric conditions these were reimplanted in a hyperbaric atmosphere. In 10 other animals which received kidneys stored identically for 48 hours but excised and reimplanted at ambient pressure there were no survivors.

The value of long-term observation and study using renal clearance tests is recommended to detect differences in methods of preservation of kidneys.

Claim is made on the originality of the data included in this thesis which consists of the successful 24-hour preservation of dog kidneys utilizing hypothermia alone and the demonstration of the superiority of hypothermic-hyperbaric method of preservation at between 5 to 10 atmospheres of oxygen. The latter technique is further supported by the survivors which received kidneys stored for 48 hours when the excision and reimplantation were performed in the operating hyperbaric chamber.

The clinical implications of the informations contained in this thesis is discussed and a suggestion is put forward that a wider use and validation of this technique should be made in organ procurement and transplantation.

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APPENDICES

Appendices

Appendix A. Master Tables:

1. Clearance of Creatinine and PAH of all Survivors.
2. Pathology of Survivors.
3. Pathology of Mortalities.

Appendix B. Photographs of Long-term Survivors.

Appendix C. Pictorial Sequences and Photomicrographs of Kidneys at Transplantation, Biopsy and Sacrifice.

APPENDIX A

Table 1. Clearances of Creatinine (C_{cr}) and Para-Amino-Hippurate (C_{pah}) of Long-term Survivors.

2. Summary of Gross and Histopathologic Features of Survivors.

3. Summary of Gross and Histopathologic Features of Mortalities.

Table I. Clearances of Creatinine (C_{cr}) and Para-Amino-Hippurate (C_{pah}) of long-term survivors.

* - sacrifice

GROUPS		No.	MONTHS 1	2	4	6	8	10	12	14	16	18	20	22	24	26	28
C	C_{cr}	3			58	110	77		s*								
	C_{pah}					182											
O	C_{cr}	4			69			s									
	C_{pah}				126												
N	C_{cr}	5					98	s									
	C_{pah}						232										
T	C_{cr}	6					116					92			105		
	C_{pah}				431	284									404		
R	C_{cr}	7					88					57			68		
	C_{pah}				246	198									230		
O	C_{cr}	8				92	104					145			97		
	C_{pah}					322	216								351		
L	C_{cr}	9				65	104		s								
	C_{pah}							403									
MEAN	C_{cr}	7															
	C_{pah}																

MONTHS

6	8	10	12	14	16	18	20	22	24	26	28	30	Average	Filtration Fraction
3 110	77	s*											82 182	0.45
69 126	s												69 126	0.55
	98 s 232												98 232	0.42
	116					92			105			s	104 280	0.37
31	284								404					
46	88 198					57			68 230			71 s 145	65 205	
92 322	104 216					145			97 351			s	110 296	0.37
65	104		s 403										85 403	0.21
													88+17 246+90	0.38

	GROUPS	No.	MONTHS 1.	2	4	6	8	10	12	14	16	18	20	22	Average	Filtra Fract
HYPOTHERMIA 4°C 24 HR.	C_{cr}	162	41				45				55			38	45	
	C_{pah}		61								119			s 64	81	0.5
	C_{cr}	164			34		45			58				35	43	
	C_{pah}				92					98				s 95	95	0.4
	C_{cr}	187	48	s											48	
	C_{pah}		155													0.3
	C_{cr}	193	57				52		s						55	
	C_{pah}		104												107	0.5
	C_{cr}	201		59	57	76	76			72			43		61	
	C_{pah}			103	157					183			s 111		139	0.4
	C_{cr}	219		55		47				62					55	
	C_{pah}			87						108			s		98	0.5
	C_{cr}	MEAN 6													51±7	
	C_{pah}														113±28	0.4

MONTHS

6	8	10	12	14	16	18	20	22	Average	Filtration Fraction
	45				55			38	45	
								s		0.56
					119			64	81	
	45			58			35		43	
							s			0.45
				98			95		95	
									48	
										0.31
	52								55	
			s							0.51
									107	
	7676			72			43		61	
							s			0.44
				183			111		139	
	47			62					55	
							s			0.56
				108					98	
									51±7	
										0.45
									113±28	

GROUPS		No.	MONTHS 2	4	6	8	10	12	14	16	18	20	Average
2 ATA-HBO 4°C 24 HRS	C_{cr}	206		65		80			67			53 s	66
	C_{pah}			124					107		133	92	114
	C_{cr}	232	99		49				35		17		50
	C_{pah}		85		15				52		28 s		45
	C_{cr}	236	17			38		23			17 s		24
	C_{pah}		28					18			28		25
	C_{cr}	238		22	36			14					24
	C_{pah}			28				31			s		30
MEAN	C_{cr}	4											41±21
	C_{pah}												54±41

MONTHS												
2	4	6	8	10	12	14	16	18	20	Average	Filtration Fraction
	65		80			67			53	66	
	124					107		133	s 92	114	0.58
		49				35		17		50	
		49				35		17s		50	0.1
		15				52		s 28		45	1.1
			38		23			17		24	
								s			0.96
					18			28		25	
2		36			14					24	
								s			0.80
8					31					30	
										41±21	
											0.76
										54±41	

GROUPS		No.	MONTHS 1	2	4	6	8	10	12	14	16	18	20	22	24	26	28	Average	Fil Fr
5 ATA-HBO	C_{cr}	13						107										107	
	C_{pah}									166			s					166	0.
4°C 24 HRS.	C_{cr}	14					74					54		131				86	
	C_{pah}						127							344			s	236	0.
	C_{cr}	16					114				86			42				81	
	C_{pah}							125						87			s	106	0.
	C_{cr}	38			22				21		37							30	
	C_{pah}				62							s						62	00.
	C_{cr}	50			153						106			74				111	
	C_{pah}				136									128			s	132	0.8
MEAN	C_{cr}	5																83±31	
	C_{pah}																	140±66	0.5

MONTHS														Average	Filtration Fraction
4	6	8	10	12	14	16	18	20	22	24	26	28			
			107											107	0.64
					166			s						166	
		74					54		131					86	0.36
		127							344			s		236	
		114				86			42					81	0.76
			125						87			s		106	
	22			21		37								30	00.48
	62						s							62	
		153				106			74					111	0.84
		136							128			s		132	
														83±31	0.59
														140±66	

GROUPS		No.	MONTHS 1	2	4	6	8	10	12	14	16	18	20	22	24	Average	Fil Fr
10 ATA-HBO	C_{cr}	87		37						31						34	
	C_{pah}			40						38		s				40	
4°C 24 HRS.	C_{cr}	100			105					101			50			85	
	C_{pah}				85								155		s	120	
	C_{cr}	106		51					109							80	
	C_{pah}			135					s							135	
	C_{cr}	109		38												38	
	C_{pah}				72											172	
	C_{cr}	112		74					100			54	86	77		78	
	C_{pah}			215								128	130	123	s	169	
	C_{cr}	127		104					72			71				82	
	C_{pah}			267								139			s	203	
MEAN	C_{cr}	6														66±24	
	C_{pah}															120±58	

MONTHS													
4	6	8	10	12	14	16	18	20	22	24	Average	Filtration Fraction
					31						34	
						s						0.85
					38						40	
105					101			50			85	
85								155		s	120	0.71
51				109							80	
35				s							135	0.59
38											38	
	72										172	0.53
				100			54	86	77		78	
							128	130	123	s	269	0.52
				72			71				82	
							139			s	203	0.46
											66±24	
											120±58	0.55

GROUPS		No.	MONTHS 1	2	4	6	8	10	12	14	16	18	20	22	24	Aver
20 ATA-HBO	C_{cr}	117			17		15									17
	C_{pah}				43				s							43
4°C 24 HRS.	C_{cr}	119			74				55			33	32			49
	C_{pah}				199							57	51	s		102
	C_{cr}	128		72				98				57			46	68
	C_{pah}			169								126			103	133
	C_{cr}	138		71				122			45					79
	C_{pah}			175							73		s			124
	C_{cr}	161		48				39			46					44
	C_{pah}			87							104			s		96
MEAN	C_{cr}	5														51±24
	C_{pah}															100±30

MONTHS													
4	6	8	10	12	14	16	18	20	22	24	Average	Filtration Fraction
17		15									17	0.40
43				s							43	
74				55			33		32		49	0.48
99							57		51	s	102	
			98				57			46	68	0.51
							126			103	133	
			122			45					79	0.64
						73		s			124	
			39			46					44	0.46
						104			8		96	
											51±24	0.51
											100±30	

GROUPS		No.	MONTHS 1	2	4	6	8	10	12	14	16	18	20	22	24	26
30 ATA-HBO 4°C 24 HRS.	C_{cr}	54					40				74			48		
	C_{pah}						88							70		
	C_{cr}	141		74					83			52				
	C_{pah}			116								145			s	
	C_{cr}	142		83				31				66		36		
	C_{pah}			250								81		97	s	
	C_{cr}	148		52				82				59				
	C_{pah}			114								176			s	
	C_{cr}	155		50							53					
	C_{pah}			125							116				s	
	C_{cr}	156			63											
	C_{pah}				126			s								
	C_{cr}	160			54		92				64					
	C_{pah}				113						197				s	
MEAN	C_{cr}	7														
	C_{pah}															

MONTHS														
2	4	6	8	10	12	14	16	18	20	22	24	26	Average	Filtration Fraction
			40				74			48			54	
			88							70			79	0.68
74					83			52					70	
116								145		s			131	0.53
83				31				66		36			54	
250								81		97	s		143	0.38
52				82				59					64	
114								176			s		145	0.44
50							53						52	
125							116				s		121	0.43
	63												63	
	126			s									126	0.50
	54			92			64						70	
	113						197				s		105	0.67
													61±10	
													121±23	0.67

GROUPS		No.	MONTHS 1.	2	4	6	8	10	12	14	16	18	20	Avera
10 ATA-HBO	C_{cr}	169			23			25		31				26
	C_{pah}				50					52				51
4°C	C_{cr}	212		11										11
	C_{pah}			44										44
48 HRS.	C_{cr}	220			44		48		40					44
	C_{pah}				26				76					51
	C_{cr}	270	48	79	40									56
	C_{pah}		100				s							100
MEAN	C_{cr}	4												34±20
	C_{pah}													62±26

MONTHS											
2	4	6	8	10	12	14	16	18	20	Average	Filtration Fraction
	23			25	31					26	
	50				52					51	0.51
	11									11	
	44									44	0025
	44		48		40					44	
	26				76					51	0.86
48	79	40								56	
)0			s							100	0.56
										34±20	
										62±26	0.55

Table 2. Gross and Microscopic Pathology of Survivors.

LEGEND - PATHOLOGY

A. GROSS

1- = 25% reduction
2- = 33% "
3- = 50% "
4- = 67% "
5- = autolysis or fibrous remnant
0 = no appreciable change
+ = 60-80 grams
2+ = 80-100 "
3+ = 100-120 "
4+ = 120 and up

B. HISTOPATH

+ = Norm
chan

2+ = Sign
atro
memb

3+ = Infl

4+ = Exte
of ne

LEGEND - PATHOLOGY

reduction
"
"
"
ysis or fibrous remnant
ppreciable change
grams
0 "
20 "
nd up

B. HISTOPATHOLOGY

+ = Normal including minimal reversible changes of glomeruli and tubular.

2+ = Significant proportions of glomeruli atrophied or hyalinized with basement membrane thickening and crescents.

3+ = Inflammation + 2

4+ = Extensive necrosis and/or fibrosis of nephron and interstitium.

GROUPS	Dog No.	COMMENTS DURING REPLANTATION	BIOPSY	UREMIA	CAUSE TECHNICAL	OF OT
CONTROLS	1	Good				
	2	Good				
	3	Good				
	4	Fair				
	5	Good				
	6	Fair	Hypertrophied			
	7	Good	Hypertrophied			

Dog No.	COMMENTS DURING REPLANTATION	BIOPSY	CAUSE OF DEATH UREMIA	TECHNICAL	OTHERS	
1	Good				+	SACRIFICE
2	Good				+	SACRIFICE
3	Good				+	SACRIFICE
4	Fair				+	SACRIFICE
5	Good				+	SACRIFICE
6	Fair	Hypertrophied			+	SACRIFICE
7	Good	Hypertrophied			+	SACRIFICE

BIOCHEMICAL TESTS PRIOR TO DEATH mg%	Ml/Min/M^2	SURVIVAL (MONTHS)	GROSS	CONTROL	PATHOLOGY HISTOPATHOLOGY BIOPSY	AUTOPSY
BUN 18 Creatinine 1.0		8	Hypertrophied*	1		
BUN 18 Creatinine 0.8		8	"	1		
BUN 16 Creatinine 1.0 C_{cr} C_{pah}	77 18	8	"	1		
BUN 16 Creatinine 0.9 C_{cr} C_{pah}	69 126	8	"	1		
BUN 12 Creatinine 0.8 C_{cr} C_{pah}	98 174	8	"	1		
BUN 14 Creatinine 0.74 C_{cr} C_{pah}	105 404	30	2+	1	1	
BUN 16 Creatinine 0.86 C_{cr} C_{pah}	68 230	30	4+	1	1	

