Int. J. Exp. Path. (1998), **79,** 55–66

Effect of anticoagulation upon nephron obstruction in experimental acute ischaemic renal failure. A morphological study

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Received for publication 1 September 1997
Accepted for publication 8 January 1998

Summary. Ischaemic-reperfusion injury as a model of acute renal failure (ARF) results in increased macromolecular permeability, tubular obstruction, and renal oedema. To investigate the role for coagulation in this model, anticoagulated and saline-pretreated rats were subjected to 60 min unilateral renal artery occlusion (RAO). After 15 min of reflow, specimens were collected for electron and light microscopic examination. Morphometry was employed to study podocyte changes and Bowman's space dilatation as measures of increased permeability and tubular obstruction, respectively. After 15 min of reflow, Bowman's space increased significantly and the podocytes were markedly widened and flattened. Rats pretreated with heparin or warfarin showed less widening of Bowman's space than saline-treated rats, whereas no significant difference was seen regarding the podocyte changes. In saline-treated rats, fibrin-positive material was seen in the tubules but not in the urine sediments collected after 90 min of reflow, either due to fibrinolysis or poor urinary elimination. The results suggest that anticoagulation does not preclude the glomerular sieving of macromolecules, but seems to reduce tubular obstruction, probably by preventing conversion of filtered fibrinogen into fibrin.

Keywords: acute renal failure, fibrin, ischaemia, reperfusion injury, morphology, tubular obstruction.

In various models of experimental acute renal failure (ARF), tubular obstruction is a frequent phenomenon (Tanner & Sophasan 1976; Burke *et al* 1983; Solez 1983; Schrier & Hensen 1988; Bratell 1990; Wolgast *et al* 1991). The post-ischaemic model of ARF has been extensively used, since it produces many of the pathological changes also seen in human ARF of different origins (Flores *et al* 1972; Venkatachalam 1981; Olsen & Hansen 1990; Racusen *et al* 1991; Racusen 1991). In this model, tubular obstruction has been suggested to play an important role for the deterioration of the renal function (Burke *et al* 1983; Wolgast 1991; Wolgast *et al* 1992; Goligorski & DiBona 1993), regardless of preceeding molecular, cellular and physiological mediators.

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In both human and experimental ARF, the kidneys are typically macroscopically swollen, and in post-ischaemic ARF, the outer medulla is almost invariably markedly congested (Karlberg *et al* 1982b; Druid & Rammer 1989). Although cellular swelling occurs in the early post-ischaemic period, it has been suggested that the renal oedema is caused mainly by an accumulation of protein-rich fluid in the tubuli, proximal to tubular obstruction (Eneström *et al* 1988; Druid & Rammer 1989, 1992; Wolgast *et al* 1991). The leakage of proteins into the tubules and renal interstitium is likely to be the result of damage to the glomerular and tubular barriers. As for the glomerular filter, podocyte changes seems to be the best morphological marker of increased macromolecular permeability (Solez *et al* 1981; Whiteside *et al* 1993; Ronco *et al* 1994).

Prolonged ischaemic insults, leading to infarction, are typically accompanied by massive fibrin deposition. However, even shorter periods of ischaemia may cause activation of the coagulation cascade, although the precise mechanism for this is unclear. Indeed, in patients developing cardiopulmonary arrest, whithout any mechanical trauma to the tissues or the blood vessels substantial fibrin formation occurred (Gando *et al* 1997), and experimental studies on ischaemic ARF by Losonczy (1985) and our laboratory (Eneström *et al* 1988), have provided evidence of local coagulation in the post-ischaemic kidney.

Pretreatment with anticoagulants both reduces the degree of fibrin deposition and the renal oedema (Druid & Rammer 1989, 1992). These changes are also paralleled by a reduction of the dilatation of proximal tubules and Bowman′s space. The effect of the anticoagulant pretreatment could either be explained by a reduction of the increased macromolecular permeability of glomeruli, or by preventing precipitation of filtered fibrinogen in the tubules. In order to establish the reason for the beneficial effect of anticoagulation on renal oedema, we decided to study the morphological markers of increased glomerular permeability and tubular obstruction by specific investigation of the glomeruli using light, immunofluorescent and electron microscopy.

Methods

Experimental animals

All procedures were approved by the Linköping Animal Care and Use Committee (No 087/1994) and were in accordance with the National Institutes of Health Guidelines for the care and use of animals. Female Sprague-Dawley rats (ALAB Farm, Stockholm), weighing 262–319 g were used. The animals had free access to food (Ewos rat pellets) and tap water.

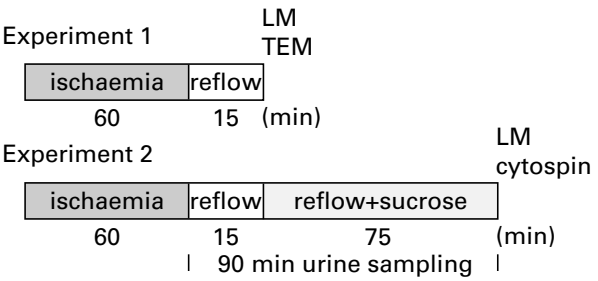

Figure 1. The procedures of experiments 1 and 2. Heparin or saline were given 5 min before clamping. Warfarin was given 24 h before clamping. LM, light microscopy; TEM, Transmission electron microscopy; cytospin, morphology on centrifuged urine samples.

Pharmacological agents

Thiopental. (Inactin, Byk-Gulden) 120 mg per kg body weight (BW) i.p. (expt 1).

Ketamin. (Ketalar, Parke-Davis) 50 mg/ml, and *Xylazin* (Rompun) 10 mg/ml were mixed 4:1 and injected i.p. in a dose of 2 ml/kg BW. Booster doses were given ad libitum during the experiment to maintain anaesthesia (expt 2).

