Virchows Arch. A Path. Anat. and Histol. 380, 273–281 (1978)

Virchows Archiv A

Pressure-Flow Relationships and Pathological Changes During Renal Preservation

Stephen W. Flax[1], Stuart J. Updike[2], Peter M. Burkholder[3], Frank D. Gutmann[2], Thomas A. Barber[3], David T. Uehling[4], and John G. Webster[1]

[1] Department of Electrical and Computer Engineering, [2] Department of Medicine
[3] Department of Pathology, [4] Department of Surgery
University of Wisconsin, Madison, Wisconsin 53706, USA

Summary. The renal pedicle of one kidney from each of four dogs was ligated for one hour. The contralateral kidney served as a control. Both kidneys were removed and perfused using the "Belzer" technique. Pressure-flow relationships were determined and biopsy samples taken. The vasculature was then injected with silicone rubber. Perfusion resistance, vascular filling with silicone rubber and observations made by electron microscopy were compared.

Key words: Renal – Perfusion – Ischemia.

Introduction

Ischaemically damaged kidneys undergoing hypothermic preservation develop increased perfusion resistance during preservation, presumably as a consequence of ischaemia. Factors such as vascular plugging (Belzer et al., 1968; Lee et al., 1961), interstitial oedema (Dougherty, 1968), vascular myogenic activity (Belzer et al., 1970; Belzer et al., 1976; Berkowitz et al., 1967; Miller et al., 1974), and cellular swelling (Enerson, 1966; Enerson et al., 1967; Flores et al., 1972; Frega et al., 1976; Leaf, 1970; Johnston et al., 1977) have been postulated as causes of the altered perfusion dynamics in the ischaemically damaged kidney. Previous experimental work in this laboratory suggested, however, that cellular swelling was the predominant factor causing these variations early in the preservation interval (Flax, 1975).

Perfusion dynamics in the kidney have previously been studied by a variety of techniques. These include inert gas washout (Thornburn et al., 1963), microsphere injections (Stein et al., 1973), silicone rubber (Microfil) injections (Burger et al., 1971), and electron or light microscopy. Leaf has presented evidence

Send offprint requests to: Dr. John Webster, Department of Electrical and Computer Engineering, University of Wisconsin, Madison, Wisconsin 53706, USA

0340-1227/78/0380/0273/$01.80

that cell swelling may play a major role in the increased perfusion resistance (Flores et al., 1972; Frega et al., 1976; Leaf, 1970) associated with ischaemic insult in brain, heart, and kidney tissues. In in vivo experiments using rat kidneys he has shown that the increased flow resistance is associated with decreased cortical perfusion and endothelial swelling, as determined by Microfil injections and electron micrography. Other investigators have not been able to reproduce this phenomenon in other animal species (Riley et al., 1975). Although Leaf's observations were based on the results of in vivo experiments our observations were made on ex vivo hypothermic perfusions which showed an apparent similiar elevation in perfusion resistance associated with ischaemia. Since our dynamic perfusion observations of ischaemically damaged dog kidneys appeared compatible with Leaf's cell swelling hypothesis, we designed the following experiments to test the hypothesis.

Experimental Design-Method

In an experimental series consisting of four anesthetized dogs (pentobarbital 30 mg/kg IV), both kidneys were exposed using a "no touch" technique (Ackerman et al., 1967). In each dog, one kidney was excised and, within one minute of vessel ligation, flushed with Ringer's lactate at 37° C for one minute. It was then placed back in the dog unperfused for one hour. Prior to the completion of the ischaemic interval for the first kidney, the second kidney was similarly removed and flushed with Ringer's lactate at 37° C. At the end of the one minute interval, this kidney was then flushed with ice cold Ringer's lactate for three minutes. At that point, the first kidney (made ischaemic for 1 h) was removed and also flushed for three minutes with ice cold Ringer's lactate. Both kidneys were placed in a Belzer LI-400 preservation system and perfused according to the Belzer protocol (Belzer et al., 1967).