* not weighed

SURVIVAL (MONTHS)	PATHOLOGY GROSS	CONTROL	HISTOPATHOLOGY BIOPSY	AUTOPSY OF SACRIFICE
8	Hypertrophied*	1		1
8	"	1		1
8	"	1		1
8	"	1		1
8	"	1		1
30	2+	1	1	1
30	4+	1	1	1

veighed

GROUPS	DOG. No.	COMMENTS DURING REPLANTATION	BIOPSY	UREMIA	CAUSE OF DEATH TECHNICAL	OTHERS	
	8	Fair	Hyper-trophied			+	SA
	9	Good				+	SA
	10	Good				+	SA
HYPOTHERMIA 4°C 24 HOURS	162	Congested	2-			+	SA
	164	"	2-		+		Di la ra no
	187	Good	0		+		De cl
	193	Reduced venous return	1-		+		Di th re ri: do
	201	Good	1-			+	SA
	219	Good	1-			+	SA

COMMENTS DURING REPLANTATION	BIOPSY	CAUSE OF DEATH UREMIA	TECHNICAL	OTHERS	
Fair	Hyper-trophied			+	SACRIFICE
Good				+	SACRIFICE
Good				+	SACRIFICE
Congested	2-			+	SACRIFICE
"	2-		+		Died following abdominal laparotomy to perform clearance studies. Dog was cyanotic during test.
Good	0		+		Deeply anesthetized during clearance studies.
Reduced venous return	1-		+		Died when it was given anesthesia to free the dog and repair a wound sustained during a fight with another dog.
Good	1-			+	SACRIFICE
Good	1-			+	SACRIFICE

BIOCHEMICAL TESTS PRIOR TO DEATH mg%	Ml/Min/M_2	SURVIVAL (MONTHS)	GROSS	CONTROL	PATHOLOGY HISTOPAT BIOPSY
BUN 17 Cr 0.77 C_{cr} 97 C_{pah} 350		30	+	1	1
BUN 19 Cr 0.9 C_{cr} 104 C_{pah} 403		8	Hypertrophied	1	
BUN 12 Cr 0.9		8	"	1	
BUN 26 Cr 1.2 C_{cr} 55 C_{pah} 119		22	2+	1	1
BUN 76 Cr 2 C_{cr} 35 C_{pah} 95		21	Bila 2+ Bilateral pulmonary congestion	1	1
BUN Cr 0.84 C_{cr} 48		6 weeks	0	1	1
BUN 17 Cr 1.2 C_{cr} 52		12		1	1

SURVIVAL (MONTHS)	PATHOLOGY GROSS	CONTROL	HISTOPATHOLOGY BIOPSY	AUTOPSY OR SACRIFICE	
30	+	1	1	1	(182)
8	Hypertrophied	1		1	(9)
8	"	1		1	(10)
22	2+	1	1	2	(162)
21	Bila 2+ Bilateral pulmonary congestion	1	1		(164)
6 weeks	0	1	1	2	(187)
12		1	1	1	(193)

BIOCHEMICAL TESTS PRIOR TO DEATH mg%	Ml/Min/M_2	SURVIVAL (MONTHS)	GROSS	PATHOLOGY CONTROL	PATHOLOGY HISTOPATHOLC BIOPSY
BUN 17 Cr 0.77 C_{cr} 97 C_{pah} 350		30	+	1	1
BUN 19 Cr 0.9 C_{cr} 104 C_{pah} 403		8	Hypertrophied	1	
BUN 12 Cr 0.9		8	"	1	
BUN 26 Cr 1.2 C_{cr} 55 C_{pah} 119		22	2+	1	1
BUN 76 Cr 2 C_{cr} 35 C_{pah} 95		21	Bila 2+ Bilateral pulmonary congestion	1	1
BUN Cr 0.84 C_{cr} 48		6 weeks	0	1	1
BUN 17 Cr 1.2 C_{cr} 52		12		1	1

n/M_2	SURVIVAL (MONTHS)	PATHOLOGY GROSS	CONTROL	HISTOPATHOLOGY BIOPSY	AUTOPSY OR SACRIFICE	
	30	+	1	1	1	(182)
	8	Hypertrophied	1		1	(9)
	8	"	1		1	(10)
	22	2+	1	1	2	(162)
	21	Bila 2+ Bilateral pulmonary congestion	1	1		(164)
	6 weeks	0	1	1	2	(187)
	12		1	1	1	(193)

BIOCHEMICAL TESTS PRIOR TO DEATH			SURVIVAL (MONTHS)	PATHOLOGY		
	mg%	Ml/min/m^2		GROSS	CONTROL	HISTOPAT BIOPSY
BUN	19					
Cr	1.1					
C_{cr}		72	21	+	1	2
C_{pah}		188				
BUN	16					
Cr	1.1					
C_{cr}		62	20	2+	1	1
C_{pah}		108				

STS mg%	Ml/min/m^2	SURVIVAL (MONTHS)	PATHOLOGY GROSS	CONTROL	HISTOPATHOLOGY BIOPSY	AUTOPSY OR SACRIFICE	
19 1.1	72 188	21	+	1	2	2	(201)
16 1.1	62 108	20	2+	1	1	1	(219)

GROUPS	DOG No.	COMMENTS DURING REPLANTATION	BIOPSY	CAUSE OF DEATH UREMIA	TECHNICAL	C
2 ATA-HBO 24 HRS. HYPOTHERMIA	206	Good	1-			
	227	Cyanotic kidney	0			
	232	Good	0			
	236	Excellent	2-			
	238	Fair	2-			
5 ATA-HBO 24 HRS. HYPOTHERMIA	13	Congested kidney chamber failure	0			+
	14	Good	1+			+
	16	Good				1+
	38		2-			+
	49	Good	0			+
	50		Enlarged			+
10 ATA-HBO 24 HOURS HYPOTHERMIA	87	Anomalous blood supply; cyanotic p-i quadrant.	Enlarged			+

COMMENTS DURING PLANTATION	BIOPSY	CAUSE OF DEATH UREMIA	TECHNICAL	OTHERS	
Good	1-			+	SACRIFICE
anotic idney	0			+	" Dog had mange and was vicious
Good	0			+	SACRIFICE
ellent	2-			+	"
air	2-			+	"
gested ney ıber .ure od	0			+	Had melena and hematemesis. No appreciable weight loss.
od	1+			+	SACRIFICE
				1+	Died following a kidney function test.
	2-			+	Could not be located. Possibly died following recent clearance studies.
d	0			+	Died in the farm during a fight with other dogs
	Enlarged			+	SACRIFICE
malous od sup- cyano- p-i lrant.	Enlarged			+	Died at McIntyre Bldg. of unascertained cause

BIOCHEMICAL TESTS PRIOR TO DEATH	mg%	Ml/min/m^2	SURVIVAL (MONTHS)	GROSS	CONTROL	PA
BUN	14					
Cr	0.61					
C_{cr}		67	20	2+	1	
C_{pah}		107				
BUN	51					
Cr	1.6		4			
BUN	21					
Cr	1.1					
C_{cr}		35	19	0	1	
C_{pah}		52				
BUN	39					
Cr	1.7					
C_{cr}		23	18	0	1	
C_{pah}		18				
BUN	22					
cr	1.5					
C_{cr}		15	18	1+	1	
C_{pah}		31				
BUN	14					
Cr	0.9					
C_{cr}	6.4	acute 6.4	21	Old blood in G-I tract hemorrhagic area on ant. surface of the kidney.	1	
C_{cr}	episode	61				
C_{pah}		166				
BUN	18					
Cr	1.0					
C_{cr}		131	29	1+	1	
C_{pah}		344				
BUN	19					
Cr	1.2					
C_{cr}		42	28		1	
C_{pah}		87				

Ml/min/m^2	SURVIVAL (MONTHS)	PATHOLOGY GROSS	CONTROL	HISTOPATHOLOGY BIOPSY	AUTOPSY	OR SACRIFICE
67 107	20	2+	1	1	2	(206)
	4					(227)
35 52	19	0	1	1	3	(232)
23 18	18	0	1		3	(236)
15 31	18	1+	1	1		(238)
acute 5.4 pisode 61 166	21	Old blood in G-I tract hemorrhagic area on ant. surface of the kidney.	1	1	1	(13)
131 344	29	1+	1	1	2	(14)
42 87	28		1	1	1	(16)

BIOCHEMICAL TESTS PRIOR TO DEATH	mg%	ml/min/m^2	SURVIVAL (MONTHS)	PATHOLOGY GROSS	HISTOPATHO CONTROL	BIOPSY
BUN Cr C_{cr}	44 2	37	19		1	1
BUN Cr	20 1.0		6	Autolyzed	1	
BUN Cr C_{cr}	22 1.0	74	28	1+	1	1
BUN Cr C_{cr}	11 0.8	37	17		1	1

ml/min/m^2	SURVIVAL (MONTHS)	PATHOLOGY GROSS	HISTOPATHOLOGY CONTROL	BIOPSY	AUTOPSY OR SACRIFICE	
37	19		1	1		(38)
	6	Autolyzed	1			(49)
74	28	1+	1	1	1	(50)
37	17		1	1	1	(87)

GROUPS	DOG No.	COMMENTS DURING REPLANTATION BIOPSY	UREMIA	CAUSE OF DEATH TECHNICAL
	100	Excellent		
	106	Good	0	
	109	Good	1-	
	112	Good	0	
	127	Excellent	Excellent	

COMMENTS DURING REPLANTATION BIOPSY		CAUSE OF DEATH			
		UREMIA	TECHNICAL	OTHERS	
Excellent				+	SACRIFICE
Good	0			+	Died at McIntyre Bldg. of undetermined cause
Good	1-			+	Lost in a private home
Good	0			+	SACRIFICE
Excellent	Excellent			+	" "

BIOCHEMICAL TESTS PRIOR TO DEATH	mg%	ml/min/m^2	SURVIVAL (MONTHS)	PATHOLOGY GROSS	HISTOPATHOLOGY CONTROL	HISTOPATHOLOGY BIOPSY	HISTOPATHOLOGY AUTOPS
BUN	11						
Cr	1.0						
C_{cr}		50	25	2+	1	1	1
C_{pah}		155					
BUN	14						
Cr	0.8						
C_{cr}		56	16		1		1
C_{pah}		148					
BUN	16						
Cr	1.0						
C_{cr}		38	13		1	1	
C_{pah}		72					
BUN	12						
Cr	1.1						
C_{cr}		54	24		1	1	1
C_{pah}		128		3+			
BUN	13						
Cr	0.7						
C_{cr}		71	24	+	1	1	1
C_{pah}		139					

ESTS H mg%	ml/min/m^2	SURVIVAL (MONTHS)	PATHOLOGY GROSS	HISTOPATHOLOGY CONTROL	BIOPSY	AUTOPSY OR SACRIFICE	
11							
1.0							(100)
	50	25	2+	1	1	1	
	155						
14							
0.8							(106)
	56	16		1		1	
	148						
16							
1.0							(109)
	38	13		1	1		
	72						
12							
1.1							(112)
	54	24		1	1	1	
	128		3+				
13							
0.7							(127)
	71	24	+	1	1	1	
	139						

GROUPS	DOG No.	COMMENTS DURING REPLANTATION	BIOPSY	CAUSE OF DEATH UREMIA	TECHNICAL	OTHERS
20 ATA-HBO 4°C 24 HOURS	117	good	0	+		
	119	"	Excellent		+	SACRI
	128	"	"		+	"
	138	Excellent	"		+	Pyohydr enlarge
	161	"	0		+	SACRIFI
30 ATA-HBO 4°C 24 HOURS	54	Dusky and congested	2-		+	SACRIFI
	141	Good	0		+	"
	142	"	Excellent		+	"

DOG No.	COMMENTS DURING REPLANTATION	BIOPSY	CAUSE OF DEATH UREMIA	TECHNICAL	OTHERS
117	good	0	+		
119	"	Excellent		+	SACRIFICE
28	"	"		+	"
38	Excellent	"		+	Pyohydronephrosis secondary to enlarge prostate
51	"	0		+	SACRIFICE
54	Dusky and congested	2-		+	SACRIFICE
41	Good	0		+	"
42	"	Excellent		+	"

BIOCHEMICAL TESTS PRIOR TO DEATH	mg%	ml/min/m^2	SURVIVAL (MONTHS)	PATHOLOGY GROSS	HISTOPATHOLOGY CONTROL	HISTOPATHOLOGY BIOPSY	HISTOPATHOLOGY AUT
BUN	40						
Cr	4		12		1		
BUN	38						
Cr	1.5						
C_{cr}		33	24	2+	1	3	
C_{pah}		57					
BUN	19						
Cr	0.9						
C_{cr}		57	24	3+	2		
C_{pah}		126					
BUN	26						
C_{cr}		45	21	Thin cortex with dilated collecting	1	2-3	
BUN	16						
Cr	1.2						
C_{cr}		46	22	2+	1	1	
C_{pah}		114					
BUN	20						
Cr	1.0						
C_{cr}		48	27	2+	1	2-3	
C_{pah}		70					
BUN	22						
Cr	1.0						
C_{cr}		52	23	2+	1	2	
C_{pah}		145					
BUN	26						
Cr	1.0						
C_{cr}		66	23	4+	2		
C_{pah}		81					

ml/min/m^2	SURVIVAL (MONTHS)	PATHOLOGY GROSS	HISTOPATHOLOGY CONTROL	BIOPSY	AUTOPSY OF SACRIFICE	
	12		1			(117)
33 57	24	2+	1	3	3	(119)
57 126	24	3+	2		2	(128)
45	21	Thin cortex with dilated collecting	1	2-3	3	(138)
46 114	22	2+	1	1	1	(161)
48 70	27	2+	1	2-3	2	(54)
52 145	23	2+	1	2	1	(141)
66 81	23	4+	2		2	(142)

