Urol Res (1991) 19:259–264

Urological Research

Changes in regional renal perfusion following ischemia/reperfusion injury to the rat kidney

G. M. Lennon[1], P. C. Ryan[1], E. F. Gaffney[2], and J. M. Fitzpatrick[1]

[1] Department of Surgery/Urology, Mater Misericordiae Hospital and University College Dublin, Ireland
[2] Department of Pathology, St. James's Hospital, Dublin, Ireland

Accepted: October 1, 1990

Summary. Post-ischemic renal failure is associated with a zone of vascular hyperaemia in the outer medulla of the kidney. The effect of this lesion on regional renal perfusion is, however, unclear. Acute unilateral renal ischemia was applied to four groups of ten adult male Wistar rats for a period of 60 min, followed by revascularisation for 0, 15, 30 or 60 min. The aorta was then clamped and Microfil was injected at a standard pressure to fill the renal vasculature. Gross and histological examinations of the renal parenchyma and vasculature were then performed. Regional renal Microfil perfusion was quantified by examination of unstained histological sections, giving rise to a vascular perfusion index (VPI) for each vascular region of the kidney. The VPIs were similar in control and ischemic kidneys that were not subjected to reflow (group 1). In contrast, the VPI was markedly decreased in the inner stripe and inner medulla in animals in which revascularisation had occurred (groups 2–4), and the vasculature in these regions was histologically shown to be packed with red blood cells. Post-ischemic renal failure is associated with hyperperfusion of the medulla resulting from blockage of the vasculature that occurs during revascularisation.

Key words: Ischemia–Reperfusion–Medullary congestion

Kidneys examined grossly following a period of ischemia and revascularisation [7, 14] have been shown to possess a characteristic discolouration at the cortico-medullary junction, and this lesion has been described as the "no reflow" phenomenon, or "blue line" [2]. On microsopic examination, it is clear that this gross appearance is due to an accumulation of red cells within the peritubular capillaries of the outer medulla but the mechanism by which this lesion develops is unclear [4, 13]. The aims of this study were to investigate the early development of this medullary congestion in the 1st h following ischemia/reperfusion injury to the rat kidney and, in addition, to quantify the effect of this lesion on regional renal perfusion using a standard-pressure casting technique and a computer-generated vascular perfusion-scoring index.

Materials and methods

Acute renal ischemia was applied to four groups of ten mature male Wistar rats. The animals were anaesthetised with halothane and, following a midline abdominal incision, the aorta and renal arteries were exposed. A non-traumatic vascular occlusive clip was applied to the left renal artery and renal ischemia was maintained for 60 min. Following this period, the animals were randomised to one of four groups, the revascularisation time being varied in each group (Table 1). The superior mesentric artery and proximal aorta were then ligated. A cannula was introduced into the distal aorta and ligated in this position, thus establishing a closed system for perfusion of the kidneys via the cannula (Fig. 1). The vascular clip was then removed and the aorta and kidneys were perfused with Microfil, a silicone polymer vascular casting material (Canton Biomedical Products Inc., Colorado). Microfil was mixed immediately prior to injection, which took place over a 2-min period with the aid of a standard-rate infusion pump. The infusion pressure was kept at <130 mmHg. This method was found to produce an even filling of the contralateral control kidney, which was exposed to identical perfusion conditions. Next, the renal pedicles were ligated and the kidneys were removed and fixed in formalin. In each case, the contralateral normal kidney served as a control.

The kidneys were subsequently bivalved and processed in the usual way for histological sectioning, the end product being a complete longitudinal hemisection of rat kidney blocked in wax, whereby the vasculature was cast in yellow Microfil and the tissues were clear due to the effects of alcohol and xylene. The renal vasculature could now be grossly examined as a three-dimensional cast, clearly observable trough the transparent renal tissues.