Within five minutes of the onset of perfusion, the initial perfusion dynamic characteristics were determined simultaneously for both kidneys. The measurement technique consists of applying a stair-step flow pattern to both kidneys simultaneously with a Harvard dual channel syringe pump (model # 600–000) while recording the corresponding pressure response on a dual channel strip recorder as measured with a matched pair of Statham (Model # 560D6) pressure transducers. Three flow rates were generated: 7.64, 15.3 and 38.2 ml/min. Each step function change in flow was applied to the kidney for 15 s. This provided time for vascular filling to occur and for the concomitant pressure to reach a steady state value. This steady state pressure value for each flow step was then plotted as a function of flow in order to describe vascular resistance. The pump flow rates were selected so as not to exceed the infusion pressure of 60 mm Hg, reported by Belzer not to damage the hypothermically perfused kidney (Belzer et al., 1967). Figure 1 shows a diagram of the system used.

Immediately following the perfusion dynamic measurement and within 10 min of the onset of perfusion, portions of the subcapsular cortex of isolated kidneys approximately 1/2 cm square by 1 mm in thickness were excised from both kidneys. These were placed in a drop of 4% glutaraldehyde buffered with 0.1 M cacodylate buffer, pH 7.4, on dental wax and diced into 1 mm or smaller cubes. The fragments were then fixed for 4 h at 4° C, washed overnight in cold 0.1 M Na cacodylate buffer containing 7.5% sucrose. Tissue blocks were secondarily fixed in S-collidine–buffered 1.0% osmium tetroxide for one hour at room temperature, dehydrated through a graded alcohol series, and infiltrated with Epon 812 resin. After embedding of tissue in polyethylene Beem capsules containing full strength Epon resin and polymerization of the resin at 60° C for 48 h, the material was sectioned and stained for light and electron microscopy. Thick (2–3 μm) sections to allow screening of tissues for light microscopy and location of glomeruli for thin sectioning were cut on an LKB 8800 ultratome and stained with toloidine blue. Selected areas were thin sectioned (500–900 Å), stained with lead citrate and uranyl acetate, and observed in a Hitachi HS-8 or Philips EM-300 transmission electron microscope at 50 or 60 kV using a 30 μm objective aperture.

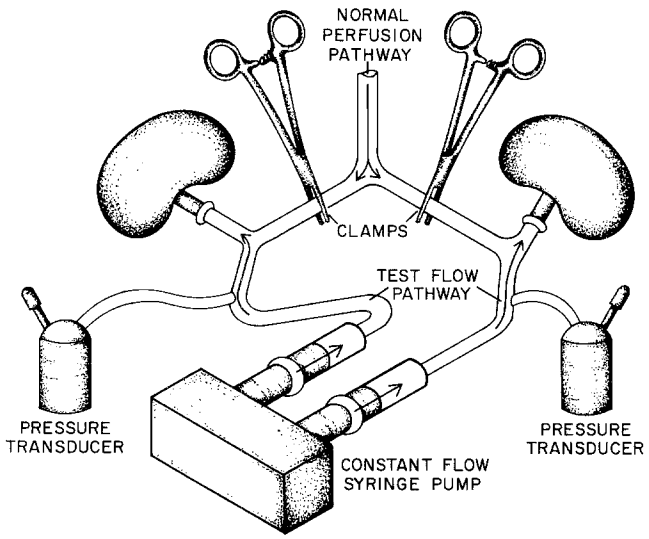

Fig. 1. A diagram of the apparatus used to quantify the perfusion dynamic response of kidneys. The syringe pump consists of two channels, which allows the measurements to be performed simultaneously when using a matched pair of pressure transducers