BIOCHEMICAL TESTS PRIOR TO DEATH	MG%	ml/min/m^2	SURVIVAL (MONTHS)	PATHOLOGY GROSS	HISTOPATHOLOGY CONTROL	BIOPSY	AUTOPSY OR S
BUN	22						
Cr	1.4						
C_{cr}		59	23	+	1	1	2
C_{pah}		176					
BUN	15						
Cr	0.8						
C_{cr}		53	22	2+	1		2
C_{pah}		116					
BUN	19						
Cr	1.0						
C_{cr}		63	10		1	2-3	
C_{pah}		123					
BUN	25						
Cr	1.1						
C_{cr}		64	22	2+	1	4	1
C_{pah}		197					
BUN	40						
Cr	1.7						
C_{cr}		31	21	+	1	2	3
C_{pah}		52					
BUN	97						
Cr	3.5						
C_{cr}		11	8		1	2	
C_{pah}		44					
BUN	27						
Cr	1.5						
C_{cr}		40	20	+	1	4	3
C_{pah}		76					
BUN	22						
Cr	0.8						
Ccr & Cpah		79, 100	9	2+	1	2	2

ml/min/m^2	SURVIVAL (MONTHS)	PATHOLOGY GROSS	HISTOPATHOLOGY CONTROL	BIOPSY	AUTOPSY OR SACRIFICE	
59 176	23	+	1	1	2	(148)
53 116	22	2+	1		2	(155)
63 123	10		1	2-3		(156)
64 197	22	2+	1	4	1	(160)
31 52	21	+	1	2	3	(169)
11 44	8		1	2		(212)
40 76	20	+	1	4	3	(220)
79, 100	9	2+	1	2	2	(270)

GROUPS	DOG No.	COMMENTS DURING REPLANTATION	BIOPSY	CAUSE OF DEATH UREMIA	TECHNICAL	OTHERS
	148	Good	0			+
	155	"	0			+
	156	Fair	0			+
	160	Mottled area	0			+
10 ATA-HBO 4°C 48 HOURS	169	Reduced blood flow	3-			+
	212	Good	3-	+		
	220	Congested	2-			+
	270	"	2-			+

DOG No.	COMMENTS DURING REPLANTATION	BIOPSY	CAUSE OF DEATH UREMIA	TECHNICAL	OTHERS	
148	Good	0			+	SACRIFICE
155	"	0			+	"
156	Fair	0			+	Dog died at McIntyre Bldg.
160	Mottled area	0			+	SACRIFICE
169	Reduced blood flow	3-			+	"
212	Good	3-	+			Dog not returned
220	Congested	2-			+	SACRIFICE
270	"	2-			+	"

Table 3. Summary of clinical course and pathological features of unsuccessful autografts, gross as well as microscopic.

GROUPS	DOG No.	PERTINENT OBSERVATIONS DURING REPLANTATION	BIOPSY	UREMIA	TECHNICAL	OTHERS	CAUS
CONTROL	305	Congested			+		PNE
	336	Good			+		EVI
TOTAL	2						
HYPOTHERMIA 4°C 24 HOURS	168	Markedly reduced venous return	2- profused at biopsy site	+			REN
	177	""	5- vessels were intact	+			SAC ON ROT
	182	Good	5- Kidney autolyzed			+	PERI
	216	Fair	4-	+			DID CONT
TOTAL	4						
2 ATA-HBO 4°C 24 HOURS	241	Good	2- pericapsular edema		+		PNEU DISC
TOTAL	1						

PERTINENT OBSERVATIONS DURING REPLANTATION	BIOPSY	UREMIA	TECHNICAL	OTHERS	CAUSE OF DEATH
Congested			+		PNEUMONIA
Good			+		EVISCERATION
Markedly reduced venous return	2- profused at biopsy site	+			RENAL FAILURE
" "	5- vessels were intact	+			SACRIFICED DURING BIOPSY ON ACCOUNT OF SMALL FIBROTIC KIDNEY REMNANT
Good	5- Kidney autolyzed			+	PERITONITIS AND PNEUMONIA
Fair	4-	+			DID NOT WAKE-UP FOLLOWING CONTRALATERAL NEPHRECTOMY
Good	2- pericapsular edema		+		PNEUMONIA-PURULENT NASAL DISCHARGE AND CYANOSIS

BIOCHEMICAL TESTS PRIOR TO DEATH	mg%	SURVIVAL (DAYS)	PATHOLOGY GROSS	CONTROL	HISTOPATHOLOGY BIOPSY	A
Creatinine	0.7	25	Good	1		
		6	Autolyzed	1		
BUN	215	11		1	4	
BUN	9	37	Fibrotic remnant	1	4	
Creatinine	1.2					
BUN	7					
Creatinine	0.7	23	Generalized peritonitis with kidney completely autolyzed			
BUN	15	21	small fibrotic kidney	1	4	
BUN	24					
Creatinine	0.9	12		1	2	

g%	SURVIVAL (DAYS)	GROSS	CONTROL	PATHOLOGY HISTOPATHOLOGY BIOPSY	AUTOPSY OR SACRIFICE
7	25	Good	1		1
	6	Autolyzed	1		
5	11		1	4	4
	37	Fibrotic remnant	1	4	4
	23	Generalized peritonitis with kidney completely autolyzed			
	21	small fibrotic kidney	1	4	4
	12		1	2	2

GROUPS	DOG No.	PERTINENT OBSERVATIONS DURING REPLANTATION	BIOPSY	UREMIA	TECHNICAL	OTHERS	
5 ATA-HBO 4°C 24 HOURS	327	Cyanotic and Congested kidney partially emptied medium	Hard enlarged fibrotic kidney		+		
	348	Accidentally decompressed before replantation				+	
	376	Anomalous blood supply	4-		+		
TOTAL	3						
10 ATA-HBO 4°C 24 HOURS	81	Good	3-	+			I
	91	Accidentally decompressed	4-		+		C
TOTAL	2						
20 ATA-HBO 4°C 24 HOURS	120	Good	0			+	D
	129	Deeply anesthetized	0		+		D
TOTAL	2						

DG o.	PERTINENT OBSERVATIONS DURING REPLANTATION	BIOPSY	UREMIA	TECHNICAL	OTHERS	CAUSE OF DEATH
27	Cyanotic and Congested kidney partially emptied medium	Hard enlarged fibrotic kidney		+		PNEUMONIA
3	Accidentally decompressed before replantation				+	DIED IN FARM
5	Anomalous blood supply	4-		+		RENAL FAILURE
	Good	3-	+			RENAL FAILURE
	Accidentally decompressed	4-		+		CLINICAL RENAL FAILURE
	Good	0			+	DIED IN FARM
	Deeply anesthetized	0		+		DID NOT WAKE UP

BIOCHEMICAL TESTS PRIOR TO DEATH	mg%	SURVIVAL (DAYS)	PATHOLOGY GROSS	HISTOPATHOLOGY CONTROL	BIOPSY	AUTOPSY OR SACRIFI
BUN Creatinine	24 0.9	12		1	2	2
Creatinine	1.4	20				
BUN Serum Creatinine	20 1.2	11				
BUN Creatinine	255 3.6	12		1	4	4
BUN Creatinine	15 1.0	5				
BUN Creatinine	79 1.94	120		1	2	
BUN Creatinine	12 0.8	16				

L TESTS EATH mg%	SURVIVAL (DAYS)	PATHOLOGY GROSS	HISTOPATHOLOGY CONTROL	BIOPSY	AUTOPSY OR SACRIFICE
24 0.9	12		1	2	2
1.4	20				
20 :i- 1.2	11				
255 3.6	12		1	4	4
15 1.0	5				
79 1.94	120		1	2	
12 0.8	16				

GROUPS	DOG No.	PERTINENT OBSERVATIONS DURING REPLANTATION	BIOPSY	UREMIA	TECHNICAL	OTHERS	CA
10 ATA-HBO 4°C 48 HOURS (AMBIENT)	231	Excellent	1-	+			RE
	233	Good	3-	+			"
	235	Congested	4-	+			"
	239	Hemorrhagic patches and congested	4-	+			"
	240		4-	+			"
	242 243 244 245 246 247 248 250	Congested	4-	+			"
TOTAL	13						
5, 20, 30 ATA-HBO 4°C 72 HOURS	345 456 207	Mottled cyanosis	4-	+			SA
TOTAL	3						

DOG No.	PERTINENT OBSERVATIONS DURING REPLANTATION	BIOPSY	UREMIA	TECHNICAL	OTHERS	CAUSE OF DEATH
231	Excellent	1-	+			RENAL FAILURE
233	Good	3-	+			" "
235	Congested	4-	+			" "
239	Hemorrhagic patches and congested	4-	+			" "
240		4-	+			" "
242 243 244 245 246 247 248 250	Congested	4-	+			" "
13						
345 456 207	Mottled cyanosis	4-	+			SACRIFICE
3						

BIOCHEMICAL TESTS PRIOR TO DEATH mg%	SURVIVAL (DAYS)	PATHOLOGY GROSS	CONTROL	HISTOPATHOLOGY BIOPSY	AUTO
BUN 255 Creatinine 3.6	12		1	4	
BUN 15 Creatinine 1.0	5				
BUN 79 Creatinine 1.94	120		1	2	
BUN 12 Creatinine 0.8	16				
			1 - 2	4	
	8	Fibrosis		4	
	6 5	Minimal bleeding at biopsy site		4	
BUN 220 Creatinine 18	6			4	
BUN 188 Creatinine 16	10			4	
BUN 125 Creatinine 10	12				
BUN 229 Creatinine 23	7			4	

TS g%	SURVIVAL (DAYS)	PATHOLOGY GROSS	CONTROL	HISTOPATHOLOGY BIOPSY	AUTOPSY OF SACRIFICE
5 3.6	12		1	4	4
5 1.0	5				
9 1.94	120		1	2	
2 68	16				
			1 - 2	4	4
	8	Fibrosis		4	4
	6 5	Minimal bleeding at biopsy site		4	4
) 3	6			4	4
	10				
	12			4	4
	7			4	4

GROUPS	DOG No.	PERTINENT OBSERVATIONS DURING REPLANTATION	BIOPSY	UREMIA	TECHNICAL	OTHERS
30 ATA-HBO 4°C 24 HOURS	152	Anomalous blood supply. Chronic interstitial cystitis	0		+	
TOTAL	1					
10 ATA-HBO 4°C 48 HOURS (TANK)*	192	Excellent	0	+		
	208	Cyanotic & congested kidney	4-	+		
	215	Dusky & congested kidney	4-	+		
	225	Mottled cyanosis impaired venous flow	1-	+		
	226	Congested	3-	+		
	229	Congested with pericapsular edema		+		
	230	Good	4-	+		
TOTAL	7					

DOG No.	PERTINENT OBSERVATIONS DURING REPLANTATION	BIOPSY	UREMIA	TECHNICAL	OTHERS	CAUSE OF DEATH
152	Anomalous blood supply. Chronic interstitial cystitis	0		+		RENAL FAILURE
1						
192	Excellent	0	+			CLINICAL RENAL FAILURE
208	Cyanotic & congested kidney	4-	+			" " "
215	Dusky & congested kidney	4-	+			" " "
225	Mottled cyanosis impaired venous flow	1-	+			" " "
226	Congested	3-	+			" " "
229	Congested with pericapsular edema		+			RENAL FAILURE
230	Good	4-	+			" "
7						

BIOCHEMICAL TESTS PRIOR TO DEATH	mg%	SURVIVAL (DAYS)	PATHOLOGY GROSS	HISTOPATHOLOGY CONTROL	BIOPSY	AUTO
				1 - 2	4	
		8	Fibrosis		4	
		6	Minimal bleeding at biopsy site		4	
		5				
BUN	220	6			4	
Creatinine	18					
BUN	188	10			4	
Creatinine						

BUN	144	8				
Creatinine	12					
BUN	127	7		1		
BUN	117	6		1	3	
BUN	186	7				
Creatinine	12			1	4	
BUN	182					
Creatinine	20				4	
					4	

ESTS I mg%	SURVIVAL (DAYS)	PATHOLOGY GROSS	HISTOPATHOLOGY CONTROL	BIOPSY	AUTOPSY OR SACRIFICE
			1 - 2	4	4
	8	Fibrosis		4	4
	6 5	Minimal bleeding at biopsy site		4	4
220 18	6			4	4
188	10			4	4
144 12	8				
127	7		1		
117	6		1	3	3
186 12 182 20	7		1	4 4	4 4
				4	4

APPENDIX B

Figure 1-a. Control Group.

1-b. Hypothermia alone (4°C) Group.

1-c. Hypothermic-Hyperbaric - 2 ATA-HBO for 24 hours.

1-d. " " 3 " " " " "

1-e. A Dog which bore 3 puppies after a Kidney was autografted which was stored by Hypothermic-Hyperbaric Method - 3 ATA-HBO for 24 Hours.

1-f. Hypothermic-Hyperbaric Method - 5 ATA-HBO for 24 Hours.

1-g. Hypothermic-Hyperbaric Method - 10 " " 24 Hours.

1-h. Hypothermic-Hyperbaric " - 20 " " 24 Hours.

1-i. Hypothermic-Hyperbaric " - 30 " " 24 Hours.

1-j. Hypothermic-Hyperbaric " - 10 " " 48 Hours.

Fig. 1-a. Control Group.

Fig. 1-b. Hypothermia (4 C) Group.