Table 1. Experimental groups

Group	Ischemia times (min)	Revascularisation time (min)
1	60	0
2	60	15
3	60	30
4	60	60

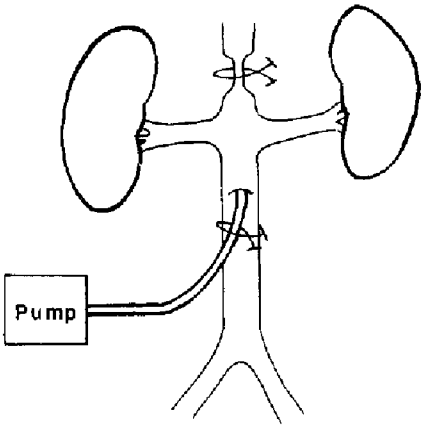


Fig. 1. Microfil perfusion system. Diagram illustrating the vasculature during Microfil perfusion. The proximal aorta is ligated between the superior mesenteric artery and the renal arteries. A perfusion cannula is ligated into the distal aorta

Histological sections were examined at low and high magnification after standard staining with haematoxylin/eosin. In addition, unstained histological sections were examined microscopically; since the casting material was non-transparent, the microvasculature in these sections could be clearly observed as a dark image against a bright background. In this way, regional renal perfusion could be assessed and the perfusion of the renal cortex and renal medulla (outer stripe, inner stripe and inner medulla) could be estimated. A vascular perfusion index (VPI) was generated for each of the four vascular regions of the kidney by quantifying the average amount of vasculature per low-power field (magnification, ×10) in ten histological sections using the Kontron Mopp Videoplan Image Analyser (Kontron Electronics, Munich). This machine, which consists of a video camera attached to a standard light microscope with a graticule in the eyepiece, enables computerised quantification of the area of a given visual field that is occupied by blood vessels. The VPI data for each vascular region of the kidney were then compared between experimental groups.

Results

The experimental procedure was well tolerated in all cases. Gross examination of control kidneys in each group showed that the renal cortex and inner stripe of the medulla were well perfused with Microfil. The outer stripe of the outer medulla was relatively poorly perfused and, similary, the inner medulla was less densely vascularised as compared with the inner stripe of the medulla, although filling of the vasa recta with Microfil was evident. Post-ischemic kidneys that were not subjected to a period of revascularisation (group 1) demonstrated Microfil perfusion similar to that observed in control kidneys on gross examination. In contrast, Microfil perfusion of the inner stripe of the outer medulla was markedly decreased, if not absent, in ischemic kidneys that had been subsequently reperfused (groups 2–4). In addition, the inner medulla appeared to be virtually devoid of Microfil in the ischemic/reperfusion groups (Fig. 2).

The normal Microfil perfusion pattern observed in unstained sections from control animals (Fig. 3a) confirmed the impression obtained from gross assessment. The cortex and inner stripe were relatively well perfused, whereas the outer stripe and inner medulla were relatively poorly perfused. The pattern was similar in the ischemic/non-reperfused kidneys (group 1). In cases in which ischemia and reperfusion had taken place (groups 2–4), the cortex and outer stripe were perfused with Microfil to the same extent observed in control kidneys, but the inner stripe of the outer medulla and the inner medulla were almost completely devoid of Microfil (Fig. 3b). Taken together with the gross findings, this latter finding suggested that a profound diminution in the blood flow in the inner stripe and inner medulla would be present in vivo.

On histological examination (HE), control kidneys demonstrated normal morphology and the normal patt-

Fig. 3.a Microfil perfusion histology: control (unstained, ×10). Histological examination of an unstained, Microfil-perfused renal section. As Microfil does not transmit light, the microvasculature is seen as a dark image against a bright background in the unstained section. Glomeruli filled with Microfil car be seen in the renal cortex. The outer stripe is poorly perfused as compared with the inner stripe, which shows a dense capillary network. Perfusion of the vasa recta is clearly visible. **b** Microfil perfusion histology: ischemia/reperfusion (group 2–4; unstained, ×10). Casting of the microvasculature in the cortex and outer medullary stripe is unchanged as compared with that in control kidneys. There is markedly deficient filling of the blood vessels in the inner stripe and inner medulla, indicating severly decreased blood flow in vivo