Heparin. (Kabi-Vitrum, Stockholm), was diluted in 0.9% NaCl to 1.25 mg/ml and given i.v. in a dose of 2.5 mg/kg BW 5 min before the renal artery occlusion. This corresponds to a dose of 400 IU/kg BW.

Warfarin. (Waran, Nycomed, Sweden) was injected i.p. 24 h before the experiment in a dose of 6.3 mg/kg BW.

Sucrose. 12% in 0.6% saline was infused in a dose of 8 ml/h (expt 2).

Antibodies. FITC-conjugated, specific goat antirat fibrinogen antibody was purchased from ICN Pharmaceuticals, Aurora, Ohio, USA for direct immunofluorescence microscopy (expt 1). Peroxidase-conjugated specific goat antirat albumin and antirat fibrinogen antibodies were purchased from Nordic laboratories, Stockholm, Sweden (expt 2).

Experimental procedure

A schematic description of the procedure of expts 1 and 2 is provided in Figure 1.

Experiment 1. Anaesthesia was induced by an i.p. injection of thiopental. The animals were placed on a heating pad to keep the temperature at 37 – 38 °C and were tracheotomized. The abdomen was opened by a midline incision. The fatty tissue (but not the fibrous capsule) around both kidneys was removed to exclude an

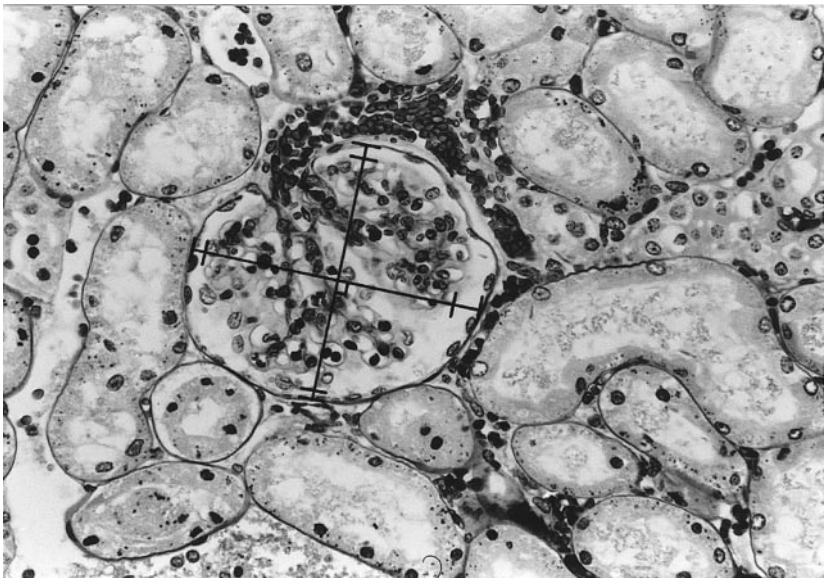

Figure 2. A glomerulus from a section stained with PASM, original magnification 320x. The technique employed for measurements of the tuft and capsule is illustrated. Glomeruli with a tuft diameter falling short of 67 μm and/or without a visible vascular pole were excluded to avoid skewness due to peripheral sectioning.

aberrant blood supply, and the pedicles were freed from surrounding tissue. The right renal pedicle was ligated and the kidney removed. The left renal artery was simultaneously clamped with a lined artery forceps, and the abdomen closed. After 60 min, blood flow was reestablished by removing the forceps. Prior to declamping, the appearance of the kidney was noted to ensure that the entire artery had been occluded and the time for complete refilling upon declamping was recorded. After 15 min of reflow the renal pedicle was reclamped and the left kidney removed. Both kidneys were cleaned with filter paper and weighed. Pieces averaging $1\times1\times2$ mm were cut from the different cortical and medullary zones and immediately fixed in glutaraldehyde. For immunofluorescence microscopy, radial sections containing all zones were snap-frozen in isopenthane. Thin, horizontal slices were fixed for light microscopy in Stieve's solution for 2 h and then transferred to formalin. Remaining material was sliced and directly formalin-fixed. The gross appearance of the kidneys was noted, and the macroscopic erythrostasis was graded from 0–3.

Mean arterial blood pressure was repeatedly measured before, during, and after RAO using a pressure transducer, amplifier and a cardiostat printer (Siemens-Elema). APTT (Activated, Partial Thromboplastin Time), and SPA (Serum Prothrombin Activity), were determined by commercial kits (Diagnostica Stago) using citrate plasma 1:9 from blood collected by aortic puncture at the end of the experiment.

Light microscopy examination. In sections, stained with periodic acid silver methenamine (PASM), the diameters of the glomerular tuft and Bowman's capsule (Figure 2) were measured by two right angle diameters, from which the means were calculated. Glomeruli from all kidneys were selected for measurements given a) the presence of a clearly visible vascular pole and b) a diameter of the tuft exceeding 67 μm (corresponding to an easily observed mark on the arbitrary scale of the eyepiece) in order to reduce skewness due to peripheral sections. Glomeruli with a large urinary pole visible were excluded. The examinations were performed without knowledge of treatment. The difference between the diameter of Bowman's capsule and the glomerular tuft was calculated, and used as a measure of Bowman's space. Formalin-fixed sections were stained with haematoxylin-eosin or PhosphoTungstic Acid Hematoxylin (PTAH).

Direct immunofluorescence microscopy examination. Small kidney slices from all rats were snap-frozen in isopentane-CO_2. Six-μm-thick cryostat sections were air dried, washed in a PBS-buffer (according to Dulbecco), pH 7.6, fixed in 95% ethanol for 10 min, and washed again in PBS-buffer. The sections were then incubated with FITC-conjugated goat IgG fraction to rat fibrinogen (Cappel, ICN Pharmaceuticals, Inc., Aurora, Ohio, USA) for 30 min in ambient temperature, diluted 1:40 with PBS-buffer. Glycerol-PBS-mounted slides were examined in a Zeiss phase contrast epi-illumination fluorescence microscope, equipped with a C35 camera. The localization of the fibrin(ogen) deposits in the sections could be precisely controlled in phase contrast.