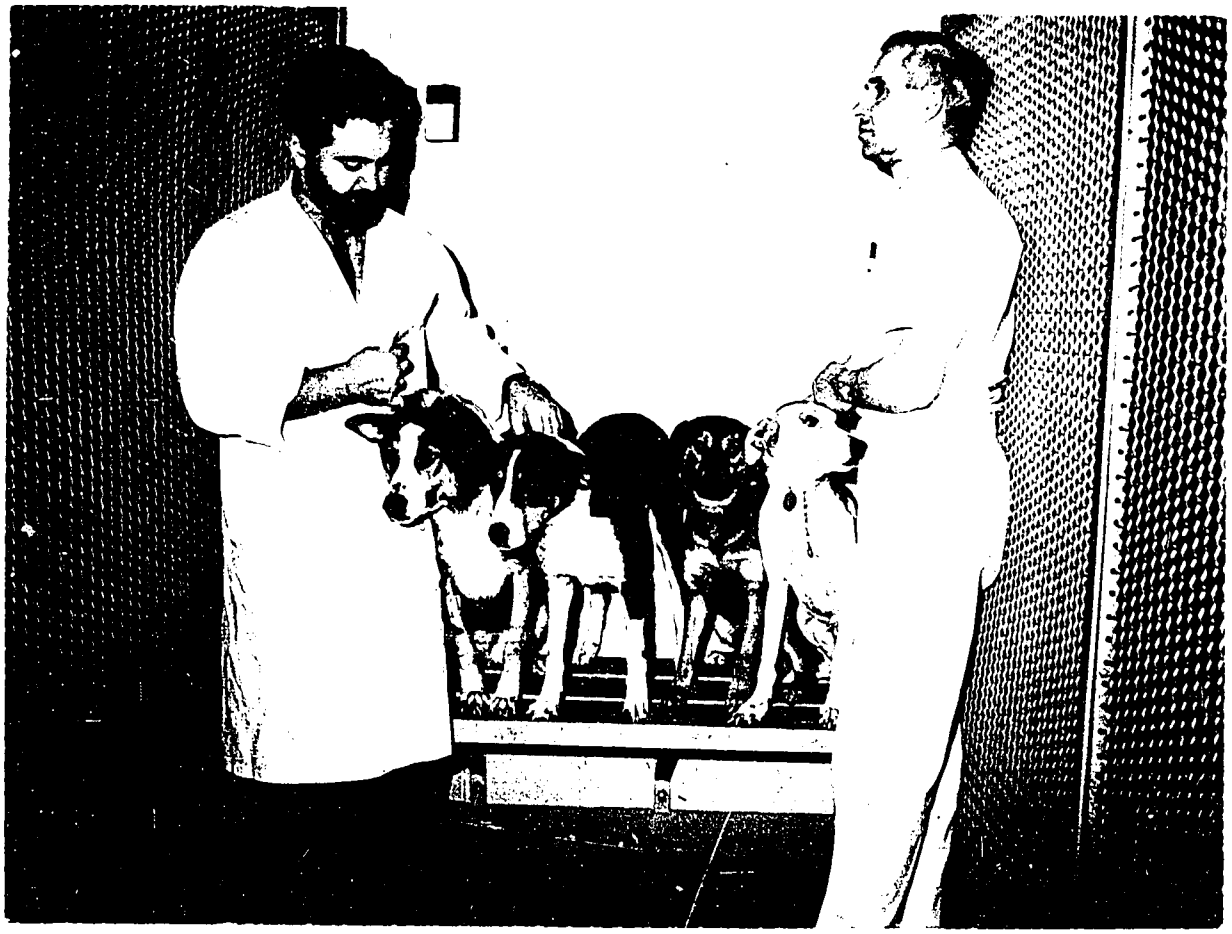

Fig. 1-c. Hyperbaric-hypothermic Preservation Group at 2 ATA-HBO for 24 hours.

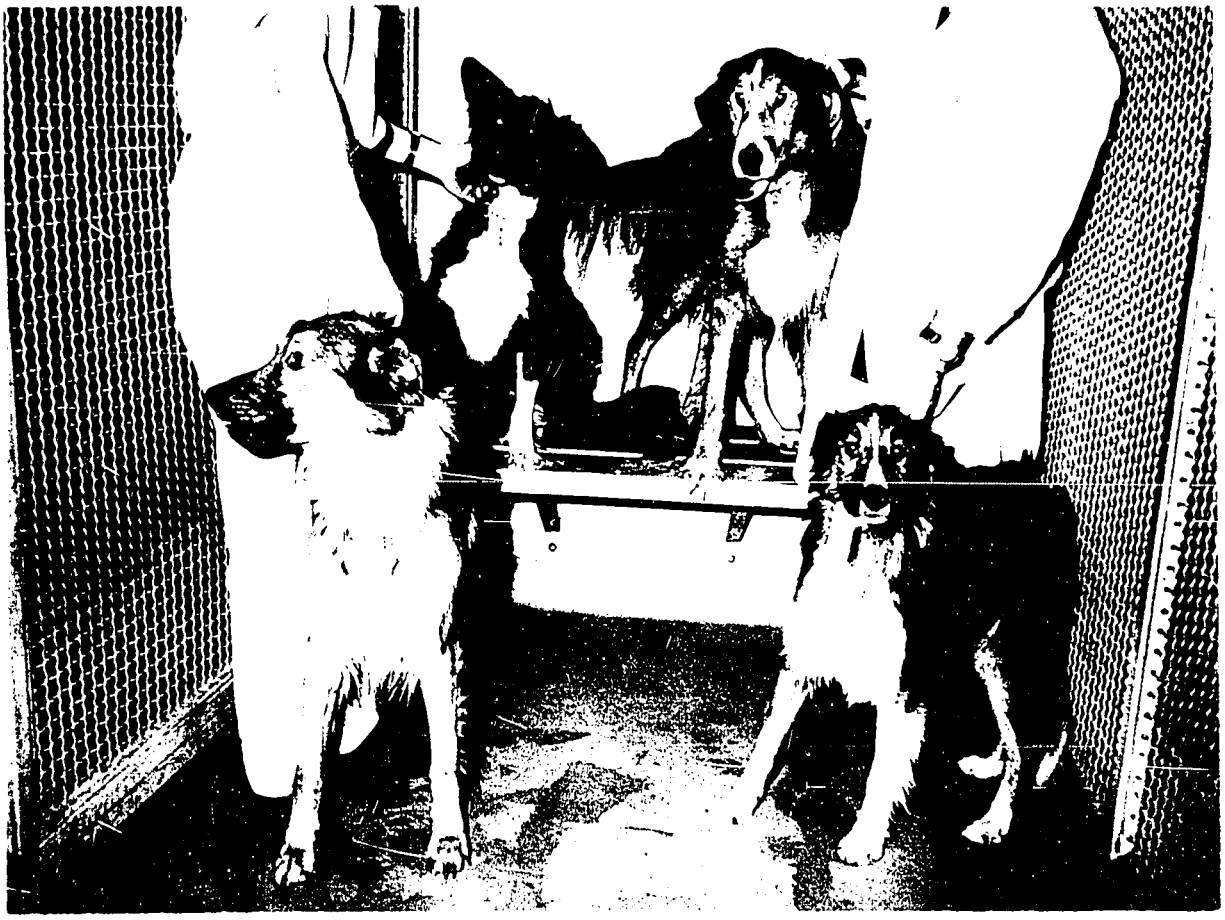

Fig. 1-f. Hyperbaric-hypothermic method of preservation at 5 ATA-280 for 24 hours.

Fig. 1-b. Hyperbaric-hypothermic preservation at 20 ATA-HBO for 24 hours.

Fig. 1-i. Hyperbaric-hypothermic method of preservation at 30 ATA-HBO for 24 hours.

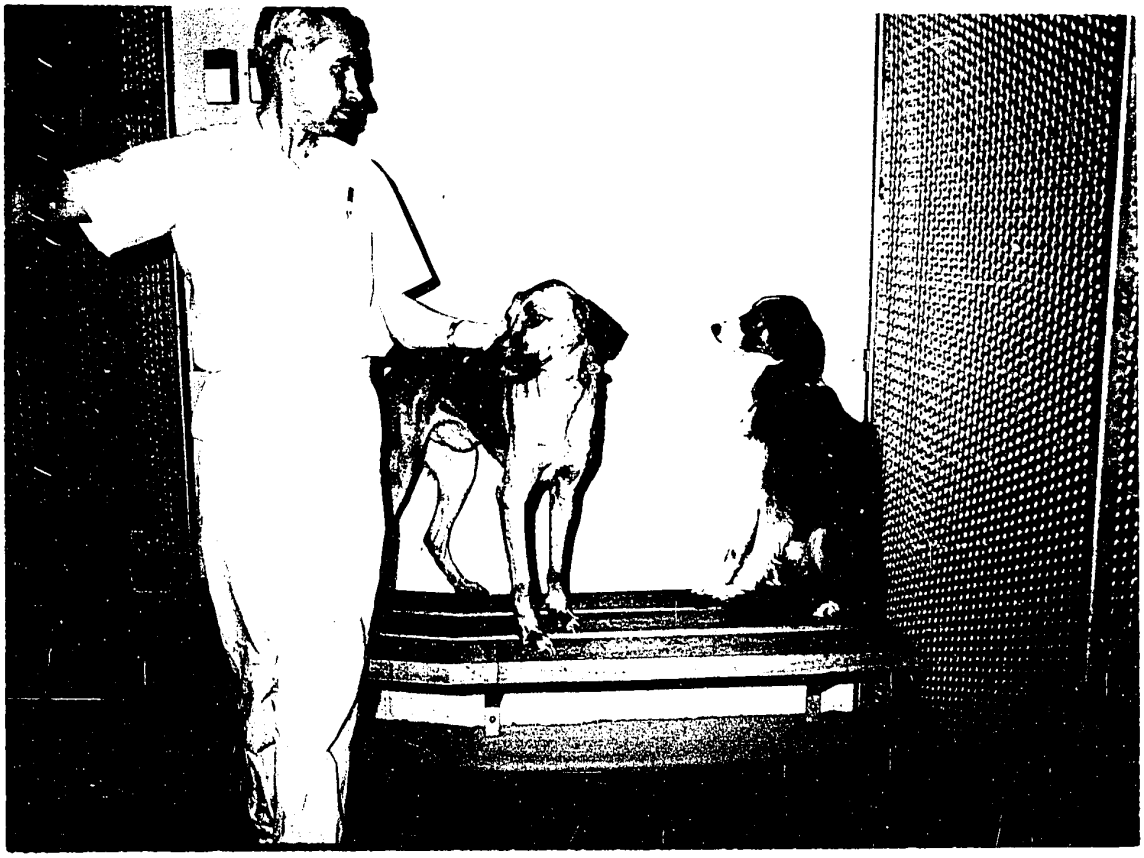

Fig. 1-j. Hyperbaric-hypothermic method of preservation at 10 ATA-HBO for 48 hours.

APPENDIX C

Gross photographs and photomicrographs of autotransplanted kidneys. The paraffin sections of unsuccessful autografts show obvious destruction (Fig. 1) and calcification (Fig. m). Preservation of renal architecture and glomerular hyperplasia are prominent features in all long-term survivors.

Figure a. Appearance of Vascular and Uretero-vesical anastomoses of an autografted kidney at 30 months.

b. Gross appearance of an Immediate Control Kidney Autograft at 2 months.

c. Comparison of Gross Photographs of Hypertrophied Unoperated and Immediately Autografted Kidney.

d. Autograft Preserved by Hypothermia alone for 24 Hours 3 weeks and 20 months respectively after Replantation.

e. Gross appearance of Kidney Autograft preserved for 24 Hours by Hypothermic-hyperbaric Method at 2 ATA-HBO at the time of replantation, biopsy and sacrifice.

f. Gross appearance of a Kidney stored for 24 Hours by Hypothermic-hyperbaric Method at 3 ATA-HBO during biopsy.

g. Appearance of a Kidney Autograft stored at 5 ATA-HBO and Hypothermia for 24 Hours during biopsy.

h. Gross appearance of a Kidney preserved at 10 ATA-HBO and Hypothermia for 24 Hours at biopsy.

i. Appearance of a Kidney preserved for 24 Hours at 20 ATA-HBO and Hypothermia during biopsy and sacrifice.

j. Gross appearance of Kidney Autograft preserved for 24 Hours by Hypothermic-hyperbaric Method of Preservation at 30 ATA-HBO at biopsy and sacrifice.

Figure k. Gross appearance of Kidney Autografts preserved for 48 Hours by Hypothermic-hyperbaric Method at 10 ATA-hbo at biopsy and sacrifice.

l. Photomicrographs of a Kidney kept for 24 Hours under 5 ATA-HBO at room temperature (27°C).

m. Photomicrographs of a biopsy material from a Kidney stored for 48 Hours at 10 ATA-HBO and Hypothermia in which excision and replantation were done at ambient pressure.

n. High power view of a Normal Kidney Biopsy.

o. Paraffin sections from materials taken at the time of biopsy and sacrifice of immediately replanted Kidney.

p. Sections from a Kidney stored for 24 Hours in Hypothermia alone (4°C) taken during biopsy and sacrifice.

q. Photomicrographs of sections taken at biopsy and sacrifice of a Kidney preserved for 24 Hours at 2 ATA-HBO and Hypothermia.

r. Photomicrographs of a biopsy material of a kidney stored for 24 Hours by Hypothermic-hyperbaric Method at 3 ATA-HBO.

s. Photomicrographs of a Kidney preserved for 24 Hours by Hypothermic-hyperbaric Method at 5 ATA-HBO.

t. Photomicrographs of renal autograft preserved for 24 Hours by Hypothermic-hyperbaric Method at 10 ATA-HBO.

u. Photomicrographs of a Kidney stored for 24 Hours by Hypothermia and Hyperbaric Oxygen at 20 ATA-HBO during biopsy and sacrifice.

v. Photomicrographs of a Kidney preserved for 24 Hours by Hypothermic-hyperbaric Method at 30 ATA-HBO during biopsy and sacrifice.

w. Photomicrographs of a Kidney preserved for 48 Hours at 10 ATA-HBO and Hypothermia during biopsy and sacrifice.

