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Nephron function in the early phase of ischemic renal failure. Significance of erythrocyte trapping

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Nephron function in the early phase of ischemic renal failure. Significance of erythrocyte trapping. Trapping of red blood cells (RBCs) in renal medulla vasculature in postischemic acute renal failure (ARF) was found to depend upon the length of the ischemic period. Thus trapping occurred after 45 minutes but not 25 minutes of ischemia. By prior hemodilution to a hematocrit (hct) of 30%, RBC trapping after 45 minutes of ischemia could be completely prevented. Likewise hemoconcentration (hct = 60%) before 25 minutes of ischemia resulted in extensive RBC trapping. By increasing or decreasing the hct, the contribution of RBC trapping to the functional defects and decrease in renal blood flow that follows minor (25 min) and more substantial (45 min) ischemia was investigated. Renal blood flow (RBF) was measured by microspheres, and vascular and tubular pressure by the micropuncture technique. Glomerular filtration rate (GFR) was estimated from inulin clearance, and tubular function from urine osmolality and sodium and potassium excretion. It was found that postischemic RBF was not correlated to RBC trapping but depended on the length of ischemia. After both 25 and 45 minutes of ischemia tubular obstructions occurred in the proximal tubules and/or loops of Henle, causing an increase in proximal tubular pressure. These obstructions were dependent on the length of ischemia but not on RBC trapping. After hemoconcentration and 25 minutes of ischemia there was an increment in distal tubular pressure, indicating that abundant RBC trapping may contribute to an increase in tubular pressure by compression of medullary tubules and thereby reduce GFR. When the damage was more severe, other factors came into play and the contribution of RBC trapping to the decrease in GFR was minimal. Thus a beneficial effect of hemodilution on 45 minutes of ischemia could not be established. Concerning tubular function, isosthenuria and potassium and sodium excretion were not dependent on RBC trapping. It is concluded that RBC trapping in the early phase of ARF has minimal influence on GFR and tubular function. Thus, standard markers of kidney function do not reflect the degree of RBC trapping.

Trapping of red blood cells (RBCs) in the vasculature of the renal medulla is a characteristic feature in the early phase of ischemic acute renal failure (ARF). Neither the cause nor the consequence of this phenomenon is completely understood. The degree of RBC trapping, however, seems to reflect the severity of the ischemic injury, since it increases with the duration of ischemia in a dose-dependent manner [1, 2].

The proposed mechanisms underlying RBC trapping include: 1) obstruction of medullary venous outflow as a consequence of dilation of tubules in the outer medulla [3], 2) cell swelling [4, 5], 3) primary plugging of capillaries by leukocytes [6, 7], and 4) increased capillary permeability leading to local hemoconcentration and increased viscous resistance [1, 8–10].

Irrespective of its cause it has been proposed that the RBC trapping may be the underlying reason for the observed incomplete return of blood flow in the renal medulla [11–14]. The isosthenuria and reduced secretion of potassium, both common features in postischemic ARF, have been considered to indicate functional impairment of the renal medulla [15] and to be results of the RBC trapping [13, 16].

A direct correlation between RBC trapping and a decrease in the glomerular filtration rate (GFR) has been suggested [5, 13, 17]. The majority of authors, however, have focused on the renal cortex in attempts to explain the functional deficit in the acute stage of renal failure. The large decrease in GFR, for example, has been ascribed to a reduction of renal blood flow (RBF) [18], tubular obstructions [19, 20], and tubular backleakage of filtered substances [21].

A contribution of the RBC trapping to long-term kidney damage has, however, recently been proposed [1].

The aim of the present study was to explore the mechanisms underlying RBC trapping and to investigate the influence of this trapping on RBF, GFR, and parameters of tubular function in the early phase of postischemic ARF. As the systemic hematocrit was found to have a profound impact on the RBC trapping, hemodilution and hemoconcentration were induced to diminish and enhance the RBC trapping, respectively. The results suggest that vascular factors are of great importance in the origin of the RBC trapping, while tubular factors are not. Contradictory to previous reports [5, 13, 17], neither the decreases in RBF and GFR, nor the impairment in tubular function seemed to be attributable to the RBC trapping to a major extent.

Methods

Animals

The experiments were performed on 50 male Sprague-Dawley rats (Anticimex, Solna, Sweden) weighing 240 to 365 g. The animals had free access to tap water and standard rat chow (R3®, Evos, Södertälje, Sweden). Anesthesia was induced by intraperitoneal injection of Inactin® (Byk Gulden, Konstanz, FRG) in a dose of 120 mg · kg^{-1} body weight. The animals were placed on a servo-controlled heating pad to maintain the body temperature at 37.5°C. Catheters were inserted in the left femoral artery and vein; the former for continuous monitoring of blood pressure and withdrawal of blood samples and the

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latter for administration of tracer substances and continuous infusion of Ringer solution containing 120 mM NaCl, 25 mM $NaHCO_3$, 2.5 mM KCl and 0.75 mM $CaCl_2$ at a rate of 5 ml · kg^{-1} body wt. The left kidney was exposed through a subcostal incision and immobilized in a lucite cup. For sampling of urine a catheter was placed in the left ureter.

Alteration of hematocrit

A low hematocrit (hct) was induced by infusion of 5 ml of a 5% albumin-Ringer solution (human albumin, Kabi, Solna, Sweden) during a 10 minute period and parallel withdrawal of an equal volume of whole blood through the femoral artery. A high hct was produced by transfusion of 5 ml of red cell concentrate (80% red blood cells in saline) during a 10 minute period. For this purpose blood was collected from a donor animal in acid-citrate-dextrose solution. The cells were sedimented by centrifugation and the plasma and leukocyte-rich "buffy coat" were removed. To prevent transfusion of physiologically active substances released from the donor animal during bleeding, the red cells were washed once in saline and resuspended to a relative packed cell volume of about 80%. It should be noted that the hemoconcentration procedure was not carried out as an exchange transfusion. The reason for this was that to obtain an increment in hct to about 60%, exchange of about 15 ml or more of RBC concentrate for whole blood would be required (compare Mayers et al [22]) and that such a large exchange transfusion may have a considerable effect on other blood-borne factors.