Transmission electron microscopy examination. Small tissue samples from 3 rats in each group were taken with a minimum of trauma. They were immediately immersion fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for 2 h at 4 °C, washed in the same buffer and postfixed in 1% osmium tetroxide in distilled water for 1 h. After dehydration in graded ethanol, the samples were embedded in Epon. Semithin sections (1 μm) were stained with toluidine blue and examined to select areas with well preserved glomeruli. Ultrathin sections (70 nm) were cut from the trimmed blocks and poststained with uranyl acetate (5% in ethanol) and lead citrate (4.4% in distilled water).

Four to eight sections from each kidney, containing 4-6 glomeruli in the ultrathin sections were examined for estimation of podocyte foot process length, separating them in those >1 μm and those <1 μm. The length of 1 μm of a well defined structure in an ultrathin section was carefully measured at a magnification of 5000 times using the measurement function of the electron microscope (JEOL 1200 EX/II) after DEF-X calibration. The same image with the measured structure was captured by the TV camera in the microscope and displayed on the monitor. A semirigid cord with a length which compared exactly to 1 μm was then tailored along the basement membrane as displayed on the monitor. This procedure thus allowed for an easy assessment of the foot process length, separating them in the two fractions: >1 μm or less. This limit has previously been used in SEM studies by Solez *et al* (1981). All measurements were performed at the same time to avoid the effect of different operating conditions.

A total of 638 podocyte foot processes were measured from 3 rats given saline, 822 from 3 rats in the heparin group, 836 from 3 rats in the warfarin group and 961 from 3 rats in the control group. The glomerular basement membrane thickness was controlled during the measurements to avoid choosing foot processes along tangentially cut capillary walls. All measurements were performed blindly. Other characteristics of the foot processes, such as the shape, 'fusion pictures' and detachment were also recorded.

Experiment 2. In separate rats (3 animals in each group), the same procedure was used as in exp 1, but Ketalar-Rompun-anaesthesia was employed, and sucrose was infused to induce osmotic diuresis. The left ureter was catheterized, and urine collected for 90 min in tubes containing 40IU heparin and 2 mg AMCA (tranexamic acid). The urine samples were then centrifuged (3000 r.p.m. for 5 min) and the pellets, totalling approximately 250 μl each, were divided into four portions and subjected to cytospin centrifugation for 7 min at 1000 r.p.m. (Cytospin 2, Shandon). The specimens were stained with peroxidase-conjugated goat antirat albumin and goat antirat fibrinogen (1:100, Nordic laboratories) or Giemsa stain. One of the four corresponding specimens was treated with normal rabbit serum, and specimens were prepared from urine collected prior to ischaemia as an additional control group. Positive controls were prepared using 1 ml rat plasma, to which 0.2 ml of thrombin solution (300NIH/ml) was added, and part of the fibrin clot was homogenized on a slide. The number of cells of different kinds were semiquantitively scored in each specimen, stained with Giemsa. The possible presence of fibrin was examined by scanning the specimens at 400 $\times$ magnification using the immunostaining technique previously described.

From all rats, small pieces were collected from the kidneys at the end of the experiment, as a control for the possible morphological changes due to the induced diuresis. The specimens were immersion-fixed in 4% buffered formalin, dehydrated, paraffin-embedded and examined by conventional light microscopy.

Statistical methods:

The raw data values of Bowman's space were used for calculation of the mean for the individual rats, and the means of the different groups were based on the means of the individual rats. Thus the raw data values were not pooled. The same applies to the calculations on the podocyte values.

Shapiro-Wilks *W*-test was employed for analysis of normal distribution. Differences between groups were tested by analysis of variance (ANOVA), and if a significant *F*-test was obtained, Tukeys HSD test (podocyte values) or Tukeys HSD-Spjotvoll & Stoline test (Bowman's space values) were used. A p-value <0.05 was considered significant.

Results

All kidneys subjected to ischaemia became swollen and displayed the typical zone of congestion (erythrostasis) in the outer medulla. These changes were, however, less severe in rats pretreted with heparin or warfarin. Distinct, fibrin positive material was seen in the tubular lumina of saline-pretreated ischaemic animals, but not in anticoagulated rats or in the right, untreated kidneys (Figure 3a-d). In all ischaemic animals, a certain degree of tubular cell detachment was present.