Immediately after the perfusion dynamic measurements were made and biopsies had been taken, the kidneys were removed from the preservation system and perfused (within 1 min) with a silicone rubber suspension (Microfil, Canton Biomedical Products, Boulder, Colorado) at an injection pressure of 50 mm Hg. After ten minutes of perfusion, the renal veins of both kidneys were clamped while an arterial pressure of 50 mm Hg was maintained until the silicone rubber had hardened (approximately 5 to 10 min). The kidneys were refrigerated for 24 h, sliced into 1 cm thick sections, then dehydrated in ethanol over a period of 5 days. The tissue was cleared by placing the dehydrated slices in methylsalicylate. The microvasculature was studied and typical areas photographed with a 35 mm camera attached to a Zeiss Tessovar Photomacrographic System. Dynamic perfusion measurements, biopsy and silicone rubber injection were performed early (within the first half hour) during hypothermic preservation, except in the fourth dog experiment in which the kidneys were perfused for 24 h. In this fourth experiment, dynamic perfusion measurements and biopsy specimens were obtained initially and after 24 h preservation. Silicone rubber was then injected as described above.

Results

Striking and consistent differences between ischaemic experimental and "normal" control kidneys were revealed by all three methods of analysis, i.e. vascular perfusion dynamics, silicone vascular injection, and ultra-structural morphology. Results from all four experiments are presented in Figure 2. The results show striking differences between the perfusion pressures of the control and ischaemic kidneys at given flow rates. Because the differences were substantial, statistical analysis was unnecessary. The perfusion pressure for the ischaemic kidneys ranged from 62 to 24.3% higher than the corresponding control kidneys, a phenomenon in agreeement with earlier observations (Flax, 1975). Comparisons

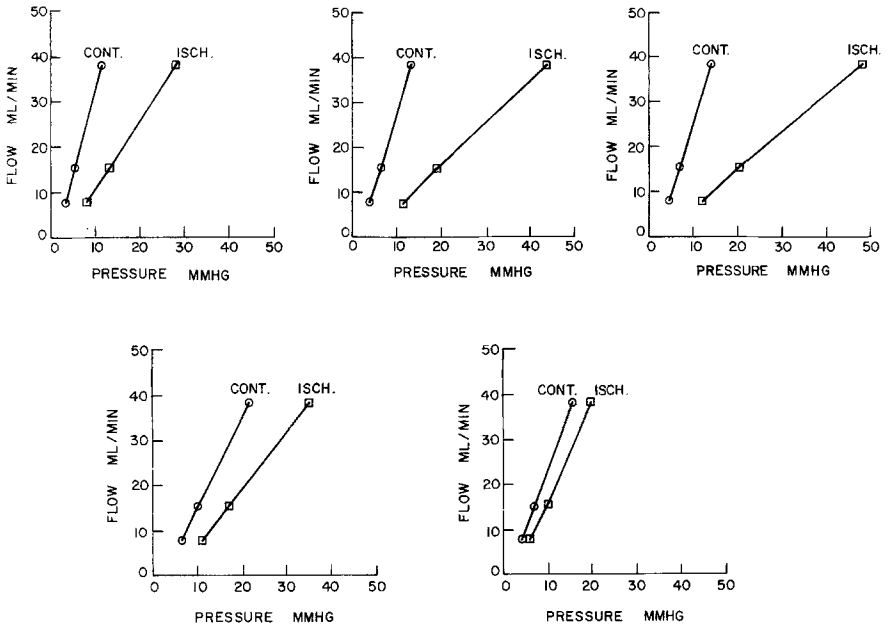

Fig. 2. A comparison between the perfusion dynamic responses of control and ischemically damaged kidneys. The top three groups are from three different kidney pairs, sampled within minutes of perfusion onset. The bottom two graphs are comparisons between a final pair of kidneys with the initial data shown at the left, and the data obtained after 24 h of perfusion shown at the right

made using the Microfil injection technique (Fig. 3) showed patchy and incomplete filling pattern in the outer cortical regions for each ischaemic kidney and a much more complete and uniform filling pattern for the control kidneys. Finally, electron microscopy (Fig. 4) revealed glomerular vascular damage, such as severely ballooned or irregular hypertrophied endothelial cells in the ischaemically damaged samples, but a comparatively unobstructed vasculature in the control kidney sections. These changes were not absolute, i.e. within any ischaemic kidney section, variable proportions of glomerular capillary loops appeared to be less impressively compromised or were not obstructed. However, sections taken from the control organs showed an almost uniformly unobstructed glomerular vasculature. Three pathologists and four nephrologists from the University of Wisconsin Medical Center examined both the microfil injected kidneys and randomly selected electron micrographs under blind conditions. Without exception they promptly identified whether a kidney had undergone ischaemic damage or was a control kidney.