Measurement of RBC trapping

The fractional erythrocyte volume in the different parts of the kidney was measured from the distribution of ^{51}Cr-labelled erythrocytes [23]. For this, 5 ml of blood was withdrawn from a donor animal into an acid-citrate-dextrose solution. The blood cells were then separated by centrifugation and the plasma fraction and the leukocyte-rich "buffy coat" were removed. About 100 μCi of sodium-^{51}chromate (Amersham International plc., Amersham, UK) was added to the red cell suspension and incubated at room temperature for one hour. The cells were then washed three times in saline. About 0.2 ml of ^{51}Cr-labelled red cell suspension (equivalent to 5 μCi) was injected intravenously before induction of ischemia. After completion of the functional studies both kidneys were removed, together with a 100 μl reference blood sample.

Using a razor blade, the kidneys were microdissected into cortex, outer and inner stripe of outer medulla, and inner medulla, as described elsewhere [23]. The pieces were weighed and then analyzed for their specific activity (CPM) in a gamma spectrophotometer. The number of red blood cells in tissue was calculated by the formula:

$$(CPM_{tissue}/CPM_{blood}) \cdot (\mu l_{RBCs}/mg_{tissue}) \cdot 100$$

This gave the number of RBCs in μl · 100 mg^{-1} tissue and, assuming a tissue density of 1, the fractional RBC volume.

Nephron function and intrarenal pressure

The hydrostatic pressures in the superficial microvasculature and tubules were measured with sharpened glass capillaries attached to a servo-nulling device as described by Wiederhielm et al [24]. The glomerular capillary pressure was measured by the indirect stop-flow technique according to the method of Gertz et al [25]: An early proximal tubular segment was punctured and castor oil was injected until filtration ceased. The stop-flow pressure was then measured through a second glass capillary inserted proximal to the oil blockade. The glomerular capillary pressure was calculated by adding the stop-flow pressure to the colloid osmotic pressure of systemic plasma; the latter was calculated from the protein concentration of systemic plasma, using the formula proposed by Landis and Pappenheimer [26], assuming an albumin/globulin ratio of 1.1 [27] in all animals.

The single nephron filtration rate (SNGFR) and whole kidney GFR were measured from the clearance of tritiated inulin. For this purpose inulin was injected intravenously at a constant rate of 50 μCi · hr^{-1}, after a single injection of 50 μCi. During ischemia the infusion rate was reduced to half. For sampling of tubular fluid a column of castor oil with a length of about five tubular diameters was injected into a proximal or distal tubular segment and this was followed by gentle suction for three minutes at a rate which kept the oil block in a fixed position. During the sampling the tubular pressure proximal to the sampling pipette was monitored continuously. The volume of the samples was measured from their length in constant-bore capillaries (Microcaps 0.5 μl, Drummond Scientific Company, Broomall, Pennsylvania, USA). For determination of plasma concentrations of inulin, blood samples were taken at regular intervals.

After ischemia, the tubules were categorized as "collapsed", "dilated" or "open" by observation of the transit of lissamine green dye injected into the tubules. The distal tubules were identified by the absence of a brush border and the higher concentration of lissamine dye injected into the corresponding proximal tubule.

Experimental scheme

After completion of the surgical procedure, the hct was either decreased by hemodilution, increased by hemoconcentration or left unaltered. One hour later the functional studies in the control period were started and proceeded for a further hour.

The left renal artery was then occluded for either 45 or 25 minutes, during which time the kidney was placed in its natural position with the abdomen closed in order to ensure a body temperature in the kidney. After ischemia the rats were allowed to recover for 60 minutes. Functional studies were then performed for about two hours, after which both kidneys were excised and analyzed for their erythrocyte content as described above.

Renal blood flow

RBF was measured by the microsphere technique in a separate series comprising 21 Sprague-Dawley rats (230 to 310 g body wt). The surgical preparations and transfusion procedures were identical to those described above. The microspheres were injected through a catheter inserted into the right carotid artery. After ischemia and 15 minutes of reperfusion, about 100,000 ^{141}Ce-labelled 15 μm NEN-TRAC® microspheres (Dupont, Heidelberg, FRG) were rapidly injected. At the same time blood was withdrawn from the femoral artery at a rate of 0.70 ml · min^{-1}, by a syringe pump. The kidneys were then

excised, weighed, and analyzed in a gamma spectrophotometer. RBF was calculated by multiplying the pump sampling rate by the ratio of the activity in the blood sample to that in the kidney specimen.

Analysis

Urine and plasma were analyzed for ^3H activity by liquid scintillation. Their sodium and potassium concentrations were measured by flame spectrophotometry, and the urine osmolality was determined by the freezing point depression method. The protein concentration in the plasma was measured by the method of Lowry et al [28]. The hematocrit and leukocyte count in the blood were determined by standard laboratory techniques.

Statistics

For statistical evaluation of the results of the micropuncture studies, the mean of the individual values in each animal was considered as one observation. To compare two groups, Student's *t*-test for independent samples was used. One factor ANOVA and the Dunnett's *t*-test were used for multiple comparison. Significance was assigned to a P value of less than 0.05.

Results

Hemodilution

The isovolemic hemodilution resulted in a decrease in hct from 47 ± 1 to 30 ± 1%. In contrast, the number of leukocytes decreased only slightly; the mononuclear leukocytes in each ml of blood decreased from 20 ± 3 · 10^6 to 16 ± 1 · 10^6 ($P < 0.05$) and the polymorphonuclear leukocytes from 19 ± 1 · 10^6 to 18 ± 2 · 10^6 (NS).

In animals with a normal hct, 45 min of ischemia resulted in substantial RBC trapping (Fig. 1) in the whole of the medulla, but this was most evident in the inner stripe of the outer medulla, where the red cell volume after two hours of reperfusion was 16.0 ± 1.4%, as against 2.3 ± 0.4% ($P < 0.001$) in the intact kidney. In the low-hct animals the RBC volumes in all areas of the medulla were not significantly higher than in the contralateral kidney. Thus hemodilution completely prevented the RBC trapping resulting from 45 minutes of ischemia. In both the low- and normal-hct groups the values in the cortex did not differ between postischemic and intact kidneys.

Compared with the contralateral kidneys, the wet weight of the ischemic kidneys increased by 27.4 ± 2.9% in the low-hct group, a value not different from that of 30.2 ± 2.1% in the normal-hct group.