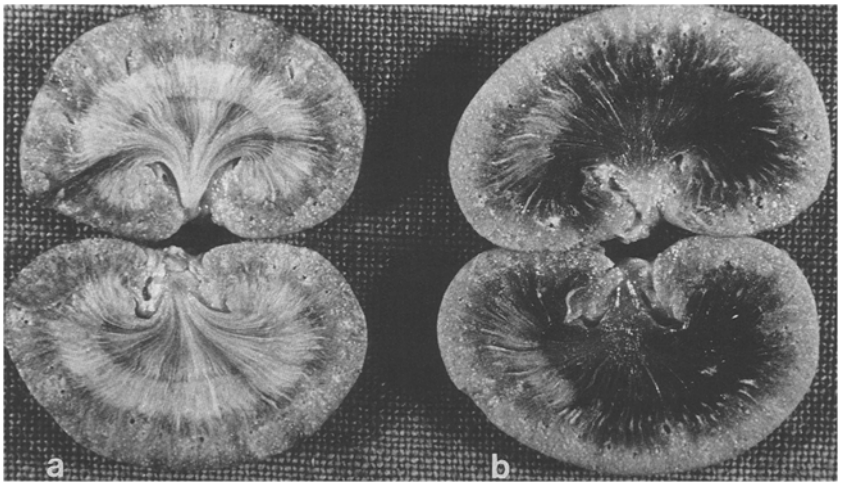
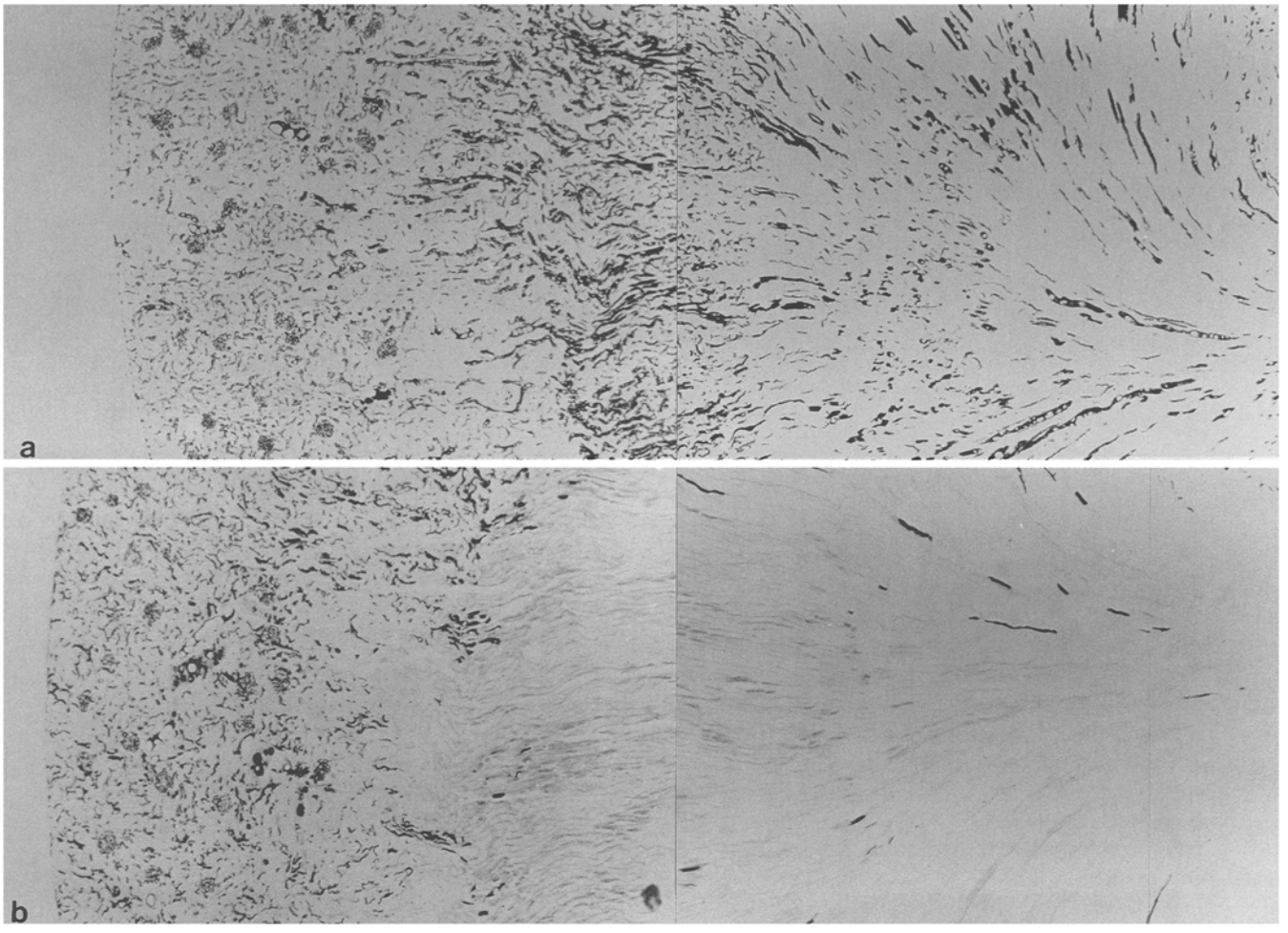