Finally, the differences between the control and ischaemic kidneys at 24 h were much less marked. The initial high resistance of the ischaemic kidney had decreased nearly to that of the control kidney at 24 h. Both kidneys exhibited a well filled microvasculature with the silastic rubber. Electron micrographic

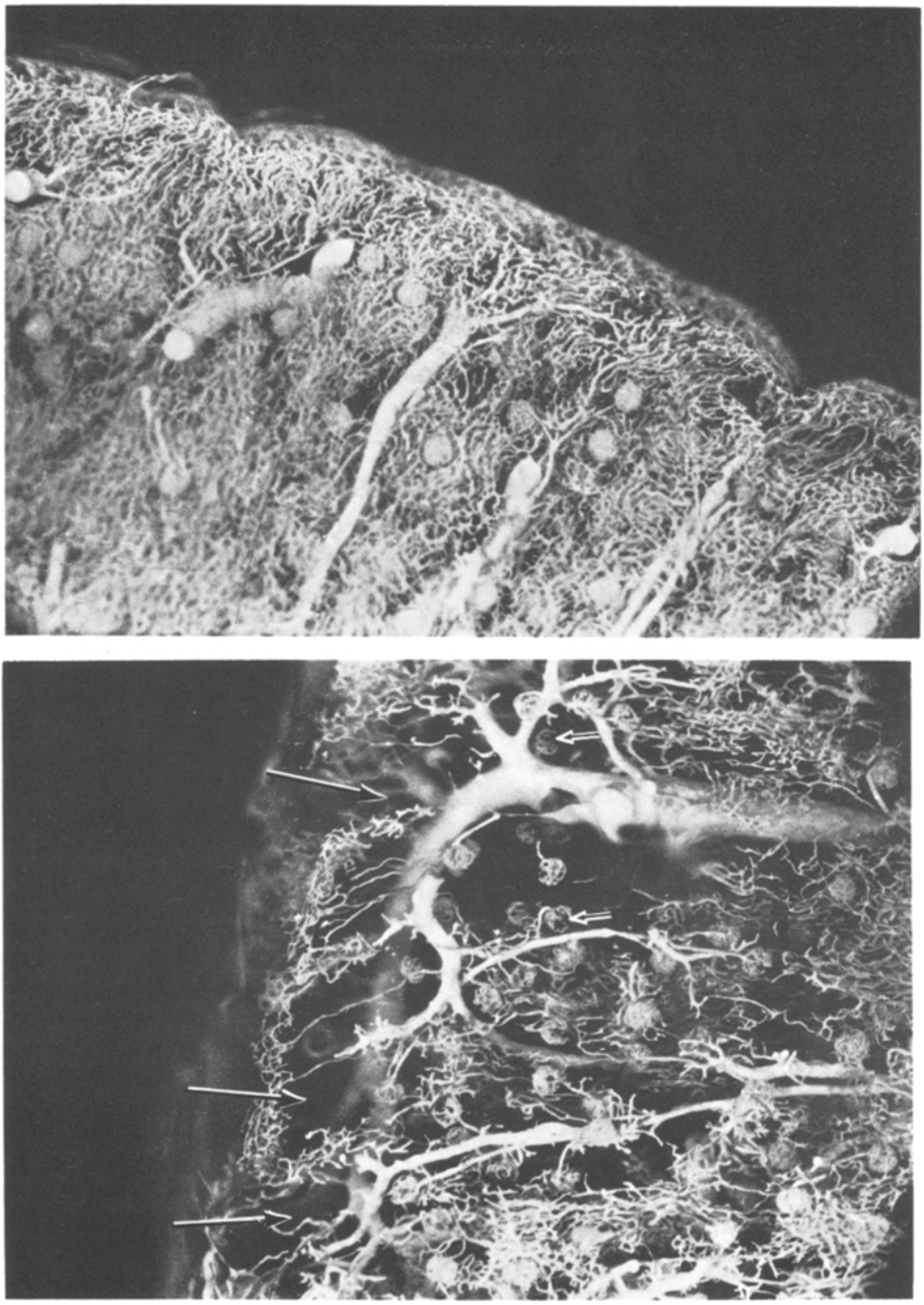

Fig. 3A and B. Silicone rubber perfused "control" (**A**) and ischemic experimental (**B**) kidneys of dog # 3 demonstrate a full pattern of vascular perfusion in the minimally ischemic control (**A**) kidney and irregular pattern of filling with incomplete subcapsular and patchy cortical (*large arrows*) vascular perfusion as well as focal