Under control (pre-ischemic) conditions the most noteworthy change resulting from hemodilution was an increase in urine volume and sodium excretion. This occurred in spite of the fact that GFR and SNGFR remained unaffected (Tables 1 and 2), and seemed to have been due to a reduction of the reabsorption in the proximal part of the nephron, since the tubular fluid to plasma concentration ratio of inulin in proximal tubules was decreased (Table 2). The arterial pressure decreased after hemodilution, while the hydrostatic pressures in the superficial renal vasculature were not affected.

Both in animals with a normal and low hct, 45 minutes of ischemia resulted in severe functional impairment, with a GFR of only a few percent of the normal, isosthenuria, reduced

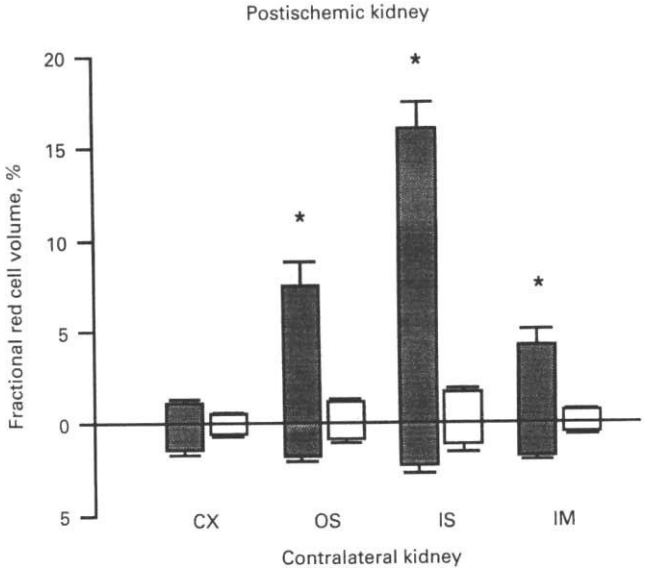

Fig. 1. *Distribution of red blood cells in the kidney after 45 minutes of ischemia and 2 hours of reperfusion.* The figures above the baseline represent the fractional volume of red blood cells in the cortex (CX), the outer stripe (OS) and inner stripe (IS) of the outer medulla, and the inner medulla (IM) in animals with a normal hematocrit of 47 ± 1% (filled bars) and a reduced hematocrit of 30 ± 1% (unfilled bars). The values under the baseline represent the contralateral kidney. Values are means ± 1 SE. (*) denotes a significant difference ($P < 0.05$) compared with the contralateral kidney.

potassium secretion and a urine sodium concentration not far from that in plasma. Although the GFR values were somewhat higher in the low-hct group, this difference was not statistically significant (Table 1).

After ischemia the kidneys appeared heterogeneous and in principal three different categories of tubules could be identified: 1) collapsed tubules with low pressure and no flow, 2) dilated tubules with high pressure and no flow, and 3) open tubules with flow. In this respect there was no apparent difference between the low and normal hct animals. In both groups most tubules were dilated without flow, some were open, and only a few were collapsed. The mean pressure in each category of tubules did not differ between the hemodilution and control group (Table 2).

The glomerular capillary pressures remained at the pre-ischemic levels, while the welling point and peritubular capillary pressure increased by about 4 mm Hg after ischemia in both groups (Table 3).

SNGFR in the open tubules was only slightly reduced, whereas that in dilated and collapsed tubules was virtually zero. However, when in the dilated tubules the pressure during sampling was deliberately reduced to normal free-flow pressure, SNGFR approached that in the open tubules (Table 2). This indicates that the glomerular ultrafiltration process per se remained intact in the dilated nephrons.

Hemoconcentration

Transfusion of 5 ml of red blood cell suspension resulted in an increase in hct from 47 ± 1% to 60 ± 1%. The concentration of polymorphonuclear leukocytes in the blood decreased at the

Table 1. Renal excretion parameters

	Body weight	V $\mu l \cdot min^{-1} \cdot 100\ g^{-1}$ body wt	U/P_{In}	C_{In} $ml \cdot min^{-1}\ 100\ g^{-1}$ body wt	U_{Osm} $mOsm \cdot kg^{-1}$	U_{Na}	U_K
						$mmol \cdot kg^{-1}$	
Pre-ischemia							
Normal hct	246 ± 7	1.4 ± 0.3	501 ± 60	0.50 ± 0.03	1848 ± 154	67 ± 11	384 ± 42
High hct	256 ± 18	6.6 ± 1.8[a]	146 ± 44[a]	0.48 ± 0.03	859 ± 196[a]	80 ± 22	123 ± 30[a]
Low hct	228 ± 4	6.0 ± 1.8[a]	176 ± 53[a]	0.52 ± 0.03	1037 ± 166[a]	148 ± 22[a]	200 ± 51[a]
25 Min ischemia							
Normal hct	265 ± 9	8.1 ± 1.7	37 ± 13	0.20 ± 0.02	461 ± 44	140 ± 8	109 ± 30
High hct	256 ± 18	3.7 ± 0.8[b]	22 ± 6	0.10 ± 0.03[b]	397 ± 34	146 ± 11	59 ± 11
45 Min ischemia							
Normal hct	230 ± 6	2.3 ± 0.9	21 ± 7	0.03 ± 0.01	387 ± 33	138 ± 6	32 ± 8
Low hct	228 ± 4	8.9 ± 2.0[b]	9 ± 2[b]	0.05 ± 0.01	363 ± 19	129 ± 3	25 ± 4

Abbreviations are: V, urine flow rate; U/P_{In}, urinary to plasma inulin concentration ratio; U_{Osm}, urine osmolality; U_{Na} and U_K, urinary sodium and potassium concentrations. Values are means ± 1 SE.

Significance level $P < 0.05$: [a] vs. normal-hematocrit (hct) group under pre-ischemic conditions and [b] vs. normal-hct group subjected to ischemia for an equal length of time.