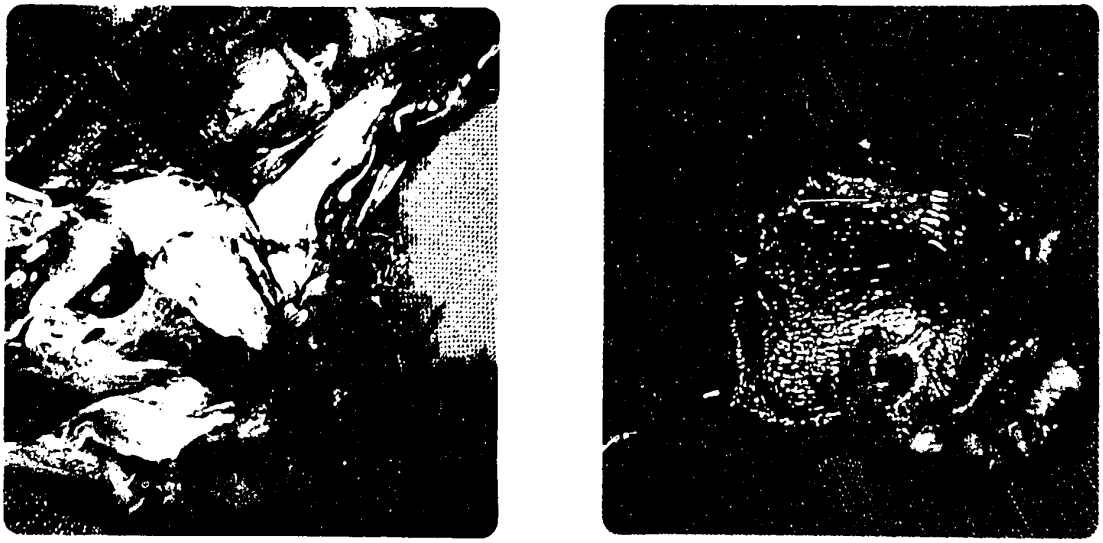

Figure a. Appearance of vascular (left) and uretero-vesical (right) anastomoses 30 months after autotransplantation. Note absence of evidence of stricture or stenosis.

Figure b. Appearance of the only remaining immediately autografted control kidney 2 months after transplantation. Note absence of scar formation or perinephric adhesion (Dog # 209).

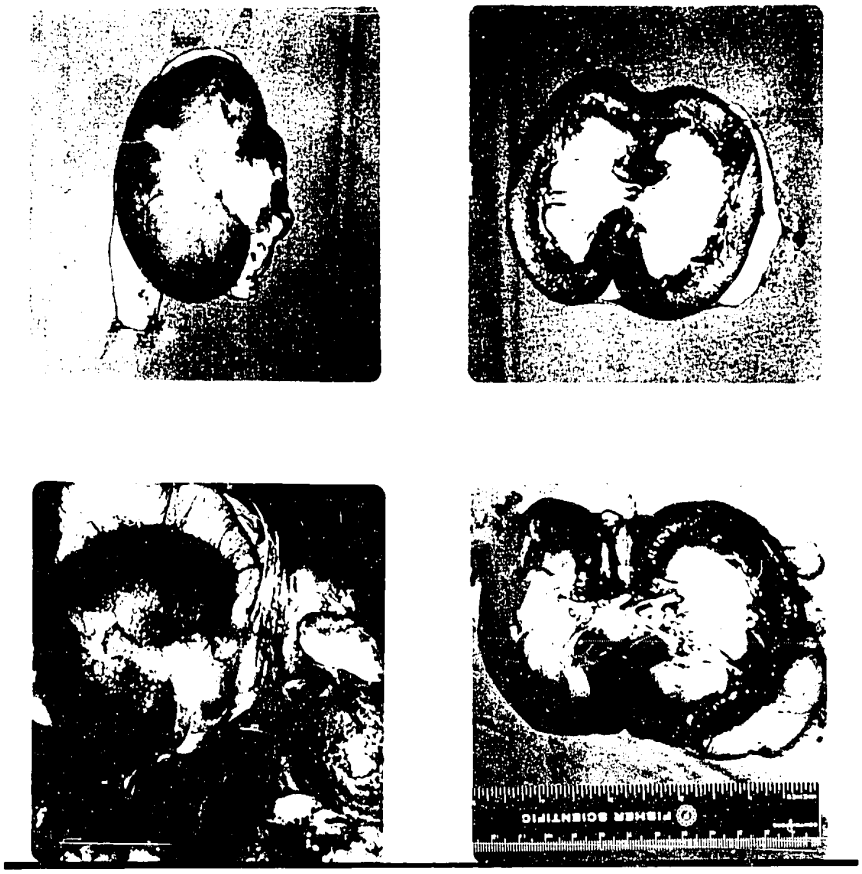

Figure c. Comparison of gross photographs of hypertrophied unoperated (Dog # 261, top) and immediately autografted control kidney (Dog # 7, bottom) at 30 months. Contra-lateral nephrectomy have been previously performed on each dog.

Figure d. Gross appearance of a kidney preserved for 24 hours at 4°C. (Dog # 201), 3 weeks (left) and 20 months (middle and right) after replantation.

Figure e. Gross appearance of an autografted kidney stored for 24 hours by hypothermic-hyperbaric method at 2 ATA-HBO, at the time of replantation (top left), 3 weeks during biopsy (top right) and 20 months (bottom) following transplantation (Dog # 206).

Figure f. Appearance of a kidney stored for 24 hours under hypothermia and hyperbaric oxygen at 3 ATA at biopsy and contralateral nephrectomy after replantation (Dog # 64).

Figure g. Appearance of a kidney autograft stored for 24 hours at 5 ATA-HBO and hypothermia during biopsy in 3 weeks (Dog # 103).

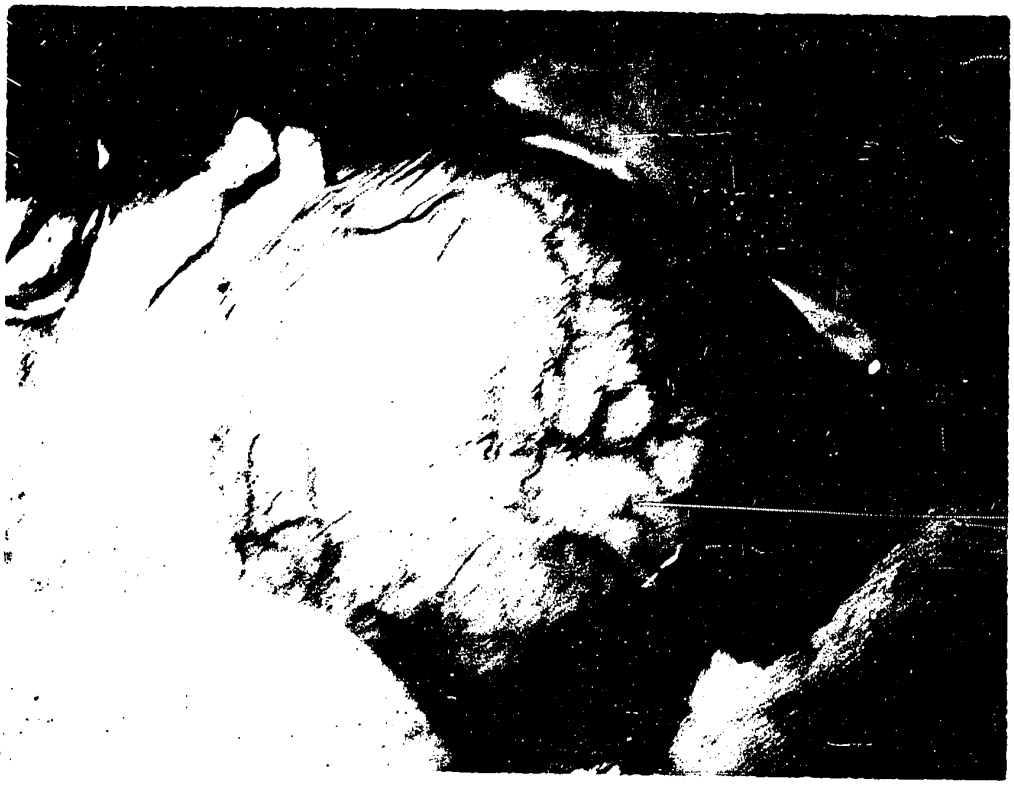

Figure h. Gross appearance of a kidney preserved at 10 ATA-HBO and hypothermia for 24 hours during biopsy 3 weeks after autografting (Dog # 127).

Figure i. Appearance of a kidney preserved for 24 hours at 20 ATA-HBO and hypothermia during biopsy (left) and sacrifice (middle and right) 24 months after contra-lateral nephrectomy (Dog # 128).

Figure j. Gross appearance of a kidney preserved for 24 hours by hypothermic-hyperbaric method at 30 ATA-HBO during biopsy (left) and sacrifice (middle and right) 23 months after replantation (Dog # 141).

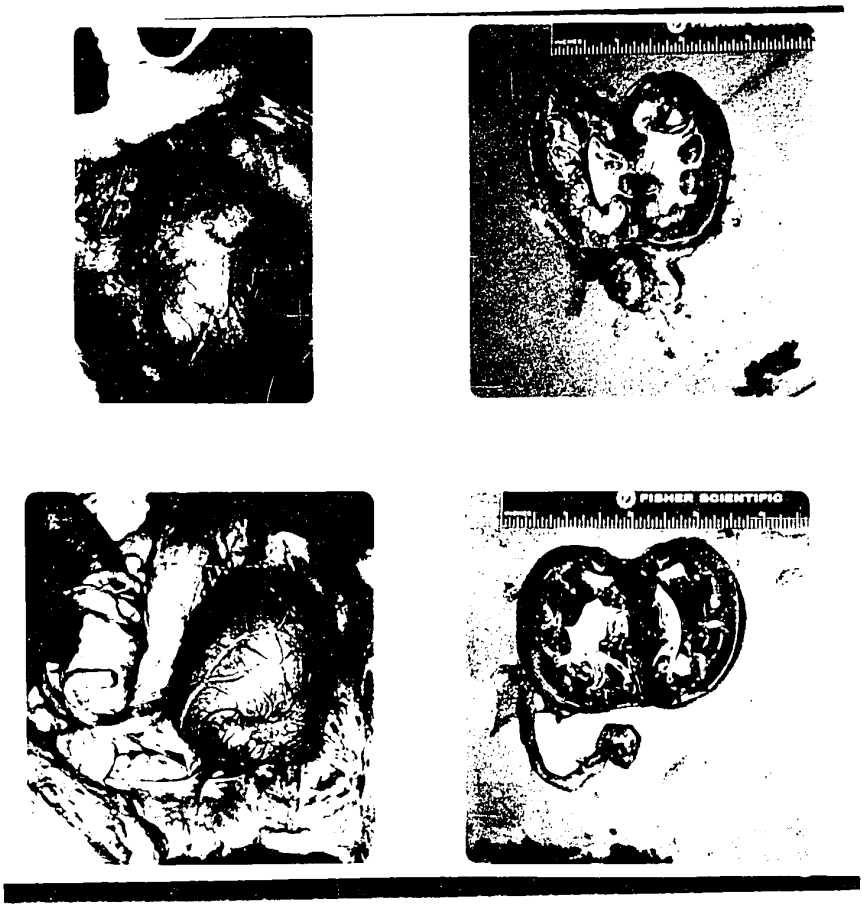

Figure k. Gross appearance of renal autografts stored for 48 hours by combined hypothermia and hyperbaric oxygen at 10 ATA during biopsy (top left) and sacrifice (top right) of Dog # 220 and Dog # 270 (bottom) 20 and 9 months respectively following transplantation.