segmental incomplete glomerular filling (*small arrows*) in the experimental ischemic (**B**) kidney ($\times 6.4$ original magnification)

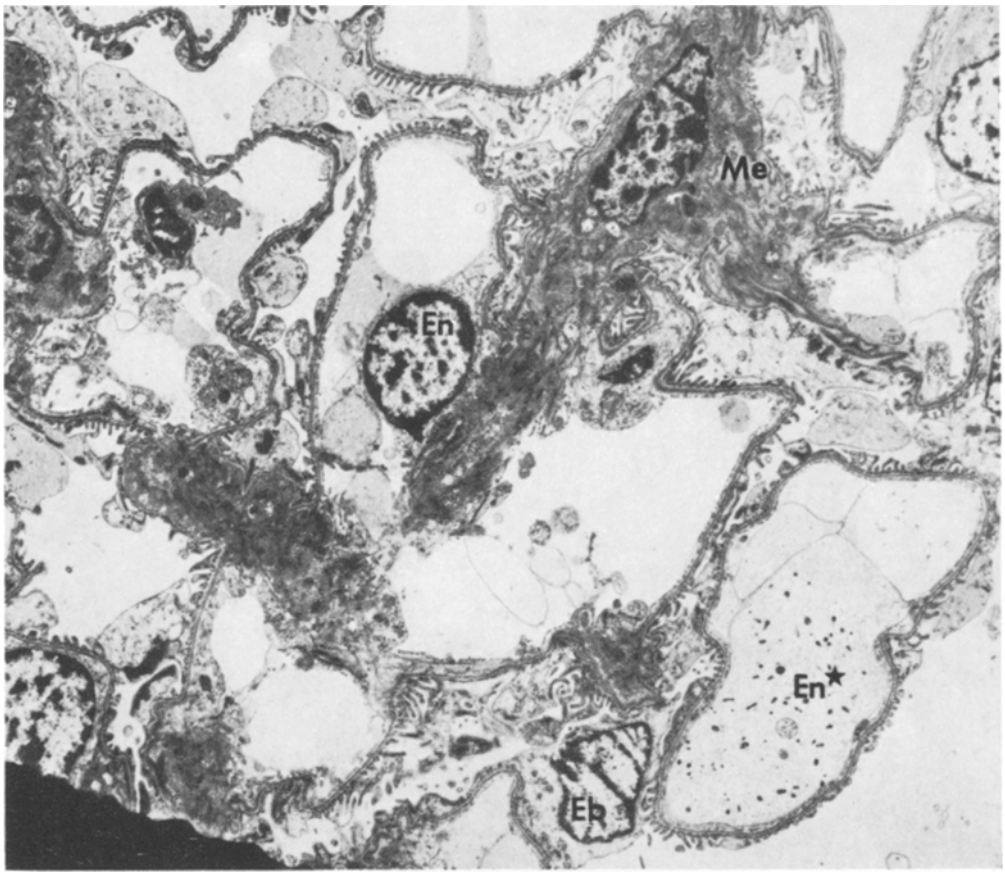

Fig. 4A and B. Comparison of control (**B**) and experimental ischemic (**A**) kidney from dog # 2 reveals widely patent glomerular capillary loops with thin capillary walls and only rarely locally ballooned segments of endothelial cells (*arrow*) in the nonischemic control (**B**) and partially compromised (*En*) or extensively obstructed (*En**) capillary loops with swollen endothelial cells in the experimental ischemic kidney (**A**). Mesangium (*Me*), Epithelium (*Ep*) (× 2300 original magnification)