Table 2. Tubular pressure and single nephron filtration rate

		Proximal tubules			Distal tubules		
	Tubule category	P_{tub} *mm Hg*	Tf/P_{In}	SNGFR $nl \cdot min^{-1} \cdot 100\ g^{-1}$ body wt	P_{tub} *mm Hg*	Tf/P_{In}	SNGFR $nl \cdot min^{-1} \cdot 100\ g^{-1}$ body wt
Pre-ischemia							
Normal hct	open	13.3 ± 0.4	1.78 ± 0.07	16.9 ± 0.70	6.4 ± 0.4	5.03 ± 0.31	15.9 ± 0.7
$N = 13$	(n = 47)				($N = 7$)		
High hct	open	13.2 ± 0.5	1.79 ± 0.08	16.7 ± 1.23	8.9 ± 1.0	5.56 ± 1.40	14.2 ± 1.1
$N = 9$	(n = 21)				($N = 5$)		
Low hct	open	15.1 ± 0.4[a]	1.43 ± 0.04[a]	18.6 ± 1.0	7.8 ± 0.4	3.30 ± 0.22	17.1 ± 0.8
$N = 8$	(n = 21)				($N = 8$)		
25 Min ischemia							
Normal hct	open	14.9 ± 0.45	1.45 ± 0.05	13.5 ± 1.05	6.9 ± 0.9	3.03 ± 0.27	10.8 ± 0.84
$N = 6$	(n = 14)				($N = 5$)		
	dilated	36.0 ± 1.9	1.60 ± 0.1	13.5 ± 1.70			
	(n = 5)						
High hct	open	30.8 ± 2.4[b]	1.55 ± 0.05	11.1 ± 0.6	19.2 ± 2.6[a]	4.53 ± 1.3	9.34 ± 1.2
$N = 9$	(n = 28)				($N = 14$)		
	dilated	48.0 ± 2.9[b]	1.93 ± 0.13	9.7 ± 1.0			
	(n = 18)						
45 Min ischemia							
Normal hct	open	20.0 ± 1.9	1.36 ± 0.14	10.4 ± 1.2	11.5 ± 1.0	3.03 ± 0.27	10.8 ± 0.84
$N = 7$	(n = 18)				($N = 13$)		
	dilated	41.5 ± 1.7	1.23 ± 0.04	9.8 ± 1.0			
	(n = 13)						
	collapsed	9.8 ± 1.0					
	(n = 5)						
Low hct	open	19.9 ± 2.5	1.04 ± 0.05[b]	12.8 ± 0.4	9.7 ± 0.6	4.43 ± 2.3	13.8 ± 1.5
$N = 8$	(n = 30)				($N = 17$)		
	dilated	40.3 ± 2.4	1.14 ± 0.38	13.4 ± 1.3			
	(n = 11)						
	collapsed	10.0 ± 1.0					
	(n = 5)						

Values are means ± 1 SE. Number of animals (N) and number of tubules (n) categorized from the transit of lissamine green as *open*, *dilated* and *collapsed*. The tubular pressure (P_{tub}), tubular fluid to plasma inulin concentration ratio (Tf/P_{In}) and single nephron filtration rate (SNGFR) were measured in proximal and distal tubules.

Significance level $P < 0.05$: [a] vs. normal-hematocrit (hct) group under pre-ischemic conditions and [b] vs. normal-hct group subjected to ischemia for an equal length of time.

same time from 24 ± 6 · 10^6 to 17 ± 5 · 10^6 · ml^{-1} ($P < 0.05$), while the mononuclear leukocyte count was not affected (22 ± 8 · 10^6 before and 21 ± 8 · 10^6 · ml^{-1} after the transfusion).

The arterial and glomerular capillary pressures increased after hemoconcentration, but the welling point and peritubular capillary pressure were unaffected (Table 3). Although the urine volume excretion increased, GFR and SNGFR remained at the control levels (Tables 1 and 2).

Figure 2 shows the fractional distribution of red blood cells after 25 minutes of ischemia and two hours of reperfusion. In the normal-hct group the values were not different from those in the intact contralateral kidney. In the high-hct group, however,

Table 3. Hydrostatic pressure in the superficial renal vasculature

	Mean arterial	Glomerular capillaries	Efferent arterioles	Peritubular capillaries
	mm Hg			
Pre-Ischemia				
Normal hct $N = 13$	119 ± 3	56.2 ± 1.0	13.6 ± 0.4	10.3 ± 0.3
High hct $N = 9$	133 ± 3[a]	63.1 ± 2.3[a]	13.9 ± 0.8	10.4 ± 0.8
Low hct $N = 8$	105 ± 3[a]	57.4 ± 1.2	13.9 ± 0.8	9.3 ± 0.9
25 Min ischemia				
Normal hct $N = 6$	104 ± 3	55.8 ± 0.27	14.4 ± 0.8	10.4 ± 1.5
High hct $N = 9$	124 ± 5[b]	70.9 ± 3.2[b]	26.1 ± 2.5[b]	14.4 ± 2.2
45 Min ischemia				
Normal hct $N = 7$	103 ± 4	51.1 ± 2.5	17.2 ± 1.5	14.8 ± 1.9
Low hct $N = 8$	102 ± 3	55.8 ± 1.7	18.1 ± 1.5	14.3 ± 1.3

Values are means ± 1 SE.
Significance level $P < 0.05$: [a] vs. normal-hematocrit (hct) group under pre-ischemic conditions and [b] vs. normal-hct group subjected to ischemia for an equal length of time.