The width of Bowman's space, as measured by light

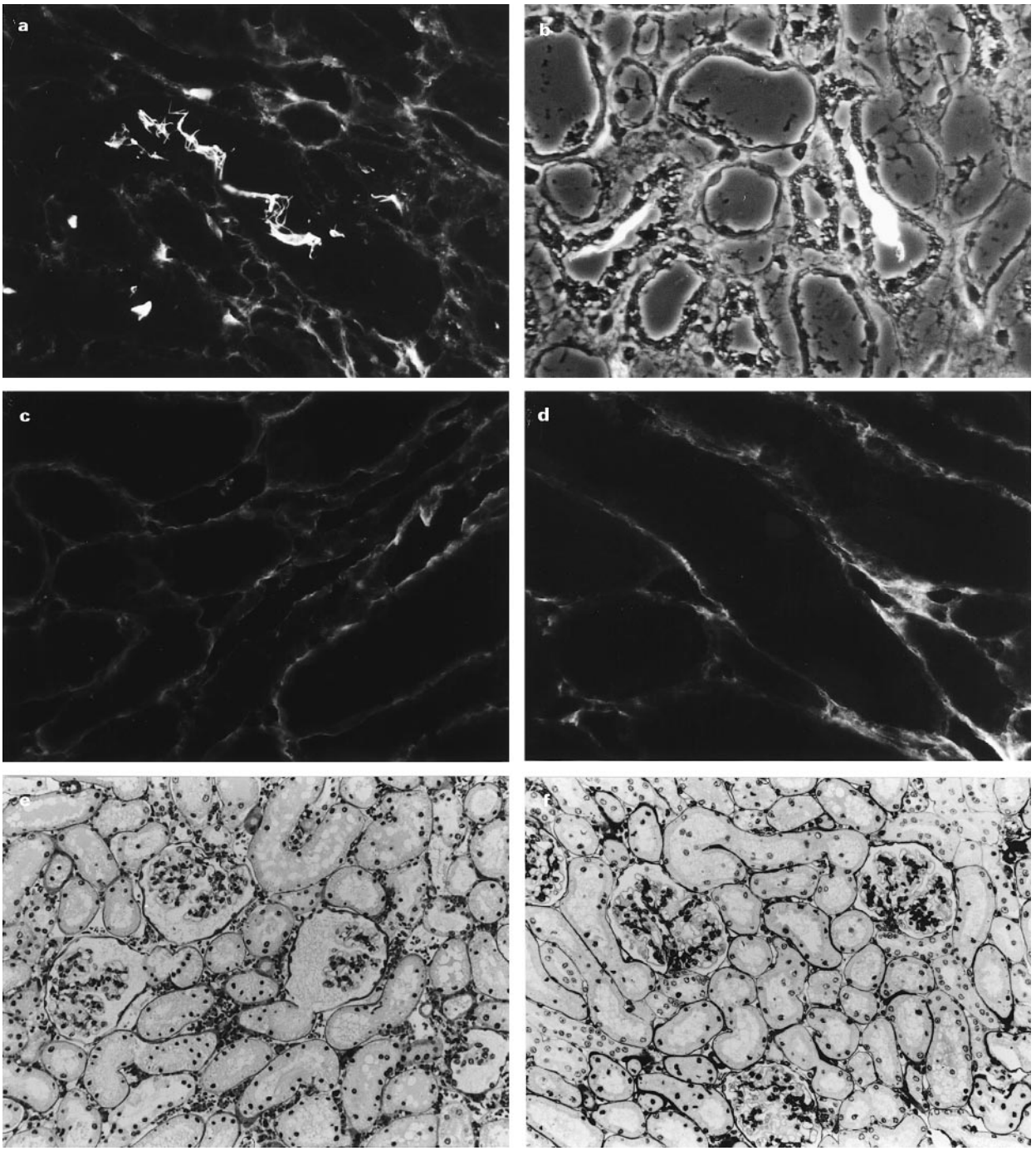

Figure 3. a, Ischaemic kidney from a saline + RAO rat showing distinct immune deposits with a filamentous texture, localized to distal and/or collecting tubules. b, Section from the same kidney showing distinct and dense fibrin deposits in distal tubules (immunofluorescence and phase contrast). No immune reactivity in the tubules could be detected in heparin + RAO (not shown) or (c) warfarin + RAO kidneys, or (d) in the right, control kidneys. None of the sections examined contained any substantial amount of immune reactive material in the blood vessels. a-d: Direct immunofluorescence with goat antirat fibrinogen. Original magnification 160x. e&f, Typical appearance of glomeruli from (e) saline + RAO and (f) heparin + RAO kidneys, original magnification 100x. PASM stain. Kidneys subjected to RAO + warfarin as well as control kidneys displayed similar glomerular appearance as RAO + heparin.

Treatment	N	Tuft	Capsule	Space
RAO + saline	6 (159)	92.0 ± 6.2	103.1 ± 8.0	11.1 ± 3.7[b]
RAO + warfarin	4 (112)	86.2 ± 6.5	90.9 ± 7.9	4.7 ± 1.4[c]
RAO + heparin	6 (167)	86.2 ± 3.3	92.9 ± 3.4[a]	6.7 ± 1.2[d]
Control	5 (157)	93.0 ± 3.6	98.4 ± 4.0	5.4 ± 0.8

All values are mean ± standard deviation. N, number of animals (glomeruli) examined; [a] $P = 0.046$ versus RAO + saline [b] $P = 0.0050$ versus control [c] $P = 0.0047$ versus RAO + saline [d] $P = 0.017$ versus RAO + saline.

Table 1. Diameters of glomerular tuft and Bowman's capsule, and width of Bowman's space (μm) for the different experimental groups

microscopy, was significantly increased in saline-pretreated ischaemic animals, whereas heparin and warfarin pretreated ischaemic animls did not differ from untreated, control kidneys (Table 1, Figure 3e,f). The distribution of the width of Bowman′s space (pooled data) was similar to controls in anticoagulated rats, whereas less than half of the glomeruli from kidneys subjected to RAO + saline were within the normal range (Figure 4).

The visceral epithelial foot processes along the glomerular basement membrane were studied by transmission electron microscopy (Figure 5). A total of 3257 foot processes were measured in the control and experimental animals, separating those below 1 μm, and those equal to or above 1 μm in length. Figure 6 thus shows the percentage of podocyte foot processes exceeding 1 μm for the different experimental groups. The percentages