studies revealed ballooned endothelial cells in both 24 h samples, but endothelial ballooning was more prominent in the ischaemic kidney.

Discussion

In these experiments the only variable distinguishing experimental kidney from control kidney was the one hour of ischaemia. The dynamic perfusion measurements, electron microscopy studies, and silicone rubber injections were all performed in a sequence within a time period of 20 min or less. The sequence of an initial dynamic perfusion measurement, then tissue biopsy and lastly

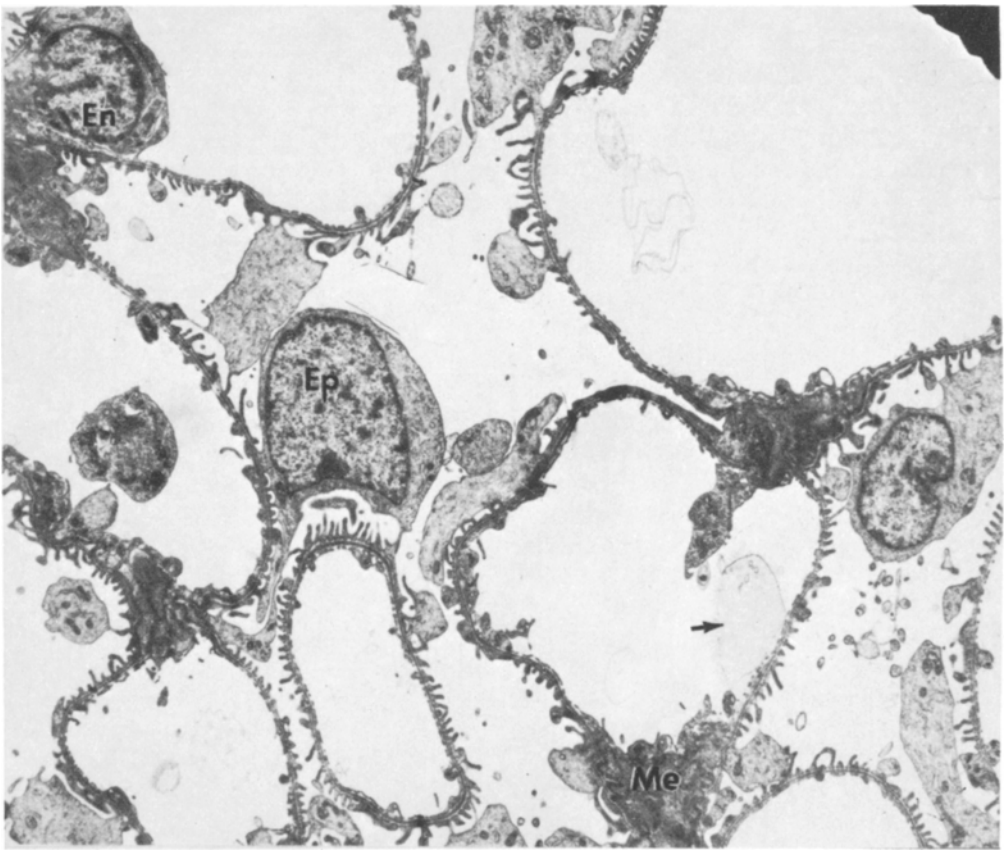

Fig. 4B

microfil injection, allowed each measurement to be performed without interference from the procedure of the previous measurement. Thus, the significance of each individual measurement is enhanced since it can be directly compared with results of the other measurements, obtained at approximately the same time but without interference. In addition, a cross comparison existed for each measurement between the control and ischaemically damaged kidney.