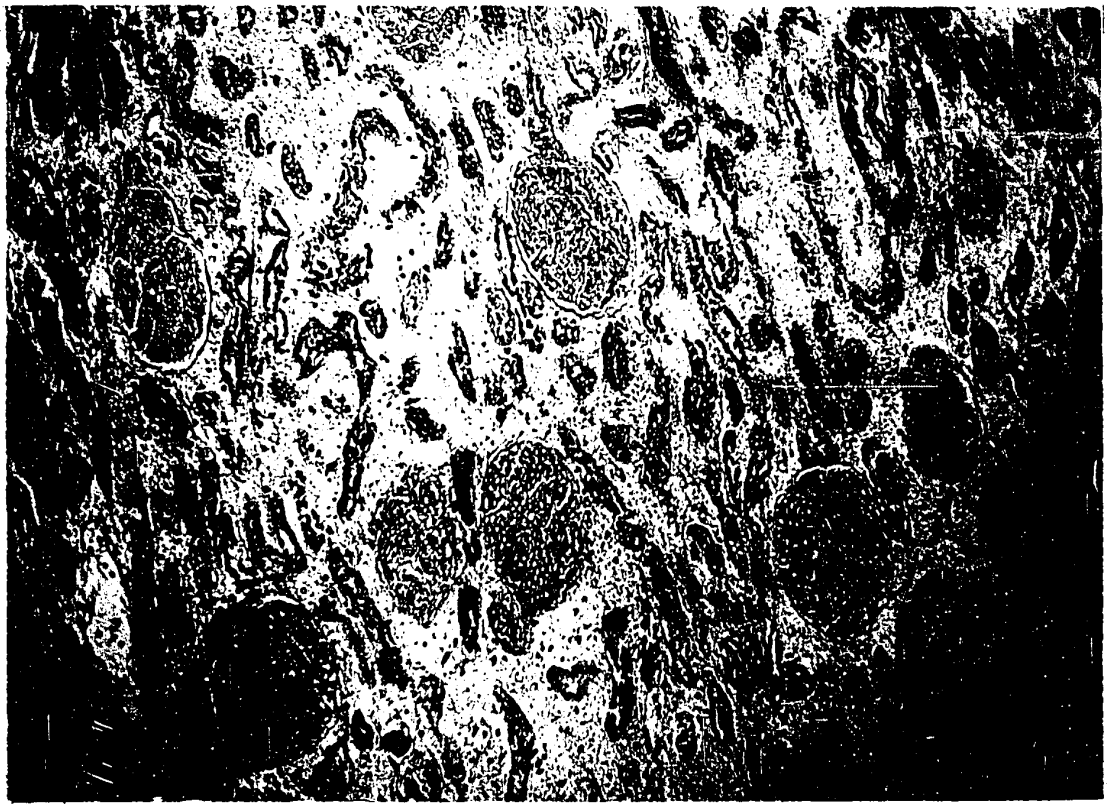

Figure 1. Photomicrograph (H & E x 100) of a kidney kept for 24 hours under 5 ATA-HBO at room temperature (27°C) during biopsy 3 weeks after replantation. Note complete destruction of renal architecture.

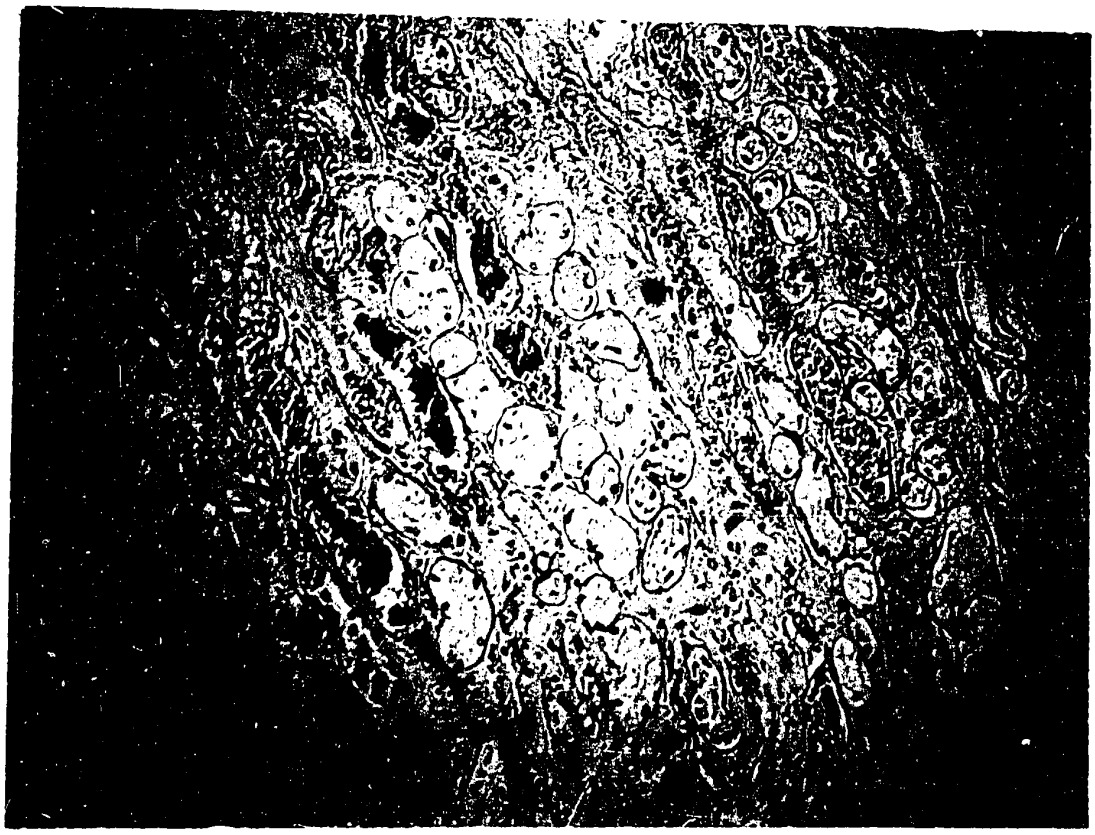

Figure m. Photomicrograph (Dog # 192, H & E x 100) of a kidney biopsy at 3 weeks. The kidney was stored for 48 hours at 10 ATA-HBO and hypothermia in which excision and replantation were performed at ambient atmospheric condition. Extensive destruction of renal architecture with calcification are evident.

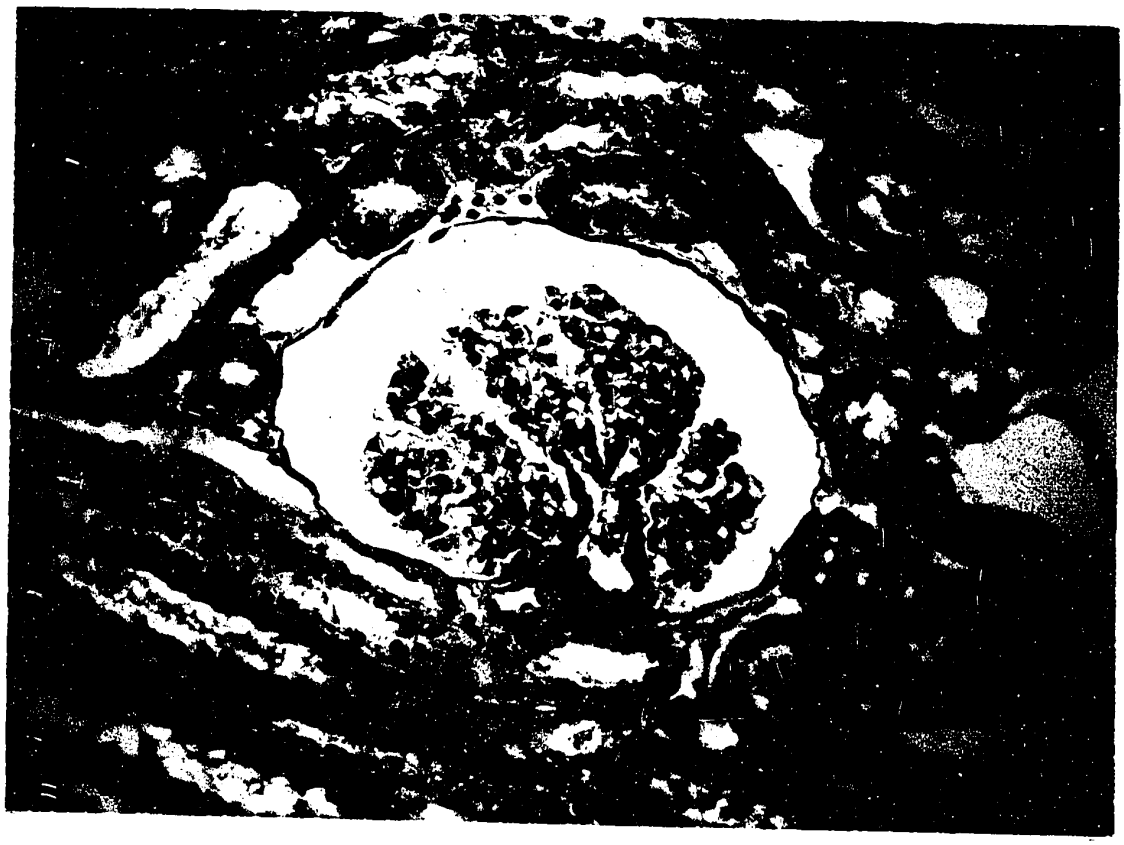

Figure n. High power view (H & E x 300) of a normal kidney biopsy.

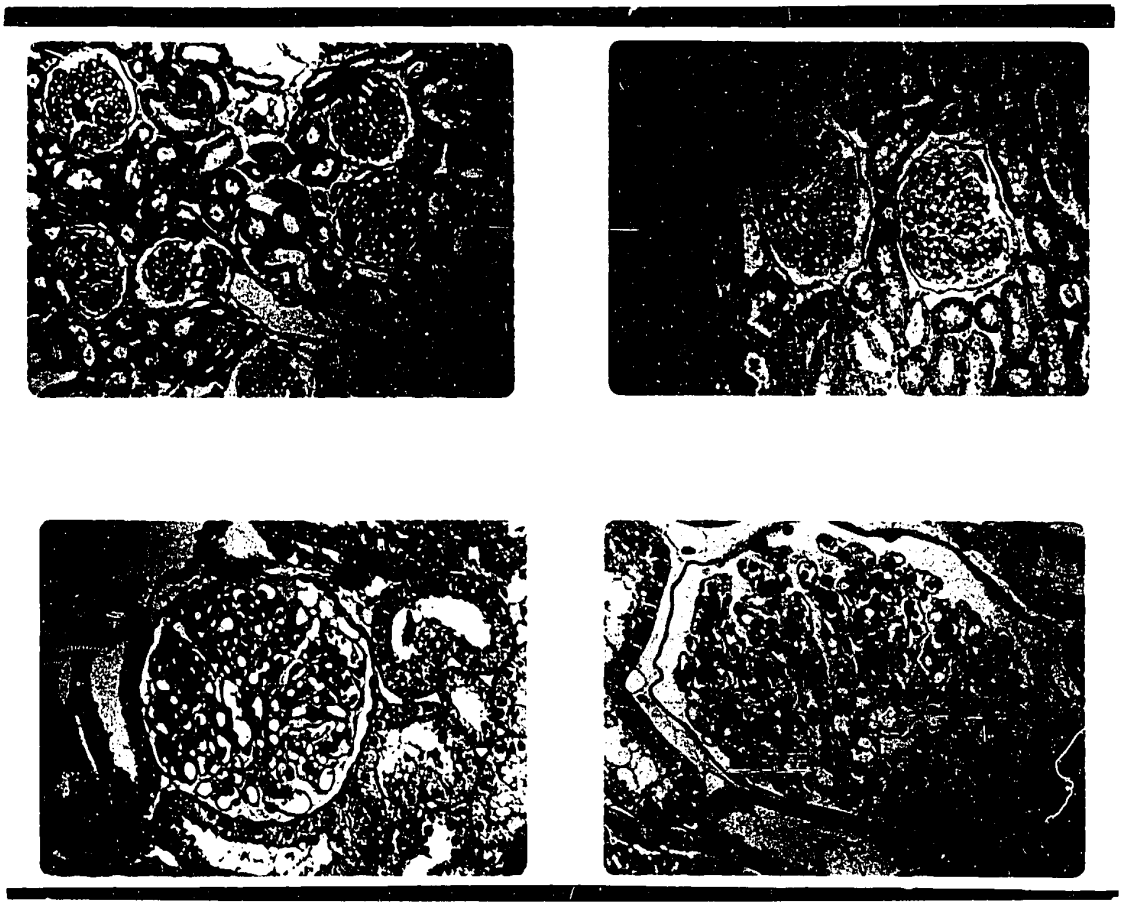

Figure o. Biopsy of an immediately replanted control kidney at 2 months (Dog # 209 - top left, PAS x 100 and bottom left x 250) and 30 months during sacrifice (Dog # 7 - H & E x 100, top right and bottom right x 250). Note preservation of normal architecture and glomerular hypertrophy.