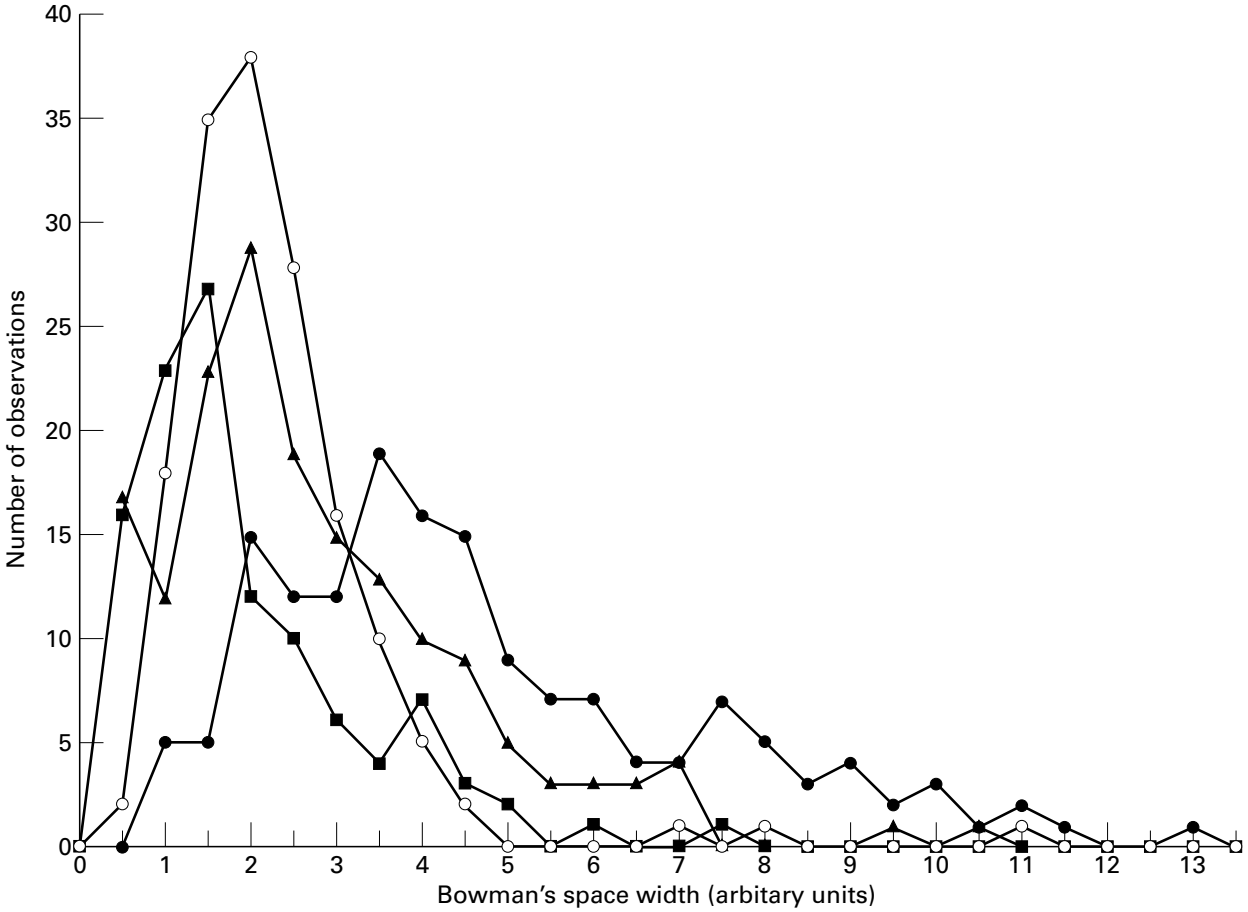

Figure 4. The frequency distribution of Bowman's space width, illustrated separately for each experimental group. ● RAO +saline; ■ RAO + warfarin; ▲ RAO + heparin; ○ Control. Note that the width is expressed in arbitrary units; μms are achieved by multiplying by 2.40.

