Kidney International, Vol. 36 (1989), pp. 753–759

LABORATORY INVESTIGATION

Role of iron in the tubulo-interstitial injury in nephrotoxic serum nephritis

ALLEN C. ALFREY, DANIEL H. FROMENT, and WILLIAM S. HAMMOND

Department of Medicine and Pathology, Renal Division, Veterans Administration Medical Center, Denver, Colorado, USA

Role of iron in the tubulo-interstitial injury in nephrotoxic serum nephritis. We studied the possibility that tubule fluid iron could be involved in the pathogenesis of the tubulo-interstitial injury associated with primary glomerular disease. Tubule fluid iron is determined by the magnitude of the glomerular leak for transferrin and the iron saturation of transferrin. To minimize tubule fluid iron in an experimental model of glomerulonephritis, iron deficiency was induced in rats prior to the induction of nephrotoxic serum nephritis. Iron deficiency did not effect the development of glomerular disease as determined by proteinuria, but had a marked effect on preventing the development of tubulo-interstitial disease and renal functional deterioration. There was also a strong correlation between the amount of functional deterioration and extent of tubulo-interstitial disease and urinary iron excretion in both the control and iron deficient animals. It is proposed that injury results from iron being dissociated from transferrin at the more acid pH of the tubule fluid. Iron, a transition element, is able to catalyze the Haber-Weiss reaction with the formation of free hydroxyl radicals which causes renal tubule cell injury. This tubulo-interstitial injury is the major determinate of progressive renal functional deterioration in this experimental model of glomerulonephritis.

There are two types of experimental renal disease that commonly cause extensive tubulo-interstitial disease and progress to end stage disease, the remnant kidney [1] and nephrotoxic serum nephritis (NSN) [2]. The remnant kidney is a hypertensive model and it is felt that glomerular hypertension resulting from systemic hypertension causes glomerular injury which produces the tubulo-interstitial disease and renal failure [3]. This is supported by the fact that conditions which increase systemic pressure accelerates the renal disease [4] whereas factors which reduce the systemic pressure are protective [5, 6]. This is further supported by the finding that renal ablation with hyperfiltration but without hypertension does not cause progressive renal damage [7].

In contrast, nephrotoxic serum nephritis is a normotensive disease which also progresses to end stage disease [8–10]. Thus, the hemodynamic events which lead to end-stage renal disease in the remnant kidney can not be evoked in the pathogenesis of the progressive destruction in nephrotoxic serum nephritis. Furthermore, in nephrotoxic serum nephritis [2], as with a number of human renal diseases [11, 12], change in renal function more closely correlates with tubulo-interstitial disease than glomerular disease. However, the mechanism by which interstitial disease results from glomerular injury is unknown.

Because of the close association between proteinuria and tubulo-interstitial disease, it has been suggested that proteinuria mediates this injury. However, the suggested mechanisms by which proteinuria could induce tubulo-interstitial disease are not totally convincing. These include excess absorption of protein by the proximal tubule cells with cellular damage from protein overload and leakage of lysosomal enzymes into the tubule lumen [13]. Another suggested cause is the formation of intraluminal protein casts which leads to the obstruction of the tubule and disruption of the tubule membrane with Tamm-Horsfall protein released into the interstitium [14]. This could result in an immune complex tubulo-interstitial nephritis mediated by auto-antibodies to Tamm-Horsfall protein [14].

That a number of experimental, as well as human, proteinuric renal diseases are not accompanied by severe tubulo-interstitial disease perhaps disputes the idea that proteinuria causes tubule injury. It may not be the proteinuria per se, but rather something which can accompany the proteinuria in some renal diseases that is responsible for the tubulo-interstitial injury.

One potential toxin that accompanies proteinuria is the transition element iron. Iron is presented to the tubular lumen in proteinuric states because of the glomerular leak for transferrin. At the pH of the tubule fluid, it seems likely that iron would be released from transferrin. Because of iron's ability to accept and donate electrons, it can catalyze the Haber-Weiss reaction promoting the formation of hydroxyl radicals [15]. The hydroxyl radicals are highly reactive and can destroy almost all known biomolecules [15] causing the tubulo-interstitial injury.

This study was undertaken to determine if iron could be involved in the pathogenesis of tubulo-interstitial disease resulting from glomerular injury. Since urinary iron excretion in glomerular disease is determined by the amount of glomerular transferrin leak and the iron saturation of transferrin, nephrotoxic serum nephritis was established in animals previously iron depleted to minimize tubule fluid iron.

