

## FUNCTIONAL SURVIVAL OF KIDNEYS SUBJECTED TO EXTRACORPOREAL FREEZING AND REIMPLANTATION\*†

E. D. MUNDTH, A. J. DEFALCO, AND Y. G. JACOBSON

*Experimental Surgery Division, Naval Medical Research Institute,  
National Naval Medical Center, Bethesda, Maryland*

Although considerable success has been attained in freeze-preservation of single cells and certain multicellular tissues, there has been little success in gaining functional survival of such complex organs as the kidney. Severe vascular damage characterized by hemorrhage throughout the cortex and medulla has been associated with extracorporeal kidney freezing and reimplantation.<sup>7</sup> Little or no renal function has been demonstrated following even brief periods of freezing.<sup>1, 2, 7</sup>

To obtain renal function following freezing, adequate renal perfusion must be re-established and maintained. Recent studies from this laboratory have shown that vascular damage is a major factor leading to tissue injury from freezing.<sup>3, 4, 6</sup> In these studies, aggregation of platelets and red blood cells producing small vessel occlusion and thrombosis were demonstrated in tissues subjected to freezing and thawing. The intravenous administration of low molecular weight dextran prior to or soon after freezing was shown to improve tissue survival following freezing, presumably because of its disaggregating effect and improvement of capillary perfusion.

The duration of normothermic or moderate hypothermic renal ischemia during preparation for freezing and reimplantation is proportional to the degree of subsequent ischemic damage to renal tubular elements. In addition, recent studies have demonstrated the importance of maintaining the intrarenal vascular volume integrity during the ischemic period of renal

transplantation.<sup>5</sup> Allowing vascular collapse during the period of total renal ischemia leads to juxtamedullary arteriovenous shunting and poor cortical perfusion. By preventing intrarenal vascular collapse, cortical perfusion was improved. With these concepts in mind, the present study was undertaken to investigate methods of preventing or minimizing the vascular damage associated with extracorporeal kidney freezing and reimplantation.

### METHODS

Fourteen adult male dogs in the weight range of 18 to 22 kg were anesthetized with sodium pentobarbital and intubated. A catheter was placed in the bladder, the bladder was emptied, and urine was collected for 1 hr for baseline urine creatinine, volume, and urinalysis. During surgery and prior to renal artery clamping, each animal received 6 g of mannitol in 1000 ml of 5% dextrose in saline intravenously. A brisk osmotic diuresis was observed in all animals. Blood samples were drawn for baseline blood urea nitrogen and serum creatinine.

After a midline laparotomy incision, the left renal pedicle was infiltrated carefully with 20 cc of 1% xylocaine and the right common iliac artery and vein were mobilized. The left kidney was then dissected out, the ureter was divided, and a ligature was placed around the renal vein. Upon cross-clamping of the renal artery, the vein ligature was simultaneously tightened to prevent renal intravascular collapse. The renal artery was divided, placing an arterial clamp proximally, and then cannulated. The kidney was then manually perfused with 200 cc of perfusate at 4°C, consisting of 4 g % low molecular weight dextran in lactated Ringer's solution, containing 2 mg % magnesium and 9 mg % calcium. The pH was adjusted to 7.4 with sodium bicarbonate after