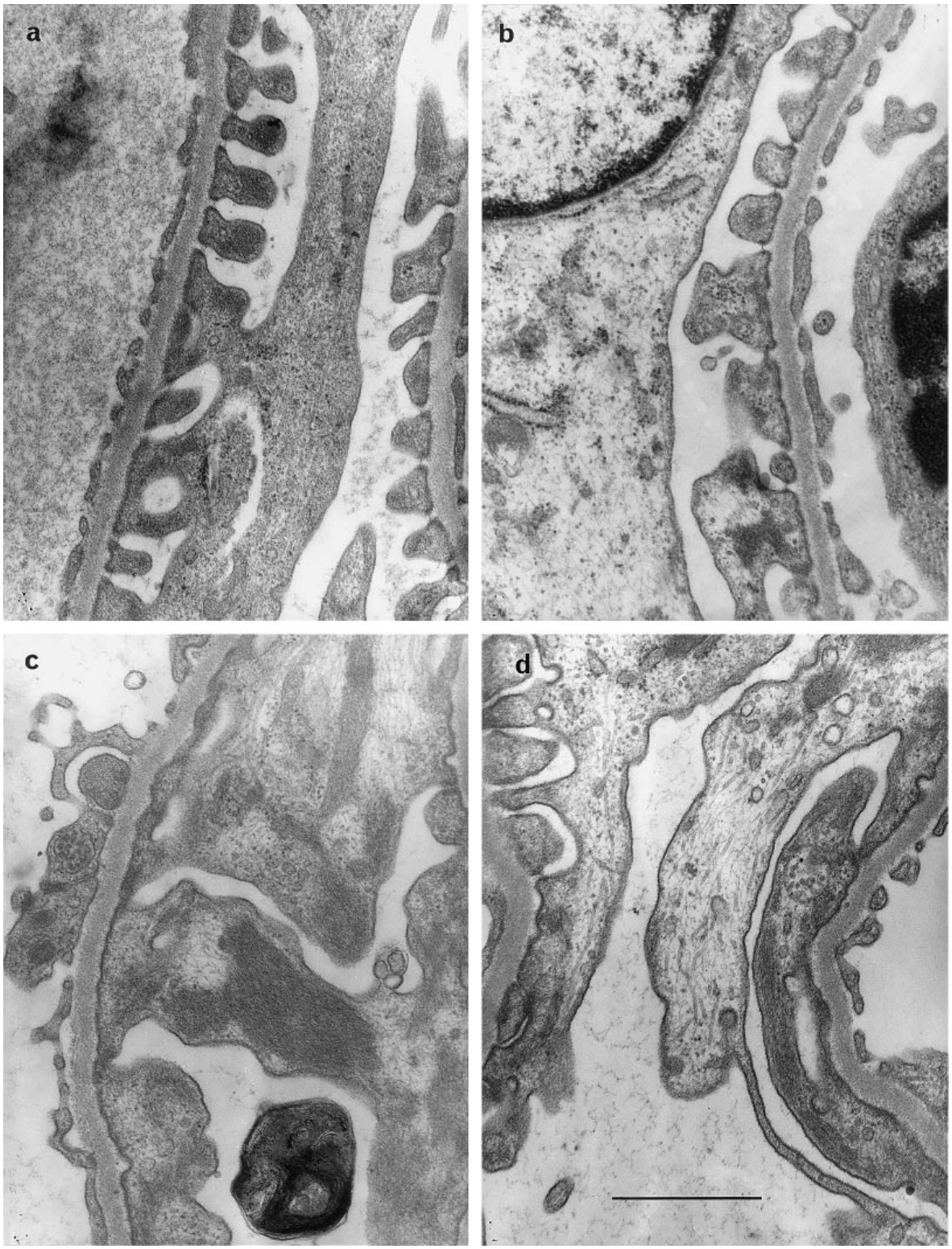

Figure 5. Portions of kidney glomerular loops from control and experimental rats. TEM, bar = 1 μm. a, control (right kidney); b, warfarin + RAO; c, heparin + RAO; d, saline + RAO. Note the extended and flattened visceral epithelial foot processes along the basement membrane of ischemic animals as opposed to the regular shape of normal foot processes (a). The severity of these changes varied from area to area, i.e. appearances as exemplified in b-d were seen in sections from all ischemic animals.

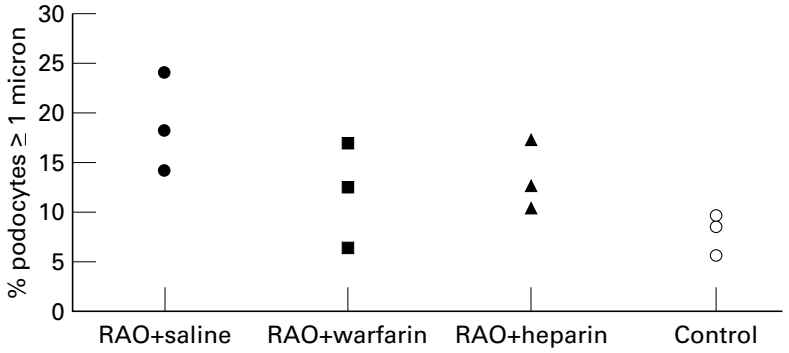

Figure 6. Proportion of podocytes exceeding 1 μm. ● RAO +saline; ■ RAO + warfarin; ▲ RAO + heparin; ○ Control. The percentages for the individual rats in each group are displayed.

did not differ significantly between groups. However, in the ischaemic animals, morphological changes were recorded, particularly flattening, even among podocytes falling short of 1 μm. Podocytes with extreme length and flattened shape were seen in all ischaemic animals. The frequency of such severely damaged foot processes was somewhat lower in RAO + warfarin rats, however, still easily distiguished from controls. Detachment of podocytes could not accurately be measured in a standardized fashion, but was considered to be equal in frequency in all ischaemic animals.

In three animals from each group, glomerular morphometry was carried out both by TEM and light microscopy (Figure 7). In the saline-pretreated ischaemic rats, the increase in number of podocyte exceeding 1 μm was paralleled by a marked increase in Bowman′s space width. The anticoagulated animals also showed severe podocyte changes, but no dilatation of Bowman′s space.

The erythrocyte volume fraction (EVF) varied between 46 and 53%, and did not differ between the experimental groups. Mean arterial blood pressures (MAP), averaging 115 + 9.5 mmHg, were also equal between groups. The weight increase (left/right kidney ratio) averaged 1.30 + 0.11, 1.12 + 0.09 and 1.16 + 0.05 for RAO + saline, RAO + warfarin and RAO + heparin, respectively. No correlation could be found between EVF or MAP and weight increase, time for macroscopic refilling, Bowman′s space width, or percentage broad podocytes.

The time for complete macroscopic refilling upon declamping averaged 27 s and did not differ significantly between the groups. APTT exceeded 5 min in all rats pretreated with heparin. SPA exceeded 5 min in all rats pretreated with warfarin. In saline-pretreated rats, APTT and SPA ranged 23-32 s and 25-37 s, respectively.

In cytospun specimens, immunological analysis failed to demonstrate recognizable fibrin. The number of cells varied greatly between different rats, and there were no differences in this regard between groups. In most specimens, the epithelial cells were gathered into aggregates of varying size and shape, but no well-formed casts were encountered. The number of erythrocytes was generally low, and only macroscopically faintly visible in one of the samples (due to bleeding from a ureteral vessel).

The light microscopic examination of the corresponding ischaemic kidneys revealed a marked distention of virtually all tubular segments in all animals, and the tubuli were fluid-rich, but devoid of identifiable cellular material (Figure 8).

Discussion

The experimental post-ischaemic model of ARF has been extensively used for evaluation of different pathogenetic mechanisms. Popular theories include the participation of oxygen free radicals, intracellular calcium accumulation, endothelin, atrial natriuretic factor (ANF), and most recently NO and adhesion molecules. Regardless of molecular mediators, medullary congestion and tubular obstruction are widely accepted as important effects of the ischaemic insult in this experimental model (Burke *et al* 1983; Wolgast *et al* 1991, 1992; Goligorski & DiBona 1993), and possibly also in some forms of human ARF (Faraggiana & Grishman 1983; Chintala *et al* 1993). An easily recognizable consequence of severe tubular obstruction is a generalized renal oedema. In previous work, we have shown that anticoagulation significantly reduces renal oedema and protein accumulation in the post-ischaemic kidney (Druid & Rammer 1989, 1992).