Fig. 2a, b. Gross finding: control and ischemic kidneys (group 2). Control kidney (**a**) demonstrates good perfusign in the cortex and inner stripe of the medulla; the outer medullary stripe and inner medulla are relatively poorly perfused. The contralateral kidney (**b**), which was exposed to ischemia and 15 min reperfusion, shows normal perfusion of the cortex and outer stripe but marked diminution in perfusion of the inner stripe and inner medulla



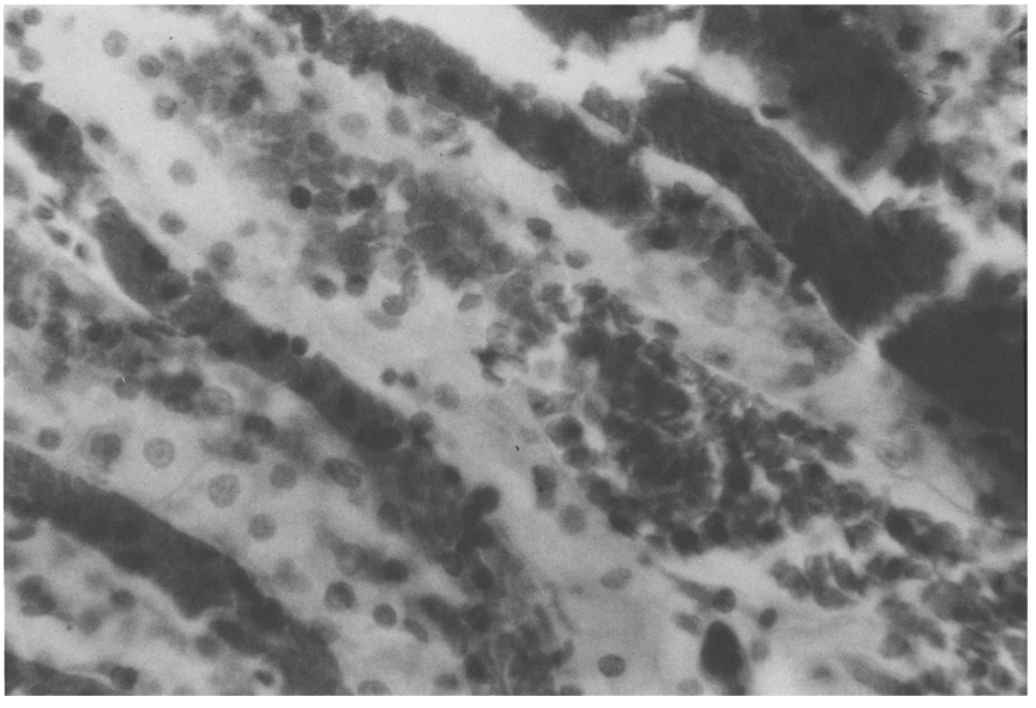


Fig. 4. Microfil perfusion histology: ischemia/reperfusion (groups 2–4; H E, ×40). At high power, it is clear that the microvasculature of the inner medulla is packed with red blood cells