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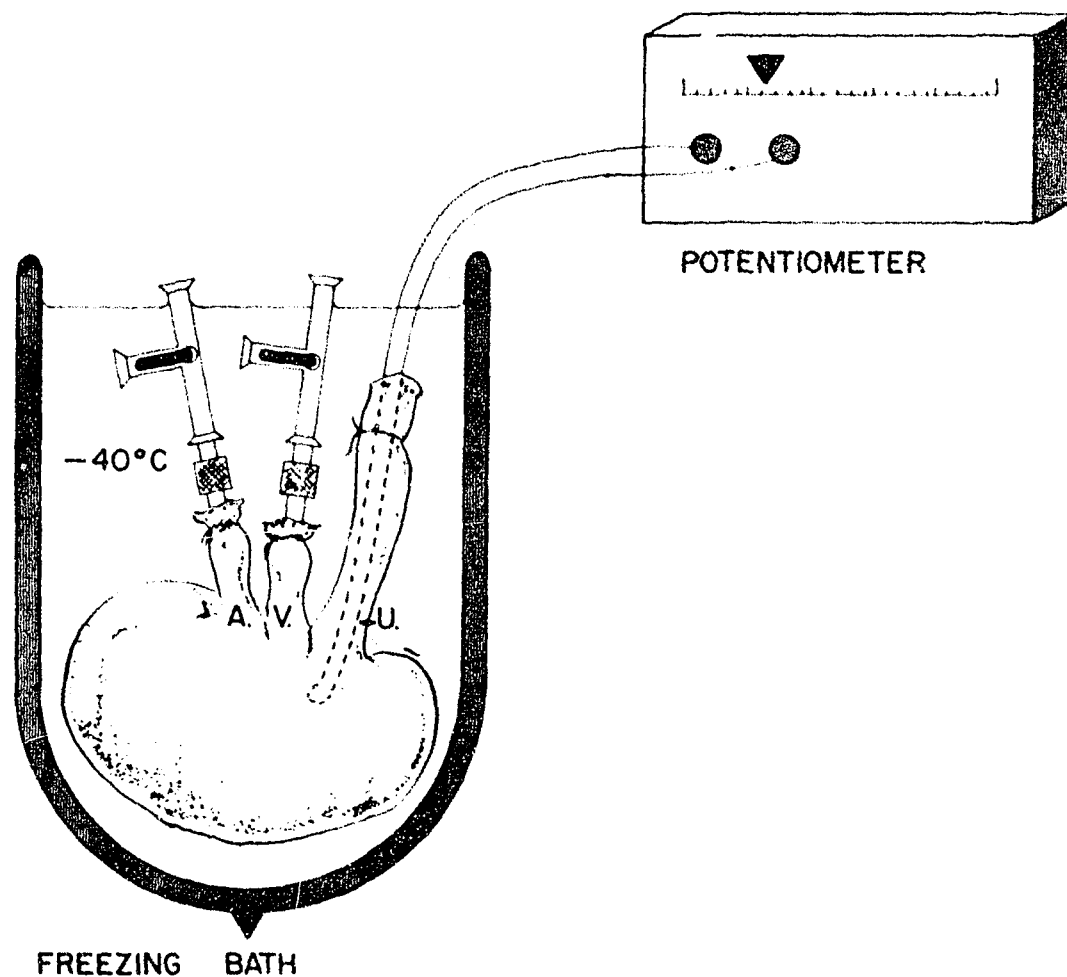


FIG. 1. Diagrammatic representation of freezing technique. Cannulas are placed in the renal artery (A.) and vein (V.), and a wire thermocouple is introduced into the intrarenal pelvis via the ureter (U.). The isolated kidney was frozen at  $-40^{\circ}\text{C}$  after perfusion.

the constituents were added. Intrarenal vascular collapse was prevented during perfusion by alternately releasing and tightening the renal vein ligature. On completion of perfusion, arterial clamps were placed proximally on the renal artery and vein, and the kidney was removed. The renal vein was then cannulated. The perfusion was then repeated *ex situ* with the same method using a similar perfusate at  $0^{\circ}\text{C}$  with the addition of 12 vol % dimethyl sulfoxide. The kidney was then placed in a saline slush at  $0^{\circ}\text{C}$  and the artery was perfused with 100% carbon dioxide gas at 2 liters per min for 10 min, again preventing intrarenal vascular collapse. A copper-constantan thermocouple (36-gauge wire) ensheathed in no. 90 polyethylene tubing was threaded into the renal pelvis through the ureter, and intrarenal temperatures were continuously recorded on a Brown potentiometer. The kidney was then placed in a sterile polyethylene container containing 100 cc of isotonic saline and immersed in a freezing bath at  $-40^{\circ}\text{C}$  (Fig. 1). The kidney was left in the freeze bath for 5 min after the intrarenal temperature reached  $-3^{\circ}$  to  $-6^{\circ}\text{C}$ . The freezing point was taken as

that point below  $0^{\circ}\text{C}$  where the slope of the tissue temperature curve abruptly changed. The mean freezing time from immersion to freezing was 7 min, and the mean time frozen, i.e., the duration between the freezing point and thawing to  $0^{\circ}\text{C}$ , was 20 min (Fig. 2). Thawing was accomplished by immersing the frozen kidney in a saline bath kept at  $25^{\circ}\text{C}$ . The right iliac vessels were transected and prepared for end-to-end anastomoses with the renal artery and vein. Arterial and vein clamps were removed simultaneously when the intrarenal temperature had reached  $0^{\circ}\text{C}$ . A contralateral nephrectomy was done. Hourly urine output, urinalysis, blood urea nitrogen, and creatinine clearance were studied at 2-day intervals following surgery. Biopsy of the reimplanted frozen kidney was obtained on two long-term survivors 6 weeks after surgery. Animals that died were autopsied promptly and the reimplanted kidney biopsied.