In the present study, we assessed the importance of coagulation in the formation of tubular blockades. The results showed that both heparin and warfarin markedly reduced the morphological signs of tubular obstruction. However, neither heparin nor warfarin provided any protection against the morphological signs of increased glomerular permeability, as judged by the podocyte

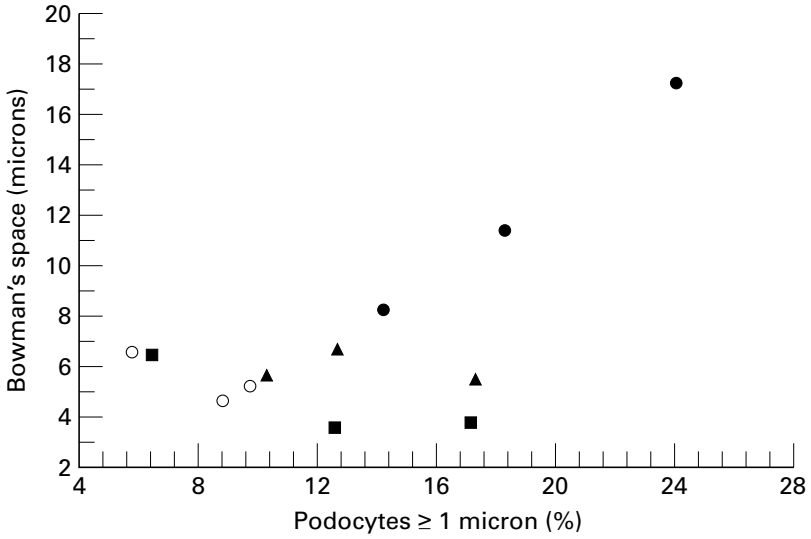

Figure 7. The illustration displays a scatterplot of the proportion of broad podocytes *vs.* Bowman′s space width. ● RAO +saline; ■ RAO + warfarin; ▲ RAO + heparin; ○ Control. Note the right-shift for the anticoagulated animals, suggesting severe damage to the podocytes without corresponding dilatation of Bowman′s space.

changes. Thus, the effect of anticoagulation on renal oedema and protein accumulation seems to be due to the prevention of fibrin formation and hence a reduction in tubular obstruction rather than protection against increased glomerular permeability.

In order to establish whether fibrin aggregates could be recovered from the urine after only a short period of reflow, urine samples were collected after sucrose infusion for *in vivo* 'flushing' of the kidneys (expt 2). The sucrose infusion was not started until 15 min after declamping, in order not to interfere with possible fibrin precipitation. In the cytospun samples, aggregates of tubular cells and cellular debris were seen, but without typical cast shape. Contrary to our expectations, no distinct fibrin aggregates were found in the urine samples, despite the occurrence of intratubular fibrin deposits in previous (Eneström *et al* 1988; Druid & Rammer 1989, 1992) and present (expt 1, Figure 3a,b) studies. This may be explained by fibrinolysis, which is supported by the detection of large amounts of radio-labelled, noncoagulable, fibrin products in the urine in the early post-ischaemic period (unpublished data). Further, despite the use of sensitive, direct-conjugated specific goat-antirat-fibrinogen antibodies, the addition of AMCA to the urine sample tubes, and the use of chrome-gelatine slides, smaller fibrin split products, and soluble

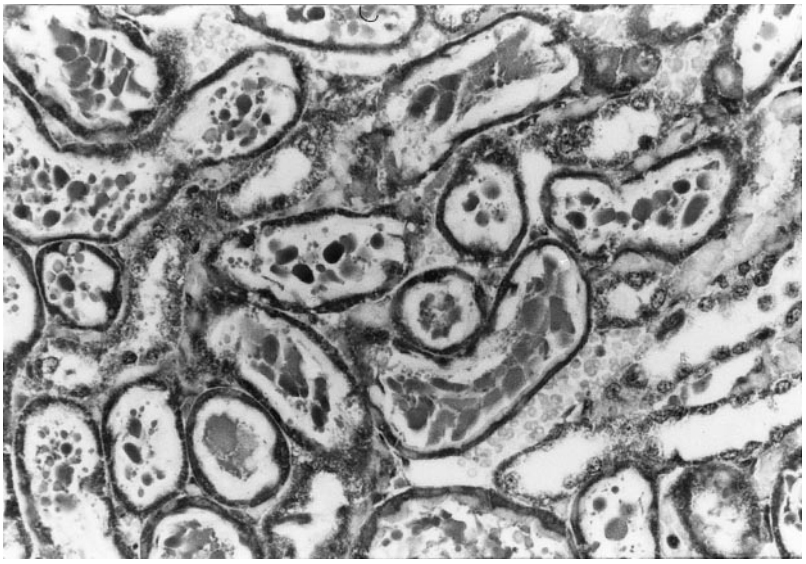

Figure 8. A mixture of proximal and distal tubules from the corticomedullary junction. The specimen is collected from a kidney subjected to 60 min of ischemia and 90 min of reflow (with sucrose infusion). The distended tubuli are filled with amorphous material, and the tubular cells generally flattened.