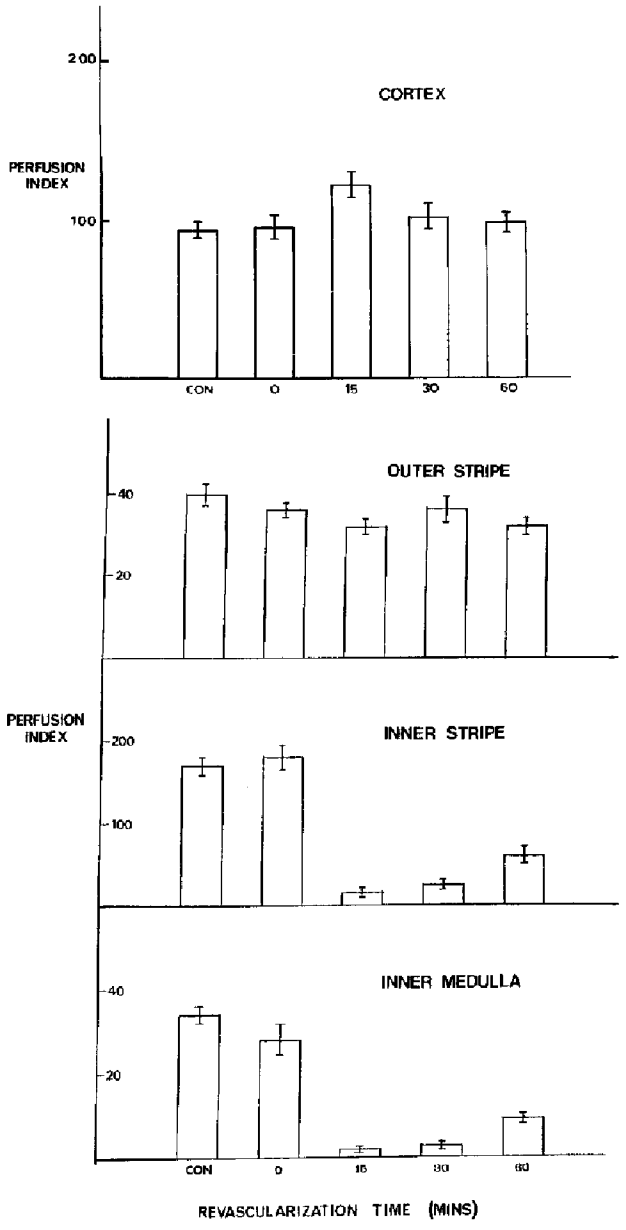


Fig. 5. Results: vascular perfusion index. Control values reflect the observations made on both gross and microscopic examination that cortex and inner medullary stripe are more densely perfused than the outer stripe and inner medulla. Distribution of Microfil was not significantly altered in kidneys that were subjected to ischemia but not reperfused (group 1). When reperfusion was allowed to occur (groups 2–4), the vascular perfusion indices in the inner stripe and inner medulla were profoundly decreased

ern of vascular filling with Microfil could be observed microscopically. Ischemic kidneys that were not reperfused (group 1) were similar to control kidneys on histological examination. Marked capillary congestion with red cells was noted in the inner stripe of the medulla in kidneys that had undergone a period of reperfusion (group 2–4; Fig. 4). Red cell congestion occurred to a lesser extent in the vasa recta of the inner medulla in the same groups.

Measurement of the VPI in control and test kidneys showed normal cortical perfusion in all groups. Vascular filling of the outer stripe of the medulla was unchanged when compared between control and ischemic kidneys in each of the groups. The VPI for the inner stripe of the medulla was markedly decreased in ischemic/reperfused kidneys as compared with controls from groups 2–4. The VPI in the inner medulla was also markedly decreased in ischemic kidneys from groups 2–4 as compared with controls (Fig. 5).

Discussion

The presence of a zone of medullary hyperaemia, or "blue line", following ischemia and reperfusion has been clearly demonstrated [2, 8]. The findings on gross examination of bivalved kidneys in the present study confirm the presence of the blue line in rat kidneys that have been subjected to ischemia and reperfusion. Histological examination has indicated that dense red blood cell congestion gives rise to this gross appearance and that the blue line is located in the inner stripe of the medulla, which exhibits a more dense capillary network than either the outer stripe or the inner medulla. The degree to which vascular congestion occurs within the medulla has been shown to be directly proportional to the impairment of renal function following acute ischemia and reperfusion [8]. However, the mechanism by which this vascular lesion causes acute renal injury is unclear.

There are a number of theories regarding the pathogenesis of renal failure following acute ischemia and reperfusion. Evidence has been presented that acute obstruction to the passage of the glomerular filtrate down the nephron occurs as a result of acute ischemia [12] and that the resulting increase in tubular diameter may cause compression on the surrounding venules, giving rise to stasis in the capillary bed [13]. Other workers agree that an increase in the diameter of the proximal straight tubule may cause compression of the drainage vessels in the inner stripe, thus leading to congestion within the capillary plexus [9].