#### RESULTS

Of 14 kidneys frozen and reimplanted, nine demonstrated some function. There were two

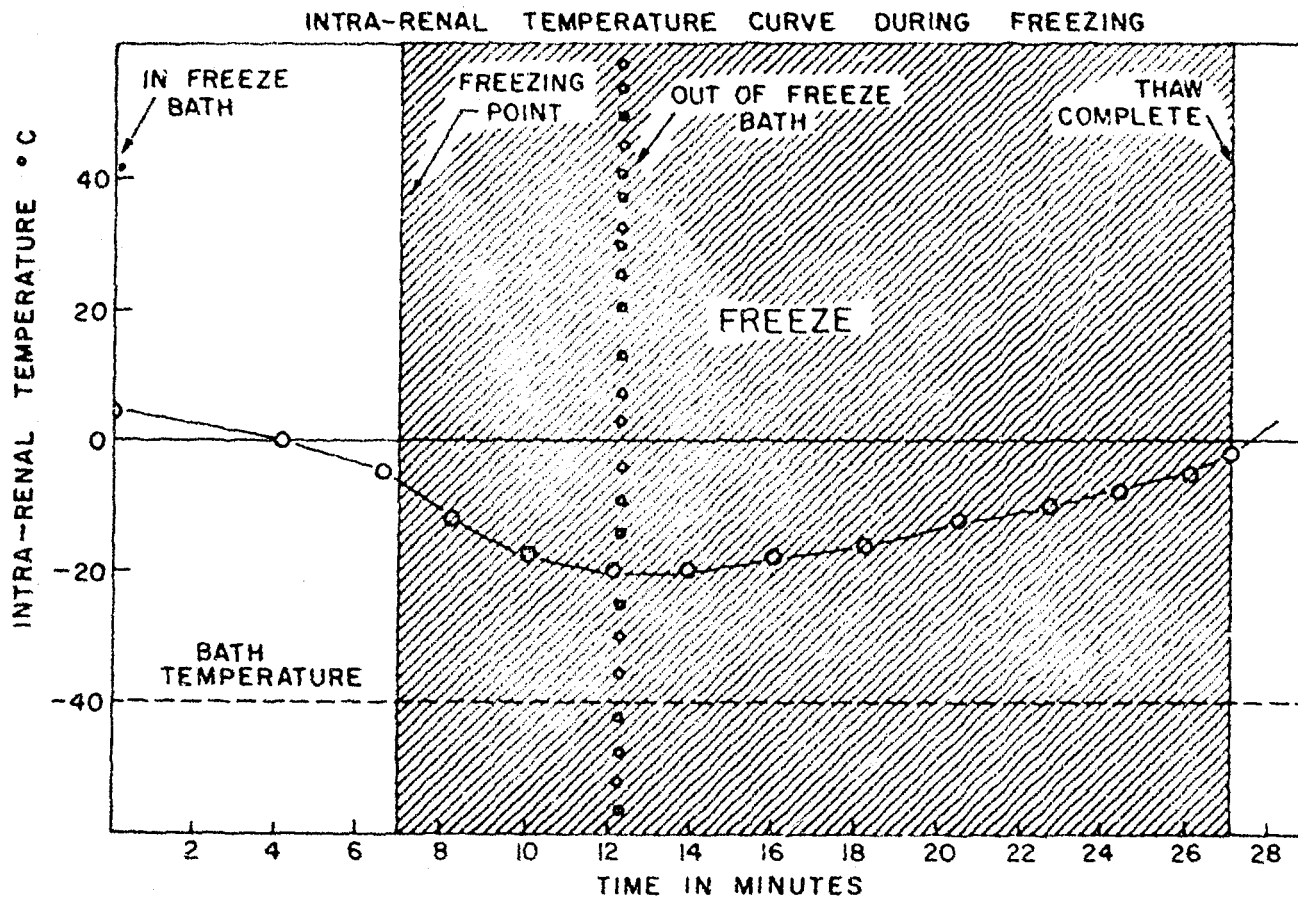


FIG. 2. Intrarenal temperature curve during freezing (mean of 14 experiments). Mean time of immersion until freezing, 7 min; mean time frozen, 20 min; mean rewarming time, 15 min.