Methods

Nephrotoxic serum nephritis was produced in 21 uninephrectomized male Sprague-Dawley rats by previously described techniques [2, 16]. Two months prior to nephrectomy and induction of nephrotoxic serum nephritis, the animals were placed on normal rat chow (AIN-76, ICN, Costa Mesa, Cali-

Received for publication November 28, 1988
and in revised form May 22, 1989
Accepted for publication May 26, 1989

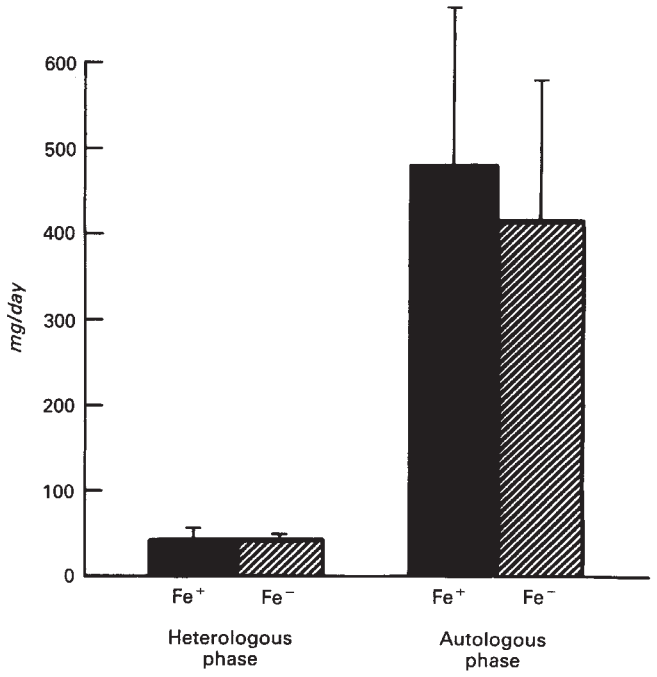

Fig. 1. *Urinary protein excretion in the heterologous (day 1) and autologous phases of nephrotoxic serum nephritis (day 28).* There was no significant difference between groups in either period. Fe^- represents low iron group and Fe^+ represents normal iron group.

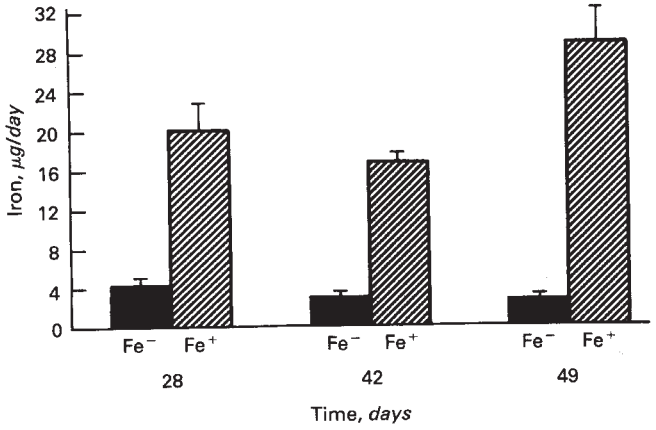

Fig. 2. *Daily urinary iron excretion at three times following nephrotoxic serum.* At each time, urinary iron was significantly less in the low iron group ($P < 0.001$). Fe^- represents low iron diet and Fe^+ represents normal iron diet.

fornia, USA, containing either 45 or 8 mg/kg iron). Iron deficiency was induced in eleven animals on the low iron diet by drawing 2 ml of blood weekly over the two month period prior to receiving nephrotoxic serum. After receiving the nephrotoxic serum, the blood drawing to induce iron deficiency was discontinued.

The rats were placed in Nalgene metabolic cages to collect 24-hour urine specimens for iron and protein determinations. For blood determinations, serial samples were obtained from the tail artery. The kidneys removed prior to induction of NSN were saved for iron determination to be compared with the kidney iron content at the termination of the study.

Analytical procedures

Protein and creatinine were measured with previously described methods [2, 16]. Urine, serum, and tissue iron were determined with a flameless atomic absorption technique which has a detection limit of 1 μg/liter. A nitric acid digest was carried out for tissue iron determinations. Serum and urine transferrin measurements were performed with a nephelometric method.

Kidney dry and wet weight were determined to calculate water content.

Systolic blood pressure measurements were made in awake animals using a rat tail blood pressure cuff.