The silicone rubber injection technique used here was modified significantly from the procedure described by Barger. Firstly, the injection pressure used was 50 mm Hg as compared to 150 mm Hg used by Barger. We considered that this caused less vascular distension and allowed the study of the vasculature as it would exist during preservation where the perfusion pressure is not allowed to exceed 60 mm Hg. Secondly, the technique of clamping the venous outflow midway through the injection procedure has not been previously used. We felt that the "stop flow" technique might help in distinguishing between apparent alterations in vascular filling patterns due to hypothetical venous sphincter constriction, rather than obstructive cellular swelling, by significantly increasing the post sphincter vascular pressure.

The present experiments on ischaemically damaged kidneys are the first studies in which a dynamic perfusion measurement technique was used concomitantly with silicone rubber injection and electron microscopy. We conclude that these three techniques utilized in concert better define ischaemic damage in the hypothermically perfused kidney. The damaged kidneys exhibited a high perfusion resistance, a "patchy" outer cortical Microfil injection pattern, and "ballooned" endothelial swelling, partially compromising a majority of the glomerular capillary loops observed. Conversely, the control kidneys exhibited a lower perfusion resistance, a uniform outer cortical Microfil injection pattern, and unobstructed glomerular capillary loops.

The fact that patchy Microfil injection patterns persisted even though the venous outflow was occluded emphasises the importance of cellular swelling rather than constricted capillary sphincters. This observation and the observation of swollen endothelial cells lends support to Leaf's hypothesis that cell swelling can cause an elevated perfusion resistance following a renal ischaemic insult.

The fact that perfusion resistance decreases with preservation may indicate that the damage is not self-sustaining, as Leaf suggsts, at least in our model. With continued perfusion, the cellular volume may approach a normovolemic state, reflected in decreased perfusion resistance. We favor this interpretation because we have observed in other work (Flax, 1975) that dog kidneys subjected to 3 h of warm ischaemia do not show a spontaneous decrease in perfusion resistance with preservation. The observation, presented by Sheil et al. (1975) that impaired perfusion does not necessarily result in transplant failure, may thus still be consistent with our observations if cellular damage is reversible with revascularization.

Finally, one important concept remains. The endothelial swelling reported here reflects gross cellular damage. We were unable to document similar consistent gross changes in other tissue cells such as tubular or arteriolar endothelial cells. Our attention focused on glomerular vascular damage simply because the damage was striking. It is also possible that the elevated perfusion resistance is a result of partial obstruction at both the arteriolar and glomerular levels when we consider the total cross-sectional area considerations of the two vascular beds. We wish to infer that a slight degree of generalized cellular swelling is probable if gross swelling can be seen in the glomerular vascular bed, and it is evident that it would be very difficult to detect a 3% cellular volume increase on an electronmicrograph, for example. Yet, if generalized cellular swelling of only 3% occurred a significant amount of vascular space could be displaced. Assuming laminar flow (a reasonable assumption in the preserved kidney as the perfusate is "cell free") perfusion resistance could be greatly increased, since flow resistance is inversely proportional to the fourth power of the radius. Thus it would be desirable to have a more sensitive means of assessing cellular swelling. The dynamic perfusion measurements fulfill this need. While this measurement technique is very sensitive, interpretation of the dynamic perfusion changes is not possible without additional techniques to characterize the nature of the change in the vascular system. However, used in conjunction with electron microscopy studies and silicone rubber injections, a rational interpretation of these perfusion dynamic changes becomes possible. The observations made in

this report suggest that elevated perfusion resistance is due to vascular obstruction, possibly due to cellular swelling.