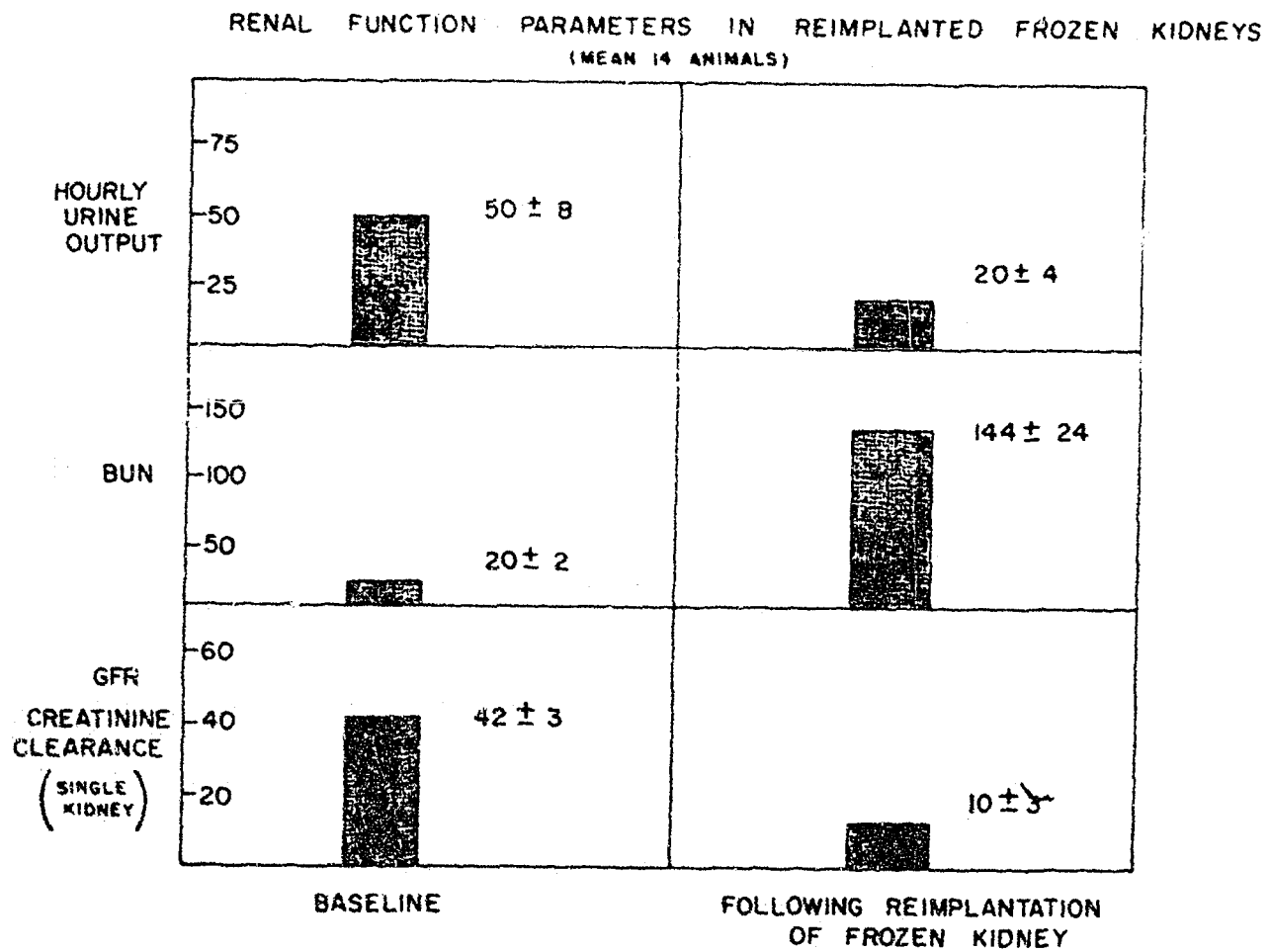


FIG. 3. Renal function parameters in reimplanted frozen kidneys. Marked renal functional impairment following freezing reflects severity of freezing injury. Two animals were long-term survivors with a single reimplanted frozen kidney and demonstrated less severe functional impairment.