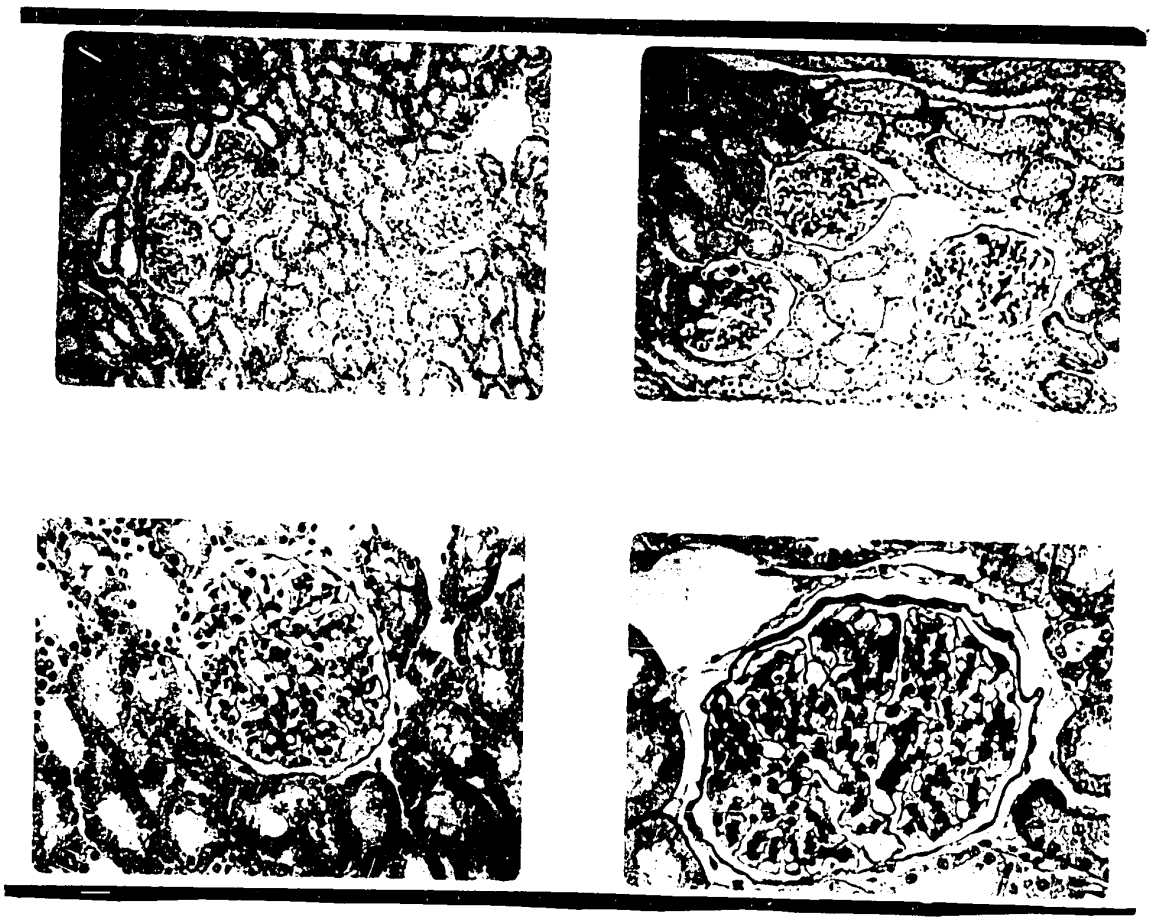

Figure p. Photomicrographs of sections of a kidney preserved in hypothermia (4°C) for 24 hours at biopsy (Dog # 201 - H & E x 100, top left and x 250 bottom left) and sacrifice 20 months after contra-lateral nephrectomy (PAS x 100, top right and x 250 bottom right). Glomerular hypertrophy and thickening of Bowman's membrane are typical findings of this group.

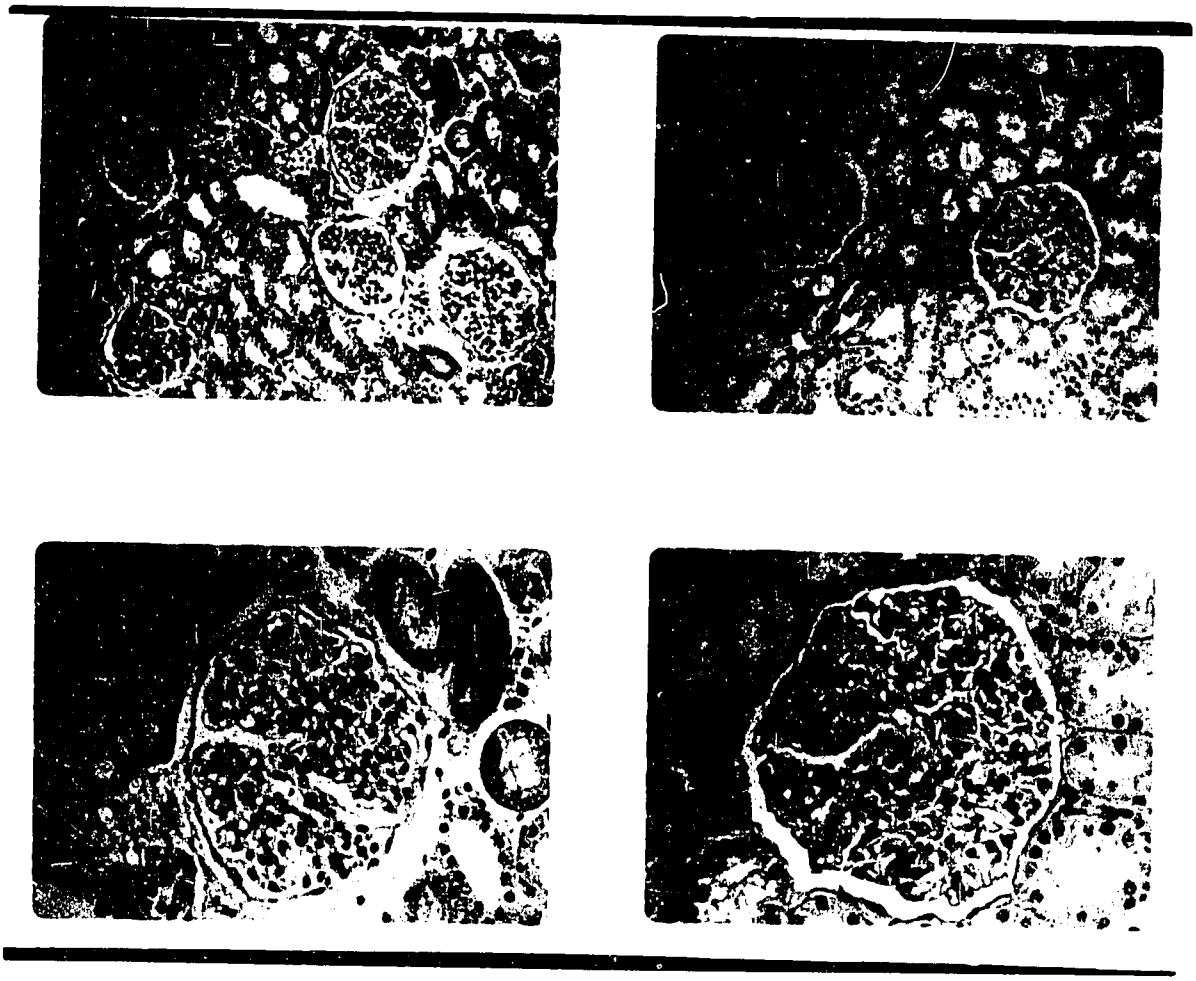

Figure q. Photomicrographs of paraffin sections of a renal autograft preserved by hypothermic-hyperbaric method at 2 ATA-HBO for 24 hours at biopsy (Dog # 206 - H & E x 100 top left and x 250 bottom left) and sacrifice (H & E x 100 top right and x 250 bottom right).

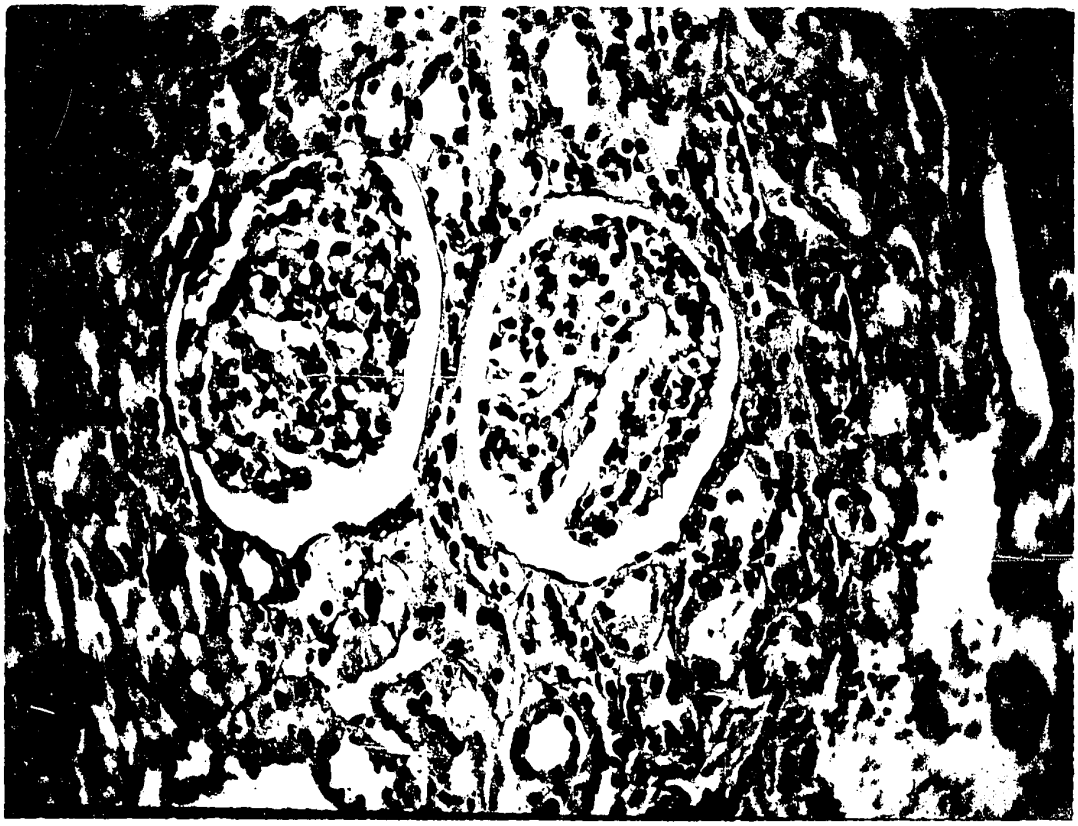

Figure r. Photomicrograph of a biopsy section of a kidney autotransplant stored for 24 hours by hypothermic-hyperbaric method at 3 ATA-HBO.

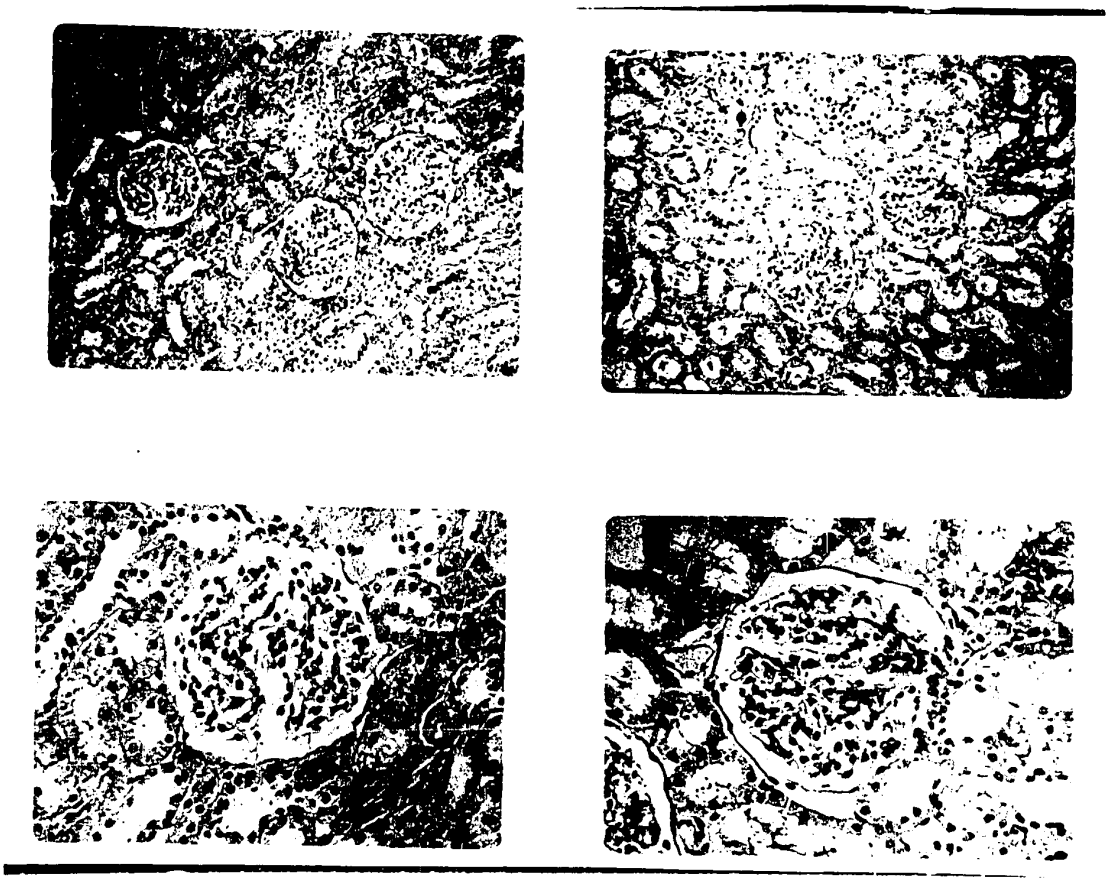

Figure s. Photomicrographs from a kidney stored at 5 ATA-HBO and hypothermia for 24 hours during biopsy (Dog # 50 - H & E x 100 top left and x 250 bottom left) and sacrifice (H & E x 100 top right and x 250 bottom right).