Routine histology was performed on all kidneys and electron microscopy on selected kidneys from both the control and iron deficient group. Kidney tissue was prepared for light microscopy by fixing overnight in 4% buffered formaldehyde and embedding in paraffin. Sections (3 μ thick) were stained with hematoxylin-eosin, periodic acid-Schiff and trichrome. For electron microscopy immersion fixation was carried out with 4% glutaraldehyde with a cacodylate buffer, pH 7.4. Electron microscopic studies were otherwise performed by standard techniques. Immunofluorescence studies for the detection of IgG and complement were also carried out. Histological grading of tubulo-interstitial and glomerular disease was made by one of the authors (W.S.H.) in a blinded fashion.

Paired and unpaired Student's *t*-test were used to compare results within and between groups, respectively. The Wilcoxon signed ranks test was used for analysis of non-parametric data. All results are expressed as mean ± 1 SEM.

Results

At the initiation of the study body weight was 351 ± 7 in the control animals vs. 325 ± 15 g in the iron deficient group (P = NS). However, during the course of NSN weight gain was less in the iron deficient group (13 ± 4 vs. 25 ± 3 g, $P < 0.05$). The initial hematocrit was 47 ± 1 vs. 34 ± 1% and serum iron 160 ± 22 vs. 37 ± 4 μg/dl in the control and iron deficient group, respectively ($P < 0.001$). At the termination of the study, the hematocrit was 39 ± 1 vs. 34 ± 1% and serum iron 115 ± 13 vs. 28 ± 3 μg/dl in the respective groups.

Urine protein in the heterologous phase (1 day following nephrotoxic serum) was 44 ± 8.5 mg/day in the control group and 44 ± 7 mg/day in the iron deficient group (P = NS). Similarly in the autologous phase (28 days following nephrotoxic serum), urine protein was not significantly different between groups, 478 ± 58 vs. 414 ± 50 mg/day (Fig. 1).

Daily urinary iron excretion was measured at three times during the study (Fig. 2). Urinary iron excretion was relatively constant in both groups being significantly lower on each occasion in the low iron group ($P < 0.001$). Urinary and serum transferrin and iron were determined at the last collection period in the control group to determine their respective ratios. The iron/transferrin ratio was 1.12 ± 0.14 in the urine as compared to 0.55 ± 0.06 μg Fe/mg transferrin in the serum ($P < 0.001$).

At day 15 and 29 of the study, plasma creatinine was similar in both groups. However, by day 50, it was significantly higher in the control group and this difference was magnified by day 58

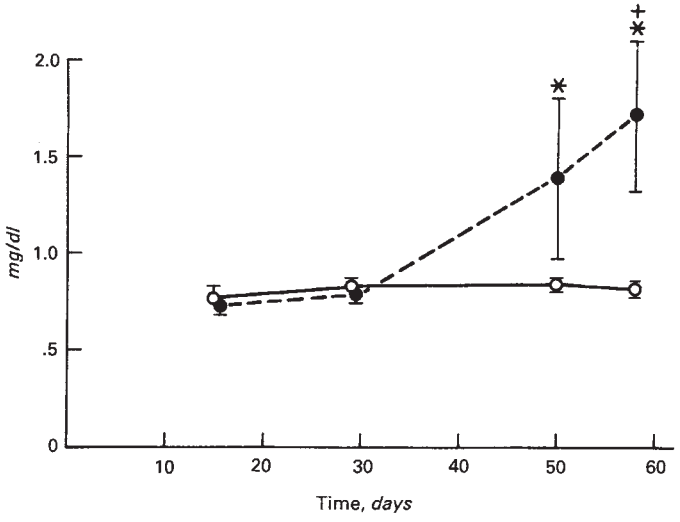

Fig. 3. *Plasma creatinine levels following nephrotoxic serum.* Values were similar in both groups at day 15 and 29. At day 50, ($P < 0.05$) and day 58 ($P < 0.01$), values were significantly higher in the normal iron group. Open circles represent low iron diet and closed circles represent normal iron diet.