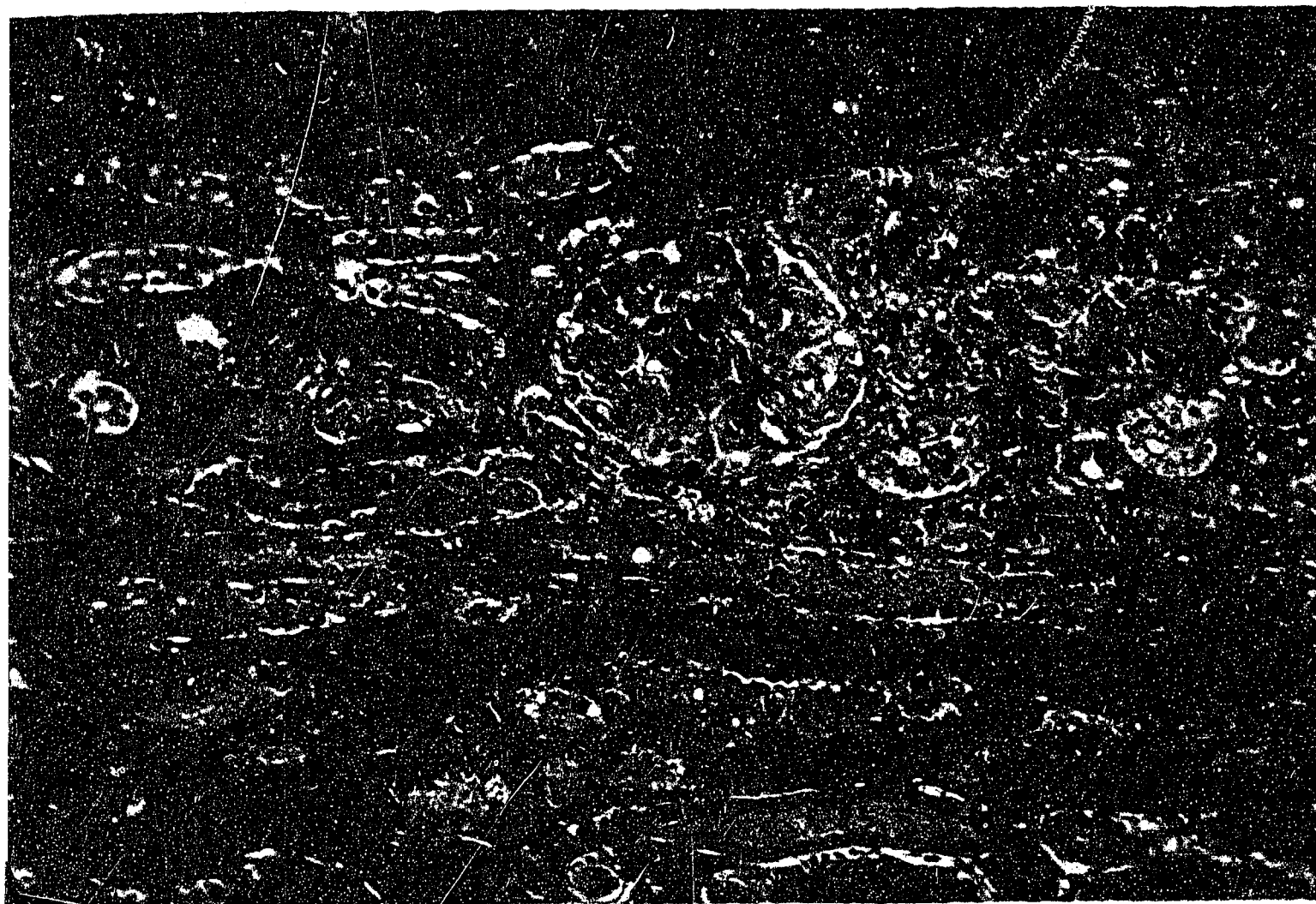


FIG. 4. Severe interstitial hemorrhage and marked tubular necrosis in reimplanted frozen kidney in animal that died 24 hr following surgery. This kidney was anuric.  $\times 200$ .

long-term survivors; one lived 6 weeks, and the other is living and well 3 months following surgery with a blood urea nitrogen of 40 mg %. Two animals died within 24 hr after surgery before renal function could be accurately assessed. In one, the frozen kidney was grossly hemorrhagic at autopsy and contained a clot in the renal artery. In the other, the kidney appeared fairly normal at autopsy and the microscopic section showed moderately severe interstitial edema, extravasation of red cells, and hydropic changes in proximal tubular cells.