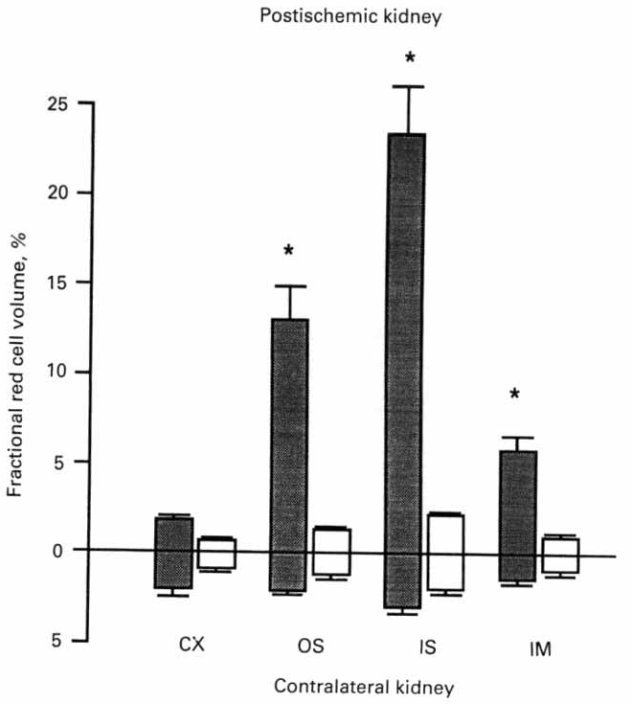

Fig. 2. *Distribution of red blood cells in the kidney after 25 minutes of ischemia and 2 hours of reperfusion.* The figures above the baseline represent the fractional volume of red blood cells in the cortex (CX), the outer stripe (OS) and inner stripe (IS) of the outer medulla and the inner medulla (IM) in animals subjected to hemoconcentration, with hct = 60 ± 1% (filled bars), and those with a normal hematocrit of 47 ± 1% (unfilled bars). The values under the baseline represent the contralateral kidney. Values are means ± 1 SE. (*) denotes a significant difference ($P < 0.05$) compared with the contralateral kidney.

this short ischemia resulted in a red cell distribution pattern very similar to that observed in animals with a normal hct exposed to 45 minutes of ischemia, that is, RBC trapping was evident in the whole of the medulla but was most pronounced in the inner stripe of the outer medulla, while the renal cortex was not affected.

In the high-hct group the wet weight of the ischemic kidney was 12.8 ± 1.7% higher than that of the contralateral kidney. This was not different from the figure of 9.5 ± 2.1% found in the normal-hct group.

After 25 minutes of ischemia both in the high-hct and normal-hct groups the majority of the tubules were open and the rest were of the dilated type. In the former group, however, the proximal and distal tubular pressures had increased by about 10 mm Hg (Table 2), indicating increased tubular fluid flow resistance in the late distal tubules or collecting ducts.

In the normal-hct group the intrarenal vascular pressures remained at the pre-ischemic levels after 25 minutes of ischemia, whereas in the high-hct group the glomerular capillary and afferent arteriolar pressures increased after ischemia, indicating increased resistance in the distal part of the vasculature (Table 3). After ischemia, GFR was reduced to a greater extent in the high-hct group than in the animals with a normal hct. In both groups the fractional reabsorption and the sodium and potassium concentrations in the urine were equal and the urine osmolality was only slightly above the plasma level (Table 1).

Renal blood flow

Table 4 summarizes the values for RBF. As measured in the contralateral intact kidneys, hemodilution caused an increase and hemoconcentration a decrease in RBF. After 45 minutes of ischemia RBC decreased to about 60% as compared with the contralateral kidney in both the normal- and low-hct group, while 25 minutes of ischemia did not cause a significant decrease in RBF either in the animals with a normal or with a high hct. Thus no major effect of the RBC trapping on total renal blood flow could be established from these results.

Table 4. Renal blood flow

	Body weight	Postischemic kidney	Contralateral kidney	Postischemic kidney/contralateral kidney
	g	*ml · min^{-1} · 100 g^{-1} body wt*		*%*
25 Min ischemia				
Normal hct $N = 5$	284 ± 25	3.39 ± 0.54	3.24 ± 0.35	103 ± 10
High hct $N = 6$	308 ± 26	2.62 ± 0.31	2.76 ± 0.27	95 ± 7
45 Min ischemia				
Normal hct $N = 5$	268 ± 29	1.86 ± 0.14[a]	3.15 ± 0.26	59 ± 3
Low hct $N = 5$	303 ± 34	2.48 ± 0.20[a]	3.97 ± 0.22	63 ± 4

Values are means ± 1 SE.
Significance limit $P < 0.05$: [a] vs. contralateral intact kidney.

Discussion

The cause of the RBC trapping in ischemic ARF is not completely understood. Mason et al observed RBC trapping or "vascular congestion" during the ischemic period [5, 13, 17], while others found that the RBC trapping first occurred during the reperfusion phase [9, 23]. Supporting the latter opinion is

the observation of RBC trapping in perfused transplanted kidneys [2, 11]. It must be considered, however, that the factors underlying the RBC trapping observed during the ischemia and those causing RBC trapping on reperfusion may not be the same.

Cell swelling has been proposed as an explanation for the vascular congestion observed during the ischemic period [5]. In the present study this cause of RBC trapping seemed less likely. For instance on hemodilution, which almost completely prevented the RBC trapping, the postischemic edema was not reduced. Furthermore, in high-hct animals the fractional RBC volume reached 20% in the inner stripe of the outer medulla, which is equivalent to the vascular space in this area of a normal kidney [29, 30], and is hence inconsistent with narrowing of the capillary lumen.

In capillaries of the intestine and heart [6, 7], primary trapping of leukocytes has been shown to cause a no-reflow phenomenon. However, the present study offers no support for this idea regarding the cause of RBC trapping in the renal vascular bed. Thus, hemodilution was associated with little, if any, decrease in the leukocyte count in the blood (probable because of continuous recruitment of leukocytes from extravascular compartments), and hemoconcentration, which enhances RBC trapping, caused no increase in the leukocyte count. Recent studies have also indicated that in contrast to RBCs, neutrophil granulocytes are found preferentially in the renal cortex after reperfusion [10].

In our view increased macromolecular permeability in the postglomerular capillaries [1, 8–10] is the main factor underlying the RBC trapping resulting from reperfusion. There is evidence to suggest that reactive oxygen metabolites [8, 31, 32] and neutrophils [10, 33] contribute to the reperfusion capillary injury. The consequent leakage of plasma would cause hemoconcentration and an increase in viscous resistance, eventually leading to complete cessation of blood flow and RBC trapping. From this viewpoint, the systemic hematocrit will play an important role. Since hemodilution has also been found to reduce RBC trapping in the transplanted perfused kidney [34], the hematocrit during reperfusion seems to be crucial.

The restriction of the trapping to the renal medulla is probably due to the substantially longer RBC transit time of the capillaries in this area [35], which will allow more plasma leakage. The lower flow velocity, which in itself increases the viscosity, may also be contributory [36].

It should be noted, however, that the RBC trapping was considerably less pronounced in the inner than in the outer medulla. This finding is in accordance with the observation by Vetterlein, Pethö and Schmidt [14] of markedly reduced perfusion in the outer medulla, except in the areas of the vasa recta. Thus the inner medullary blood flow may be well preserved after ischemia [37, 38], while the blood flow in the juxtamedullar nephrons, which reflects that in the whole of the medulla, may be reduced [11, 13].