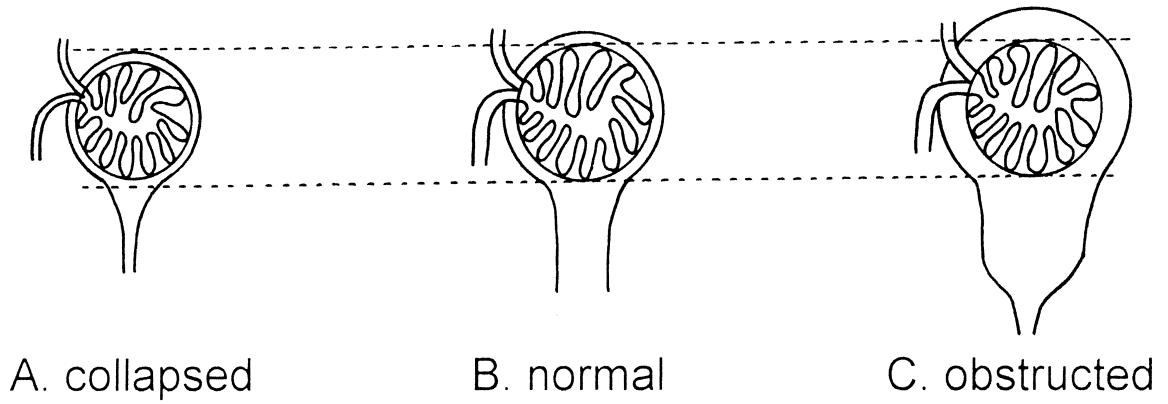

Figure 9. Schematic description of the different categories of nephrons encountered in all ischemic animals. A, collapsed type; B, normal, presumably filtering type; C, obstructed type. Types A and C were virtually absent in controls. Type C was seen in a substantial number in RAO + saline, but only occasionally in RAO + heparin and RAO + warfarin. Type A were equal in number in all ischaemic groups. Note that the tufts were not found to be compressed in the obstructed nephrons.

fibrin mono- and oligomers, might not be likely to stay in place during the indispensible washing steps of the preparation. Also, back-leak of filtrate may include smaller fibrin products, which thus could be eliminated by the lymph or the peritubular capillaries.

The normalization of the Bowman′s space in anticoagulated ischaemic rats could theoretically be explained by a reduction of renal blood flow and decreased filtration pressure, due to blood loss in these animals. However, all small bleedings were carefully monitored, and the MAP did not differ between the experimental groups. Further, any possible contributory beneficial effect on oedema and medullary congestion by lowered EVF (Wolgast *et al* 1992) could be excluded by identical EVF values of the animals in the different groups.

There seems to be a general agreement that increased macromolecular permeability of the glomerular barrier ensues in the ischaemic ARF model (Tanner & Sophasan 1976; Vetterlein 1986; Bratell 1990; Hellberg 1990; Chintala *et al* 1993), but the morphological counterpart in the glomerular barrier has been difficult to identify. However, most electron microscopic studies on glomerular permeability indicate that swelling, flattening and detachment of podocyte foot processes are the changes which correlate best to proteinuric conditions (Solez *et al* 1981; Faraggiana & Grishman 1983; Whiteside *et al* 1993; Ronco *et al* 1994). These changes are fairly easy to recognize for an experienced observer, but less simple to objectively quantify. In most morphometric studies on podocyte changes in conditions with increased permeability, SEM has been employed. However, by this method, the glomerular basement membrane cannot be studied and the occurrence of podocyte foot process detachment not be assessed. Instead, we decided to measure, by TEM, the proportion of foot processes exceeding 1 μm, well aware of the limitations of a single marker. Therefore, in the final interpretation of the TEM examinations we also took into account a number of other factors, which were recorded in a less standardized fashion, such as shape, maximal length, detachment pictures, and more. One striking feature of virtually all ischaemic foot processes was the loss of a typical waist. This character, like the occurrence of detatchment pictures, was, however, difficult to objectively assess. Taken together, the TEM pictures of the glomerular changes were grossly the same in all ischaemic kidneys, and markedly different from untreated kidneys.

Effort was put on making the light microscopic glomerular measurements as accurate and representative as possible. Although computerized image analysers have been increasingly used in recent years, manual measurement of glomeruli still seems to be more reliable (Romano *et al* 1996). The necessity of excluding too small glomeruli is easily realized by looking at Figure 3(e). Yet, a considerable variation in diameter of tuft and capsule was found. In ischaemic, saline-pretreated kidneys, a substantial number of the glomeruli showed very dilated capsules, whereas the tufts were not compressed. This is in contrast to the result obtained by Bayati *et al* (1990). After 45 min of ischaemia and 20 min of reflow, they found both a compression of the tuft and an enlargement of the capsule. However, this discrepancy is most likely due to the morphometric techniques employed.

Measurements of tubular pressures of post-ischaemic kidneys have revealed three types of nephrons;

collapsed, normal, and obstructed (Karlberg *et al* 1982a). In the present study, the majority of small tufts encountered (in all treatment groups) were found in glomeruli with small capsule diameter, consistent with collapsed nephrons, whereas glomeruli with large capsules did not show obvious signs of tuft compression. The glomeruli thus seem to fall into the morphological categories depicted in Figure 9. The tendency towards smaller tuft diameters in ischaemic, anticoagulated kidneys than in controls may therefore be explained by absence of obstructed nephrons (Figure 9A) and presence of collapsed nephrons (Figure 9B). The occurrence of the latter was suggested by Karlberg *et al* 1982a) to be due to afferent arteriolar constriction. According to the present study, anticoagulation did not affect this phenomenon.

In recent publications, the effect of adhesion molecules for both neutrofil adhesion in the renal vessels and for cellular aggregation in the tubuli has been studied (Goligorski & Di Bona 1993; Noiri *et al* 1994; Kelly *et al* 1994). Hence, Noiri *et al* (1994) demonstrated effectiveness of cyclic RDG peptides in ameliorating ischaemic renal failure, presumably by the prevention of tubular blockades, which they suggested was due to an inhibition of cell-cell adhesion among exfoliated tubular cells. In the present study, a similar effect was found by anticoagulant pretreatment, probably due to preventing filtered fibrinogen converting to a fibrin network.

Acknowledgements

This study was supported by grants from the National Board of Forensic Medicine, Stockholm, Sweden and from the Forensic Science Center, Linköping, Sweden.

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