The mean survival time of the 13 animals that succumbed was 9.8 days. Uremia undoubtedly caused or contributed to death in 11 of these. In all of these the frozen and reimplanted kidney continued to function, although inadequately, until death. Renal function tended to be stable in each animal; i.e., the initial renal function as determined by urine output, creatinine clearance, and blood urea nitrogen persisted until death with little change. In the case of the two long-term survivors, renal

function tended to improve gradually with time (Fig. 3).

Histological studies of the frozen and reimplanted kidneys at autopsy revealed microscopic damage proportional to the degree of renal function depression. In those animals dying soon after surgery with very poor renal function, severe gross interstitial hemorrhage and marked tubular necrosis were noted (Fig. 4). Microscopic studies of renal biopsies of the two long-term survivors showed considerable proximal tubular necrosis and interstitial fibrosis with evidence of active tubular epithelial regeneration. Hyaline casts of sloughed tubular epithelium were noted in lumens of many of the tubules (Fig. 5).

#### DISCUSSION

In freezing tissue of considerable mass, such as the kidney, it is essential to monitor the intrarenal temperature in order to ascertain whether freezing of the entire kidney has actually taken place and to establish the time

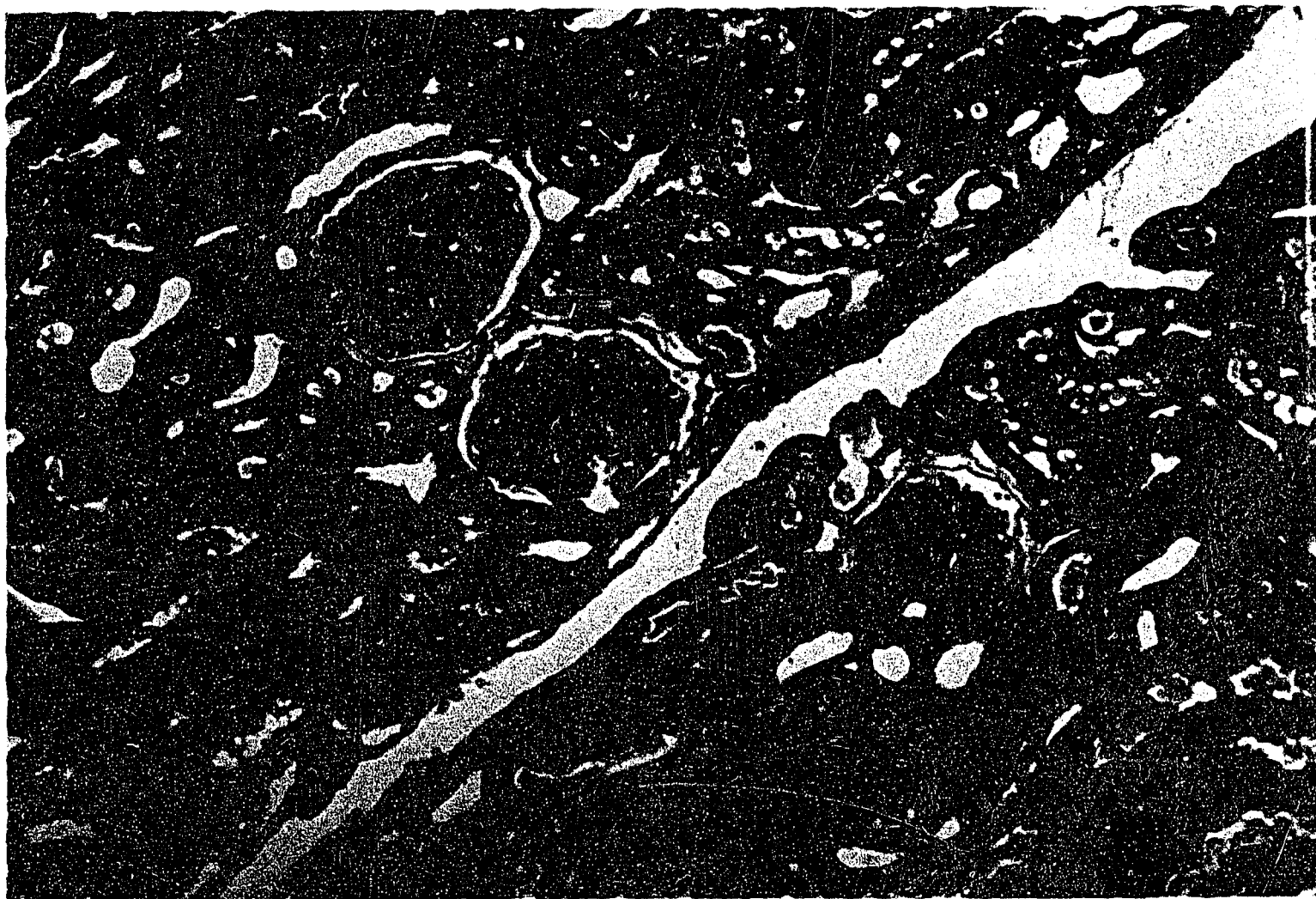


FIG. 5. Frozen reimplanted kidney of long-term survivor. Biopsy at sacrifice 4 months after kidney was frozen and reimplanted. Blood urea nitrogen, 40 mg% at time of sacrifice. Note moderate interstitial edema and fibrosis and tubular cellular regeneration.  $\times 150$ .

frozen. We have found that a kidney may appear to be frozen and yet have an intrarenal temperature above  $0^{\circ}\text{C}$ . If such a kidney is "thawed" and reimplanted, surprisingly good renal function may be evident, which reflects the fact that the kidney was supercooled rather than actually frozen. Studies in which kidney freezing has been substantiated by intrarenal temperature monitoring have been very discouraging in terms of obtaining renal function following reimplantation. Hemorrhagic necrosis of the corticomedullary zone and massive tubular necrosis reflect the severity of the vascular damage incurred by freezing and thawing.

Undoubtedly there are many factors leading to renal damage following freezing and reimplantation. In this study we attempted to prevent the severe vascular injury associated with freezing by several approaches: 1) infiltration of the renal pedicle with local anesthesia prior to dissection to help prevent the reflex intrarenal vasoconstriction characteristic of the isolated kidney preparation; 2) prevention of