Contribution of RBC trapping to the functional defects in postischemic ARF

It was found previously that the degree of RBC trapping after 15 minutes of reperfusion was related to the length of ischemia [1]. The same observation was made in the present study after two hours of reperfusion. Thus RBC trapping was evident after 45 minutes but not 25 minutes of ischemia. As has also been shown previously, the systemic hematocrit has a profound influence on RBC trapping [1, 17, 34]. For instance the trapping caused by 45 minutes of ischemia could be abolished by prior hemodilution. Likewise 25 minutes of ischemia preceded by hemoconcentration was followed by extensive RBC trapping. This finding may allow an estimation of the contribution of RBC trapping to the functional defects that follow minor (25 min) and more substantial (45 min) renal ischemia.

However, considerations must first be paid to the fact that hemodilution and hemoconcentration *per se* may influence the nephron function. In the present study it was found that under pre-ischemic conditions, hemodilution caused an increase and hemoconcentration a decrease in RBF, but no major changes in GFR, implying a change in the filtration fraction [22]. Moreover, hemodilution caused reduced fractional absorption in proximal tubules, as indicated by the reduced TF/P inulin ratio. The consequent increase in tubular fluid flow in the proximal part of the nephron may be one of many possible explanations for the increased urine volume and sodium excretion. The latter effects of isovolemic hemodilution have been observed previously [39, 40].

Regarding hemoconcentration, it should be noted that this was accompanied by a volume expansion. The latter may thus explain the increase in urine volume and sodium output and may partially have contributed to the increases in arterial and glomerular capillary pressure, which have also been observed on isovolemic hemoconcentration [22].

It has been proposed that almost all of the decrease in RBF and, consequently, as much as half of the decrease in GFR may be attributed to vascular congestion [5, 13, 17]. In the present study there appeared to be a correlation between RBC trapping and RBF. Considering the fact that both hemodilution and hemoconcentration *per se* influenced RBF, however, it seems clear that the relative decrease in RBF resulting from ischemia was not related to the RBC trapping but was entirely dependent upon the length of the ischemic period. Thus after 25 minutes of ischemia RBF was not affected in either the high- or normal-hct group, while 45 minutes of ischemia resulted in an approximately equal decrease in RBF to about 60% of normal in both normal- and low-hct groups. Since the RBC trapping was restricted to the medulla, a major effect on total renal blood flow would also seem less likely.

Thus the decrease in GFR resulting from 25 minutes of ischemia clearly cannot be due to a decrease in RBF. Likewise after 45 minutes of ischemia the GFR reduction to about 5% of normal cannot be explained by a decrease in RBF to 60% of normal. A major effect on the ultrafiltration coefficient (K_f) can also be excluded, since there was only a minimal decrease in SNGFR.

It thus seems evident that an increase in tubular pressure is the main cause of the reduced GFR both after 25 and 45 minutes of ischemia. This study suggests that such an increase may have two different mechanisms, as illustrated in Figure 3.

One is the presence of tubular casts, which occurs independently of RBC trapping, that is, also in low-hct animals. These casts seem to depend on the duration of ischemia and probably reflect damage to either of the "loci minores", that is, the straight segment of the proximal tubules [41], and the thick

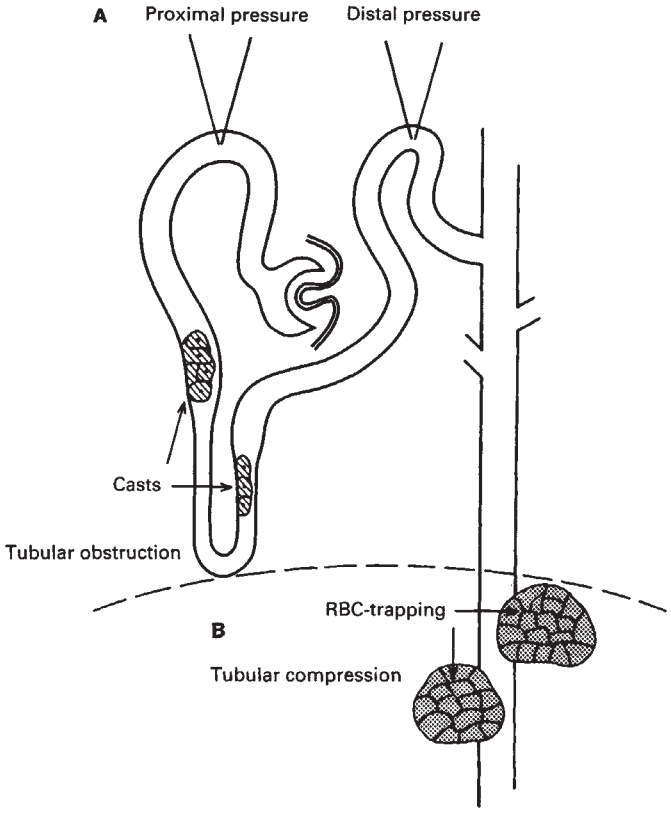

Fig. 3. *Proposed mechanisms responsible for the increase in superficial tubular pressure in postischemic ARF.* **A.** Casts consisting of tubular cells and cell debri originating from the proximal tubules will cause an increase in tubular fluid flow resistance in proximal tubules and loops of Henle. This in turn will increase proximal tubular pressure but not distal. The occurrence of these casts seems to depend on the duration of ischemia and not on RBC trapping. **B.** Compression of collecting duct in the outer medulla by capillaries dilated by RBC trapping. Provided that tubular fluid flow is not obstructed by casts, this compression will increase both proximal and distal tubular pressure.

ascending limb of the loop of Henle [15]. Because of their location, these casts will cause an increase in proximal, but not distal, tubular pressure.

The other possible cause of an increase in tubular pressure is compression of the medullary tubules exerted by capillaries dilated by RBC trapping. As the collecting ducts will be compressed, this will result in an increase in both the distal and proximal tubular pressure in the superficial nephrons, as was found in fact in high-hct but not in the normal-hct group after 25 minutes of ischemia.