It has also been suggested that the vascular architecture of the capillary plexus in the inner stripe may play an important role: the descending vasa rectae divide into a multitude of capillary branches, resulting in a low velocity of blood flow with consequent sludging of erythrocytes [8]. A factor that might promote this latter process involves damage to the vascular endothelium in the medulla due to ischemia, resulting in loss of intravascular fluid, increased viscosity and decreased blood flow [13, 16]. Also, decreased reabsorption of the glomerular filtrate by ischemic tubular cells contributes to increased viscosity within the vasa rectae and peritubular capillaries [2]. Some workers believe that this order of events is reversed, whereby red blood cell congestion within the capillaries is the primary phenomenon and the resulting expansion of capillary volume causes compression on the surrounding nephron, giving rise to obstruction and urinary stasis [12].

Renal venous ammonia levels are known to be markedly increased following acute ischemia [15]. Experimental

work in dogs has suggested that prevention of ammonia formation by alkalinisation may protect against ischemic renal damage [2]. The mechanism by which ammonia might cause red cell congestion is unknown, but architectural deformity in red blood cells was noted on electron microscopy in the present study.

The importance of local hypoxia in determining of tubular cell viability is evident from experiments on isolated, perfused rat kidneys. Cell necrosis increases with distance from the arterial vasa rectae in the vascular bundles traversing the outer medulla. However, the addition of erythrocytes to the perfusion medium can prevent such necrosis [10]. In the post-ischemic kidney, the sludging of erythrocytes in the peritubular capillaries of the inner stripe may be associated with significant tubular cell hypoxia, which may in part explain the correlation between the degree of red cell congestion and the subsequent impairment of renal function [8].

As regards the timing of the development of the blue line, the present study appears to show clearly that the lesion is not present immediately after ischemia but develops during reperfusion. These data indicate that sludging of red cells during arrested blood flow is not the sole factor that causes the blue line. There must therefore be some mechanism by which red cell congestion is produced during the kidney's attempt to recover from the ischemic episode. Our findings are in agreement with previous studies showing that the vascular congestion of the outer medulla is a post-reflow phenomenon [3, 11], but they conflict with evidence from another investigation suggesting that vascular red cell congestion develops while the kidney is ischemic [16].

If, as our data suggest, the perfusion defect develops following recirculation, its occurrence could be explained by the release of some noxious agent or agents following reperfusion of the kidney. Oxygen free radicals, which are known to have the potential to cause cellular structural damage, are normally generated during purine metabolism when hypoxanthine is oxidised to xanthine, a reaction that is catalysed by the enzyme xanthine oxidase. During ischemia the reaction is interrupted, leading to hypoxanthine accumulation. When oxygen once again becomes available following reperfusion the reaction recommences and the formation of oxygen free radicals may exceed the capacity of the cells' scavenger system, leading to tissue damage [5].

Damage to cell membranes by oxygen free radicals has been shown to cause an increase in cell permeability [1]. Radical-mediated damage to the vascular endothelium of the capillaries in the inner stripe could cause an increase in vascular permeability, leading to plasma leakage, increased haematocrit and sludging of red blood cells in the outer medulla. Inhibition of the xanthine oxidase reaction by the inhibitor allopurinol has been shown to prevent not only hypoxanthine accumulation during ischemia (and, by implication, the production of oxygen free radicals) but also the characteristic accumulation of red blood cells in the inner stripe of the outer medulla [14]. These observations suggest that oxygen free radicals may play a significant role in the production of the blue line, or "no reflow", phenomenon.

The implication of the blue line for renal medullary perfusion are clearly defined by the gross and microscopic findings in Microfil vascular casts in the present study. The ischemic non-reperfused kidney demonstrated perfusion at each level of the renal medullary vasculature: outer stripe, inner stripe and inner medulla. After 15 min reperfusion, perfusion of the outer stripe with Microfil was not significantly altered, but the inner stripe and inner medulla showed virtually no perfusion with Microfil. Following 60 min reperfusion, Microfil casts indicated some recovery in potential blood flow to the inner stripe and inner medulla, but the VPIs remained markedly decreased as compared with those in controls. These findings are in agreement with rubidium measurements of renal blood flow, which demonstrate not only a reduction in total medullary blood flow but also a strikingly similar reduction in the percertage of flow to the three vascular regions of the medulla [6]. Whatever the exact pathogenesis of the blue line lesion may be, its significance for renal perfusion is clearly defined in these experiments.