intrarenal vascular collapse during the period of total arterial ischemia to mitigate the establishment of corticomedullary arteriovenous shunts and subsequent deficient cortical perfusion; 3) use of a cryoprotective solute (dimethyl sulfoxide) to prevent plasmolysis and intracellular enzyme denaturation; 4) use of 100% carbon dioxide perfusion during hypothermia to obtain a plastic intravascular space during freezing and to increase tissue  $p_{\text{CO}_2}$ , a measure which Smith<sup>8</sup> has shown to improve survival of animals subjected to periods of profound hypothermia; 5) use of a moderate freezing rate of  $1^{\circ}$  to  $2^{\circ}\text{C}$  per min, which in our experience prevents rupture of the kidney; and 6) use of a moderate rate of external rewarming (bath temperature  $25^{\circ}\text{C}$ ) to prevent excessive rewarming of the ischemic cortex prior to re-establishment of renal perfusion.

Although severe morphological and functional damage occurred in kidneys subjected to this method of freeze-preservation, the fact

that any degree of renal function was obtained after reimplantation was encouraging and suggests that freeze-preservation of complex organs such as kidney may be feasible.

#### SUMMARY

This study was undertaken to investigate the functional viability of canine kidneys subjected to extracorporeal freezing and reimplantation. Although considerable success has been attained in freeze-preservation of single cells and certain multicellular tissues, there has been little success in obtaining functional survival of such complex organs as the kidney. Severe vascular damage characterized by hemorrhage throughout the cortex and medulla has been associated with extracorporeal kidney freezing and reimplantation. A new method for preparation of the kidney for freezing has been developed. Using this method, the left kidneys of 14 dogs were removed and frozen for periods of 10 to 30 min, thawed, and reimplanted in the right iliac fossa. Intrarenal temperatures were monitored throughout the freezing and thawing period. An immediate contralateral nephrectomy was done. Renal function was assessed periodically postoperatively, including blood urea nitrogen, creatine clearance, urine osmolarity, and urinalyses. Nine of the 14 dogs had some renal functional survival following freezing. There were two long-term survivors, one living 6 weeks and the other sacrificed 4 months following surgery. Histological studies of the frozen and reimplanted kidneys at autopsy revealed microscopic damage proportional to the degree of renal functional depres-

sion. Although there was rather severe impairment of renal function postoperatively, the appearance of renal function in the majority of animals and the long-term survival of two was encouraging and suggests that freeze-preservation of complex organs such as kidney may be feasible.

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