On 45 minutes of ischemia, however, the abundance of tubular casts in the proximal tubules will allow only minor tubular fluid flow in the distal portions of the nephron. The RBC trapping-induced compression of the collecting ducts will then be of little importance, that is, the distal tubular pressure will not increase substantially.

DeRougemont et al observed a correlation between surface tubular pressure and capillary diameter in the inner stripe of the outer medulla [3]. These authors concluded that a cause-effect relation between these parameters could be due either to compression of venules draining the medulla by high-pressure tubules or, conversely, compression of the tubules by dilated capillaries. The latter concept is supported by observations on scanning electron microscopy [1, 9], that grossly widened capillaries seemed to compress the medullary tubules. This interpretation is also in line with the present findings that the massive RBC trapping in high-hct animals after 25 minutes of ischemia was accompanied by an increase in tubular pressure, whereas the increase in tubular pressure in the low-hct animals after 45 minutes of ischemia was not followed by RBC trapping.

A strong cause-effect relation between RBC trapping and the reduction in GFR has been proposed [5, 13, 17], on the basis of the observation that intervention against RBC trapping, either by increasing the renal perfusion pressure or by pre-ischemic hemodilution, was accompanied by an increase in C_{In} [17]. No such beneficial effect of hemodilution was observed in the present study. These results may not be as contradictory as they seem. In the former study there was a reduction in C_{In} to 20 to 40% of normal after 45 minutes of ischemia, while in the present study C_{In} was reduced to only a few percent of normal. Thus in our study the contribution of RBC trapping to the functional defects may have been overruled by quantitatively more important factors resulting from the ischemic injury *per se*, for example, back-leakage of inulin [21] and tubular obstructions by cast [19, 20].

On the other hand, efforts to reduce the RBC trapping may, independently of their effect in this respect, also affect other features of ARF. It seems very possible, for instance, that the increase in tubular fluid flow resulting from hemodilution may prevent the formation of tubular casts. Furthermore, an increase in tubular fluid flow will reduce the transit time for inulin in the tubules and hence the magnitude of tubular leakage after ischemia, which may lead to higher values for inulin clearance without affecting GFR. Analogously, not only may an increase in perfusion pressure cause wash-out of the trapped RBCs [17] but the consequent increase in tubular pressure may also lead to wash-out of tubular casts [20]. A lack of a direct relationship between inulin clearance and RBC trapping is also illustrated by the observation that administration of a hyperosmolar component such as mannitol [42], sucrose [32] or contrast medium [43] after the ischemic injury caused an increase in inulin clearance without reducing the RBC trapping.

Two classical features of ARF, namely the disturbance of the urinary concentration process and impaired potassium secretion, have been proposed as arguments for a functional deficit in the renal medulla in ischemic ARF [13, 15, 16]. The present findings do not indicate, however, that the RBC trapping plays a major role in this respect, since neither of these parameters was correlated to this trapping.

The main conclusion that may be drawn from the present study is that both RBC trapping and an alteration in nephron function reflect the degree of primary injury to the kidney. However, there seems to be little direct correlation between these parameters. This lack of correlation is most notable in the light of previous reports on an improved survival rate in unilaterally nephrectomized dogs subjected to hemodilution and complete renal ischemia in the remaining kidney [44]. In a recent study at our laboratory, prevention of the RBC trapping by hemodilution or its enhancement by hemoconcentration was found to have a substantial effect on the long-term outcome [1]. Thus, in the acute stage of ischemic ARF, standard markers of nephron function may not reflect the RBC trapping, whereas

this trapping may cause additional hypoxia in the outer medulla and subsequent kidney damage.