As described in the present study, the VPI is a useful means of measuring potential renal blood flow in a histological specimen. The vasculature in control and test sections is exposed to an identical standard perfusion pressure and its patency can be accurately measured. In this regard, the use of the Kontron Mopp Videoplan Image Analyser provides a reliable and reproducible means of measuring potential renal blood flow in the kidney and other organ systems.

Experimental renal ischemia followed by reperfusion gives rise to a vascular lesion comprising a dense aggregation of red cells that is most marked in the inner stripe of the renal medulla. This lesion develops during reperfusion and was found to be maximal after 15 min in the present study. The consequences are marked hypoperfusion of the inner stripe and inner medulla, which recovers to some extent after 1 reperfusion. The mechanism by which this lesion develops, wheter biochemical or otherwise, requires further assessment.

References

1. Bjork J, Del Maestro RF, Arfors KE (1980) Evidence for participation of hydroxyl radical in increased microvascular permeability. Agents Action [Suppl] 7:208
2. Fitzpatrick JM, Monson JRT, Gunter PA, Watkinson LE, Wickham JEA (1982) Renal accumulation of ammonia: the cause of post-ischeamic functional loss and the "blue line". Br J Urol 54:608
3. Flores J, diBona DR, Beck CH, Leaf A (1972) The role of cell swelling in ischemic renal damage and the protective effect of hypertonic solute. J Clin Invest 51:118
4. Frega NS, DiBona DR, Guertler B, Leaf A (1976) Ischemic renal renal injury. Kidney Int 10 [Suppl 6]:17
5. Hansson R, Gustafsson B, Jonsson O, Lundstam S, S Pettersson, Schersten T, Waldenstrom J (1982) Effect of xanthine oxidase inhibition on renal circulation after ischemia. Transplant Proc 14:51
6. Karlberg L, Norlen BJ, Ojteg G, Wolgast M (1983) Impaired medullary circulation in post-ischemic acute renal failure. Acta Physiol Scand 118:11

7. Mason J, Welsch J (1982) Glomerular filtration of the deeper lying nephrons after ischemic injury. Nephron 31:304
8. Mason J, Torhorst J, Welsch J (1984) Role of the medullary perfusion defect in the pathogenesis of ischemic renal failure. Kidney Int 26:283
9. Rougemont D de, Brunner FP, Torhorst J, Wunderlich PF, Thiel G (1982) Superficial nephron obstruction and medullary congestion after ischemic injury: effect of protective treatments. Nephron 31:310
10. Schurek HJ, Kriz W (1985) Morphologic and functional evidence for oxygen deficiency in the isolated perfused rat kidney. Lab Invest 53:145
11. Summers WK, Jamison RL (1971) The no reflow phenomenon in renal ischemia. Lab Invest 25:635
12. Thiel G, Rougemont D de, Torhorst J (1980) Importance of tubular obstruction and its prevention in ischemic acute renal failure in the rat. In: Leaf, Giebisch, Bolis, Gorini (eds) Recent advances in renal pathophysiology. Raven Press, New York, p 223
13. Thiel G, Rougemont D de, Kriz W, Mason J, Torhorst J, Wolgast M (1982) The role of reduced medullary perfusion in the genesis of acute ischemic renal failure. Nephron 31:321
14. Torhorst J, Rougemont D de, Brunner FP, Thiel G (1982) Morphology of the renal medulla in ischemic acute renal failure in the rat. Nephron 31:296
15. Wickham JEA, Sharma GP (1965) Endogenous ammonia formation in experimental renal ischemia. Lancet I: 195
16. Wolgast M, Karlberg L, Kallskog O, Norlen BJ, Nygren N, Ojteg O (1982) Haemodynamic alterations in ischemic acute renal failure. Nephron 31:301

Prof. J. M. Fitzpatrick
Department of Surgery/Urology
Mater Misericordiae Hospital
47 Eales Street
Dublin 7
Ireland