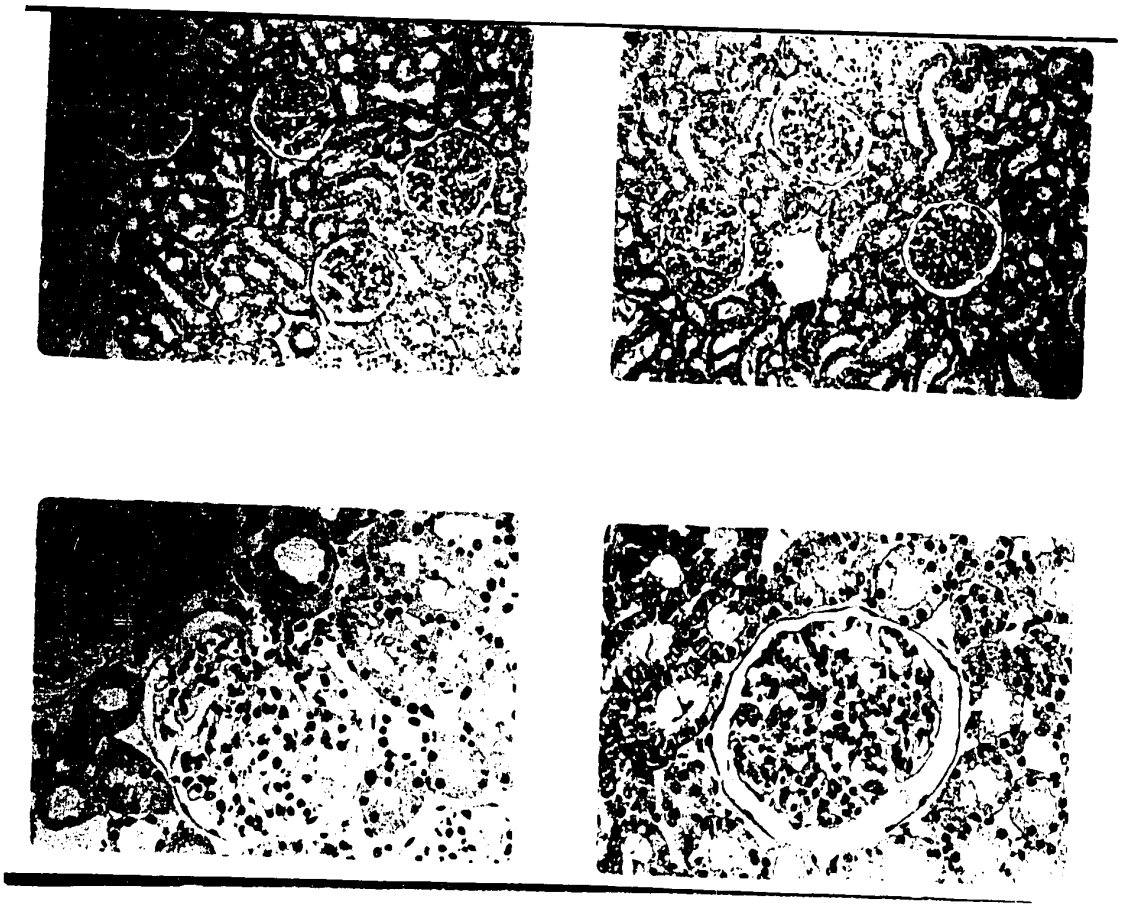

Figure t. Photomicrographs of a kidney autograft preserved for 24 hours by hypothermic-hyperbaric method at 10 ATA-HBO during biopsy (Dog # 127 - H & E x 100 top left and x 250 bottom left) and sacrifice (H & E x 100 top right and x 250 bottom right).

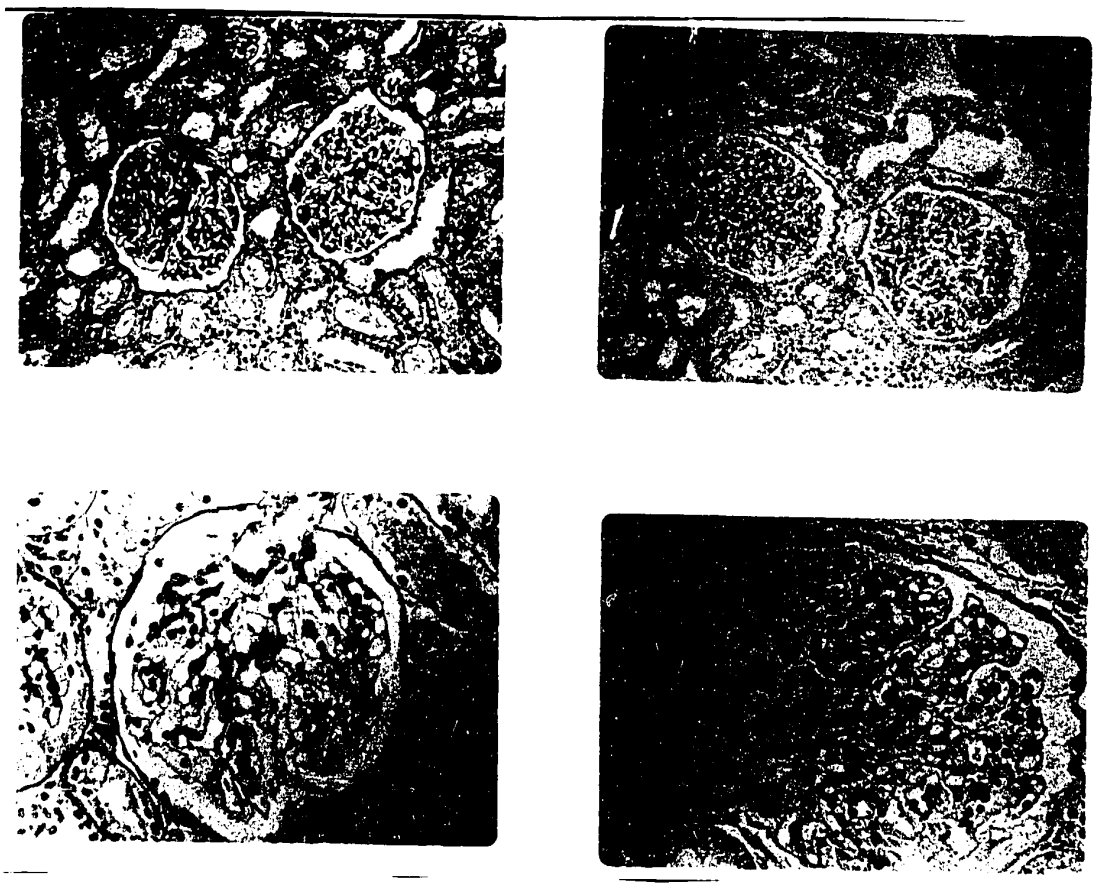

Figure u. Photomicrographs of sections of a kidney preserved for 24 hours by hypothermic-hyperbaric method at 20 ATA-HBO taken during biopsy (Dog # 128 - PAS x 100 top left and x 250 bottom left) and sacrifice (H & E x 100 top right and x 250 bottom right). Thickening or Bowman's membrane is noted in section at bottom right.

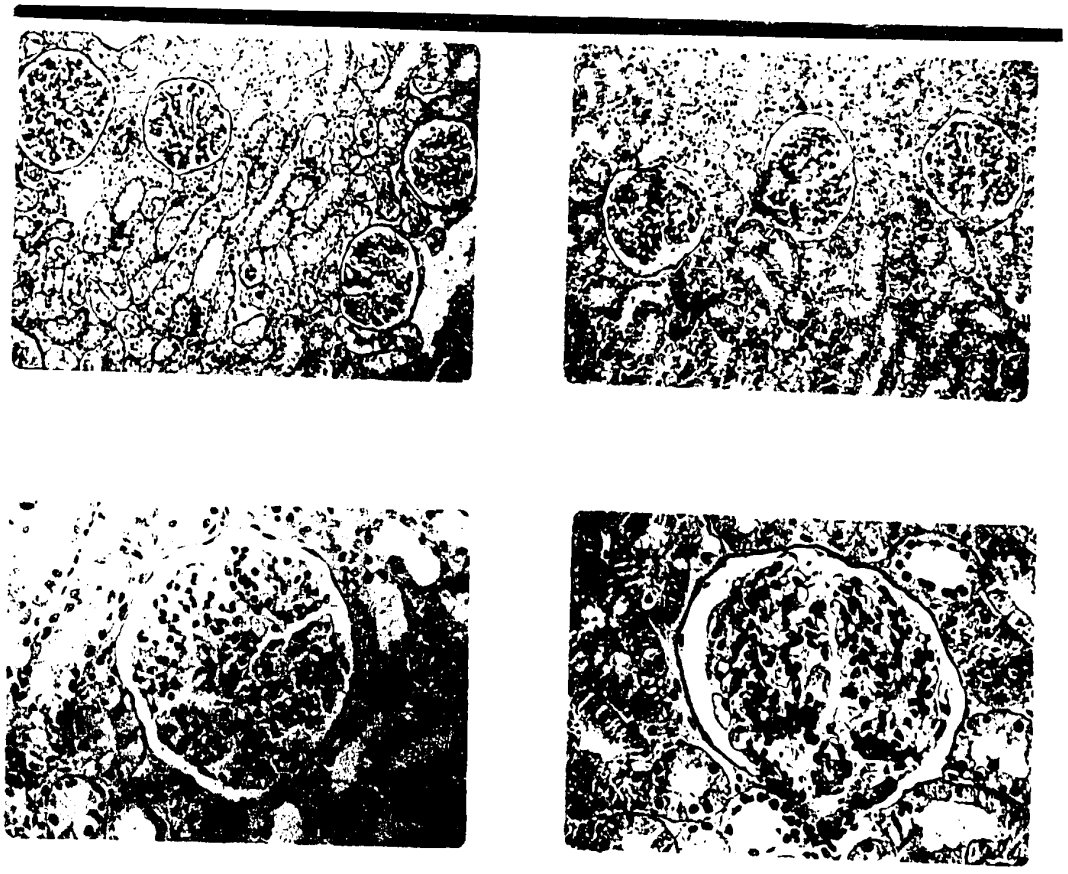

Figure v. Photomicrographs of a kidney stored at 30 ATA-HBO and hypothermia for 24 hours during biopsy (Dog # 141 - H & E x 100 top left and x 250 bottom left) and sacrifice (H & E x 100 top right and x 250 bottom right).

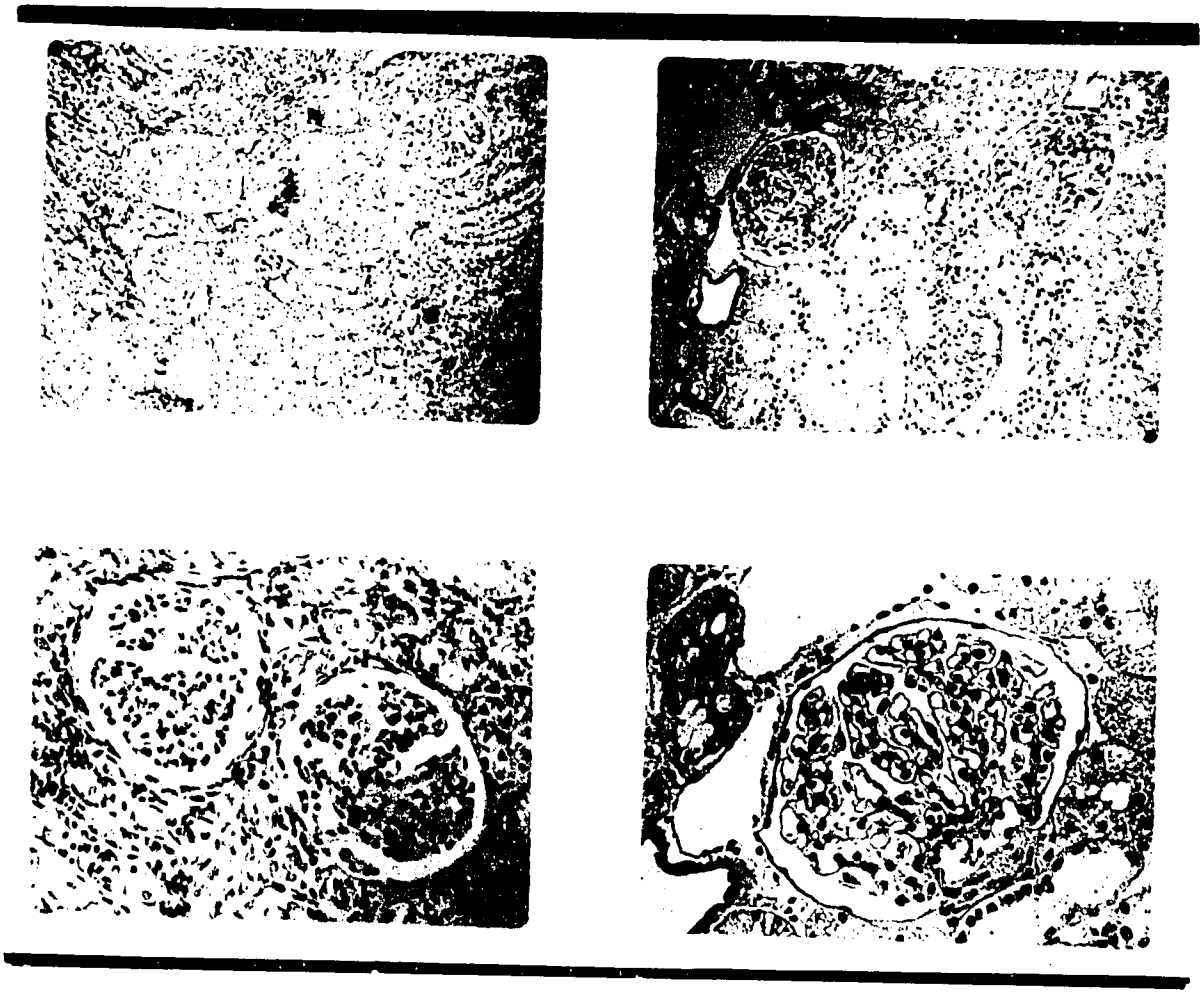

Figure w. Photomicrographs of a kidney stored for 48 hours by hypothermic-hyperbaric method at 10 ATA-HBO during biopsy (Dog # 270 - H & E x 100 top left and x 250 bottom left) and sacrifice (H & E x 100 top right and x 250 bottom right).