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References

1. HELLBERG POA, BAYATI A, KÄLLSKOG Ö, WOLGAST M: Red cell trapping after ischemia and long term kidney damage. Influence of hematocrit. *Kidney Int* 37:1240–1247, 1990
2. JACOBSSON J, ODLIND B, TUFVESON G, WAHLBERG J: Effects of cold ischemia and reperfusion on trapping of erythrocytes in the rat kidney. *Transplant Int* 1:75–79, 1988
3. DE ROUGEMONT D, BRUNNER P, TORHOST J, WUNDERLICH P, THIEL G: Superficial nephron obstruction and medullary congestion after ischemic injury: Effects of protective treatment. *Nephron* 31:310–320, 1982
4. FLORES J, DIBONA DR, BECK CH, LEAF A: The role of cell swelling in ischemic renal damage and the protective effect of hypertonic solute. *J Clin Invest* 51:118–126, 1972
5. MASON J, JOERIS B, WELSCH J, KRITZ W: Vascular congestion in ischemic renal failure: The role of cell swelling. *Miner Electrol Metab* 15:114–124, 1989
6. ENGLER R, SCHMID-SCHÖNBAIN G, PAVELEC R: Leukocyte capillary plugging in myocardial ischemia and reperfusion in the dog. *Am J Pathol* 111:98–111, 1983
7. BAROSSO-ARANDA J, SCHMID-SCHÖNBEIN GW, ZWEIFACH BW, ENGLER RL: Granulocytes and no-reflow phenomenon in irreversible hemorrhagic shock. *Circ Rec* 63:437–447, 1988
8. ÖJTEG G, BAYATI A, KÄLLSKOG Ö, WOLGAST M: Renal capillary permeability and intravascular red cell aggregation after ischaemia. I. Effects of xanthine oxidase activity. *Acta Physiol Scand* 129:295–304, 1988
9. BAYATI A, CHRISTOFFERSON R, KÄLLSKOG Ö, WOLGAST M: Mechanism of erythrocyte trapping in ischaemic acute renal failure. *Acta Physiol Scand* 138:13–23, 1990
10. HELLBERG POA, KÄLLSKOG Ö, WOLGAST M, ÖJTEG G: Peritubular capillary permeability and intravascular red cell aggregation after ischemia. Influence of neutrophils. *Am J Physiol* 350:F1018–F1025, 1990
11. NORLEN BJ, ENGBERG A, KÄLLSKOG Ö, WOLGAST M: Intrarenal hemodynamics in the transplanted rat kidney. *Kidney Int* 14:1–9, 1978
12. KARLBERG L, NORLEN BJ, ÖJTEG G, WOLGAST M: Impaired medullary circulation in postischemic acute renal failure. *Acta Physiol Scand* 118:11–17, 1983
13. MASON J, THORUST J, WELSCH J: Role of the medullary perfusion deficit in the pathogenesis of ischemic renal failure. *Kidney Int* 26:283–293, 1984
14. VETTERLEIN F, PETHÖ A, SCHMIDT G: Distribution of blood flow in rat kidney during postischemic renal failure. *Am J Physiol* 251:H510–H519, 1986
15. BREZIS M, ROSEN S, SILVA P, EPSTEIN F: Renal ischemia: A new perspective. *Kidney Int* 26:375–383, 1984
16. KARLBERG L, KÄLLSKOG Ö, NORLEN BJ, WOLGAST M: Nephron function in postischemic renal failure. *Scand J Urol Nephrol* 16:167–172, 1982
17. MASON J, WELSCH J, THORHORST J: The contribution of vascular obstruction to the functional defect that follows renal ischemia. *Kidney Int* 31:65–71, 1987
18. DAUGHARTY TM, BRENNER BM: Reversible hemodynamic defect in glomerular filtration rate after ischemic injury. *Am J Physiol* 228:1436–1448, 1975
19. ARENDHORST W, FINN W, GOTTSHALK C: Pathogenesis of acute renal failure following temporary ischemia in the rat. *Circ Res* 37:558–568, 1975
20. TANNER GA, STEINHAUSEN M: Tubular obstruction in ischemia-induced acute renal failure in the rat. *Kidney Int* 10:65–73, 1976
21. DONOHOE J, VENKATACHALAM M, BERNARD D, LEWINSKY N: Tubular leakage and obstructions after renal ischemia: Structural and functional correlation. *Kidney Int* 13:208–222, 1978
22. MYERS BD, DEEN WM, ROBERTSON CR, BRENNER BM: Dynamics of glomerular ultrafiltration in the rat. Effects of hematocrit. *Circ Res* 36:425–435, 1975
23. KARLBERG L, NORLEN BJ, ÖJTEG G, WOLGAST M: Erythrocyte and albumin distribution in the kidney following warm ischemia. *Scand J Urol Nephrol* 16:173–177, 1982
24. WIEDERHIELM CA, WOODBURY J, KIRK S, RUCHMER RF: Pulsative pressure in the microcirculation of frog mesentery. *Am J Physiol* 207:173–176, 1964
25. GERTZ KH, MANGOS JA, BRAUN G, PAGEL HD: Pressure in the glomerular capillaries and its relationship to arterial pressure. *Pflügers Arch* 288:369–374, 1960
26. LANDIS EM, PAPPENHEIMER JP: Exchange of substances through the capillary wall, in *Handbook of Physiology*, edited by W.F. HAMILTON, New York, Waverly Press Ltd, 1963, vol. 3, pp. 961–1034
27. KÄLLSKOG Ö, WOLGAST M: Driving forces over the peritubular capillary membrane in the rat kidney during antidiuresis and saline expansion. *Acta Physiol Scand* 89:116–125, 1973
28. LOWRY O, ROSENBROUGH N, FARR A, RANDALL R: Protein measurement with the folic phenol reagent. *J Biol Chem* 193:265–275, 1951
29. RASSMUSEN SN: Intrarenal red cell and plasma volumes in the non-diuretic rat. *Pflügers Arch* 342:61–72, 1973
30. PFALLER W, RITTINGER M: Quantitative morphology of the rat kidney. *Int J Biochem* 12:17–22, 1980
31. DEL MAESTRO RF, BJÖRK J, ARFORS K-E: Increase in microvascular permeability induced by enzymatically generated free radicals. I. In vivo study. *Microvasc Res* 22:239–245, 1981
32. BAYATI A, HELLBERG POA, ODLIND B, WOLGAST M: Prevention of acute renal failure with superoxide dismutase and sucrose. *Acta Physiol Scand* 130:367–372, 1987
33. HERNANDEZ LA, GRISHAM M, TWOHIG B, ARFORS K, HARLAN J, GRANGER N: Role of neutrophils in ischemia-reperfusion induced microvascular injury. *Am J Physiol* 253:H699–H703, 1987
34. JACOBSSON J, ODLIND B, TUFVESON G, WAHLBERG J: The effects of type of preservation solution and hemodilution of recipient on postischemic erythrocyte trapping in kidney grafts; An experimental study in the rat. *Transplantation* 47:876–879, 1989
35. WOLGAST M: Renal medullary red cell and plasma flow as studied with labelled indicators and intrarenal detection. *Acta Physiol Scand* 88:212–225, 1973
36. MERRILL EW: Rheology of blood. *Physiol Rev* 49:863–888, 1969
37. BÖTTCHER W, STEINHAUSEN M: Microcirculation of the papilla of rats under control conditions and temporary ischemia. *Kidney Int* 10:74–80, 1976
38. YAGIL Y, MIAMOTO M, JAMISON RL: Inner medullary blood flow in postischemic acute renal failure in the rat. *Am J Physiol* 256:F456–F461, 1989
39. BAHLMAN J, MCDONALD S, DUNNINGHAM J, DEWARDNER H: The effect on sodium excretion of altering the packed cell volume with albumin solutions without changing the blood volume in the dog. *Clin Sci* 32:395–402, 1967
40. NASHAT F, SCHOLEFIELD F, TAPPIN J, WILCOX C: The effect of changes in hematocrit in the anaesthetized dog on the volume and character of the urine. *J Physiol* 205:305–316, 1969
41. VENKATACHALAM M, BERNAD D, DONOHOE J, LEVINSKY N: Ischemic damage and repair in the rat proximal tubule: Differences among the S1, S2 and S3 segments. *Kidney Int* 14:31–40, 1978
42. HELLBERG POA, NYGREN A, HANSELL P, FASCHING A: Post-ischaemic administration of hyperosmolar mannitol enhances erythrocyte trapping in outer medullary vasculature in the rat kidney. *Renal Physiol* (in press)
43. NYGREN A, HANSELL P, HELLBERG O, ERIKSON U: Red cell congestion in renal microvasculature induced by low osmolar contrast media and mannitol, in Contrast media and regional renal blood flow. Thesis by NYGREN A, Uppsala, 1989, II, pp. 1–13.
44. RAJAGOPALAN PR, REINES HD: Reversal of acute renal failure using hemodilution with hydroxyethyl starch. *J Trauma* 23:795–800, 1983