References

Ackermann, J.R., Hoskinson, W.I., Murphy, D.M.G., Kenyon, J.R.: Successful 24–48 hour canine kidney preservation. Advancement in transplantation, p. 743, Baltimore: Williams and Wilkins 1967

Barger, A.C., Herd, J.A.: The renal circulation. New Eng. J. Med. **284**, 482 (1971)

Belzer, F.O., Ashby, B.S., Dunphy, J.E.: 24 hour preservation of canine kidneys. Lancet **2**, 536 (1967)

Belzer, F.O., Ashby, B.S., Huang, J.S., Dunphy, J.E.: Etiology of rising perfusion pressure in isolated organ perfusion. Ann. Surg. **168**, 383 (1968)

Belzer, F.O., Reed, T.W., Pryor, J.P., Kountz, S.L., Dunphy, J.E.: Cause of renal injury in kidneys obtained from cadaver donors. Surg. **130**, 467 (1970)

Belzer, F.O., Salvatierra, O.: Renal transplantation: Organ procurement, preservation, and surgical management. In: The kidney, (Brenner and Rector, eds.), chapt. 14. Baltimore: W.B. Saunders 1976

Berkowitz, H.D., Miller, L.D., Itskovitz, H.D.: Renal function and the renin-angiotensin system in the isolated perfused kidney. Am. J. Physiol. **213** (1967)

Dougherty, J.C., Panossian, A., Nehlsen, S.L., Torres, M., Veith, F.J.: Distribution of blood flow in isolated kidneys perfused with blood, plasma, and crystalloids. Organ perfusion and preservation, New York: Appleton-Century Crofts 1968

Enerson, D.M.: Cellular swelling. I. Hypothermia graded hypoxia and osmotic effects of low molecular weight dextran on isolated tissues. Ann. Surg., **163**, 169 (1966)

Enerson, D.M., Berman, H.M.: Cellular swelling. II. Effects of hypotonicity, low molecular weight dextran addition and pH changes on oxygen consumption of isolated tissues. Ann. Surg. **163**, 537 (1967)

Flax, S.W.: A computer model of the perfusion dynamic behavior of the kidney during preservation. Ph.D. thesis University of Wisconsin, 1975

Flores, J., DiBona, D.R., Beck, C.H., Leaf, A.: The role of cell swelling in ischemic renal damage and the protective effects of hypertonic solute. J. Clin. Invest. **51**, 118 (1972)

Frega, N.S., DiBona, D.R., Guertler, B., Leaf, A.: Ischemic renal injury. Kidney Internat. **10**, S17 (1976)

Johnston, W.H., Latta, H.: Glomerular mesangial and endothelial cell swelling following temporary renal ischemia and its role in the no-reflow phenomenon. Am. J. Path. **89**, 153 (1977)

Leaf, A.: Regulation of intracellular fluid volume and disease. Am. J. Med. **49**, 291 (1970)

Lee, W.H., Jr., Krumhaar, D., Fonkalstud, E.W., Schjeide, O.A., Maloney, J.V., Jr.: Denaturation of plasma proteins as a cause of morbidity and death after intracardiac operations. Surg. **50**, 29 (1961)

Miller, H.C., Alexander, J.W., Smith, E.J., Fidler, J.P.: Salutary effect of phentolamine (regitine) on renal vasoconstriction in donor kidneys. Transplant. **17**, 201 (1974)

Riley, A.L., Alexander, E.A., Migdal, S., Levinsky, N.G.: The effect of ischemia on renal blood flow in the dog. Kidney Internat. **7**, 27 (1975)

Sheil, A.G.R., Drummond, J.M., Rogers, J.H., Boulas, J., May, J., Storey, B.G.: A controlled clinical trial of machine perfusion of cadaveric donor renal allografts. Lancet **2**, 287 (1975)

Stein, J.H., Boonjarern, S., Mark, R.C., Ferris, T.F.: Mechanism of the redistribution of renal cortical blood flow during hemorrhagic hypotension in the dog. J.C.I. **52**, (1973)

Thornburn, G.D., Kopald, H.H., Herd, J.A., Hollenberg, Mi, O'Morchoe, C.C.C., Barger, A.C.: Intrarenal distribution of nutrient blood flow determined with 85 Krypton in the unanesthetized dog. Circ. Res. **13**, 290 (1963)

Received June 20, 1978