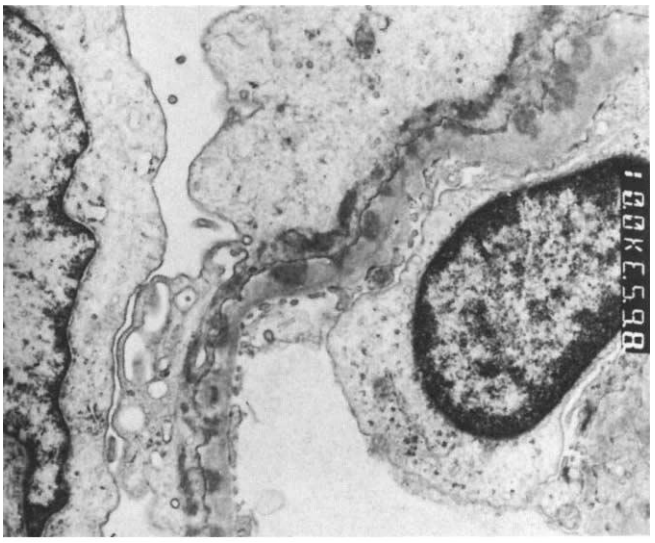

Fig. 4. *Electron micrograph of a glomerulus obtained from an animal in the low iron diet group at the termination of the study (day 59).* There are numerous subepithelial deposits with effacement of epithelial foot processes but no leukocytic infiltration or inflammatory features (× 14,000).

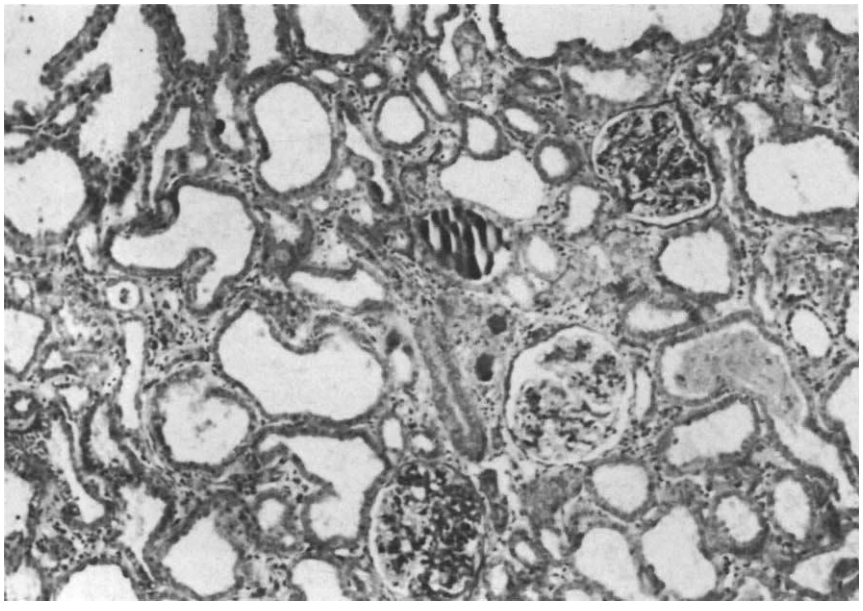

Fig. 5. *Light microscopic features of a kidney obtained from an animal maintained on a normal iron diet.* There is extensive tubulo-interstitial disease and mild sclerosis of the glomeruli. The kidney weighted 5.17 grams and was graded as 3+ tubulo-interstitial disease.

(Fig. 3; $P < 0.001$). Similarly creatinine clearance was unchanged between day 30 (0.24 ± 0.02) and day 58 (0.22 ± 0.02 ml/100 g body wt, P = NS) in the low iron group, in contrast to the control group where there was a significant decrease in creatinine clearance during this period (0.23 ± 0.02 vs. 0.15 ± 0.01 ml/100 g body wt, $P < 0.01$).

Systolic blood pressure measurements obtained between day 50 and 58, at a time when there was progressive functional deterioration, was 114 ± 5 in the control animals and 108 ± 8 mm Hg in the iron deficient group (P = NS).

At the termination of the study, single kidney weight was 5.14 ± 0.82 in the controls versus 2.22 ± 0.24 grams in the iron deficient rats (normal uninephrectomized animal kidney 1.8 ± 0.08 g). As found by other investigators [17, 18] the autologous phase of NSN in the rat is usually a model of membranous nephritis. Although basement membrane thickening was not obvious on light microscopy, electron microscopy revealed numerous subepithelial deposits with effacement of epithelial foot processes (Fig. 4). Immunofluorescence showed a granular deposit of IgG and complement. In the protected animals this histological pattern of only membranous nephritis persisted throughout the course of the disease. In contrast in the non-protected animals extensive tubulo-interstitial disease occurred with a variable amount of sclerosis of the glomeruli (Fig. 5).

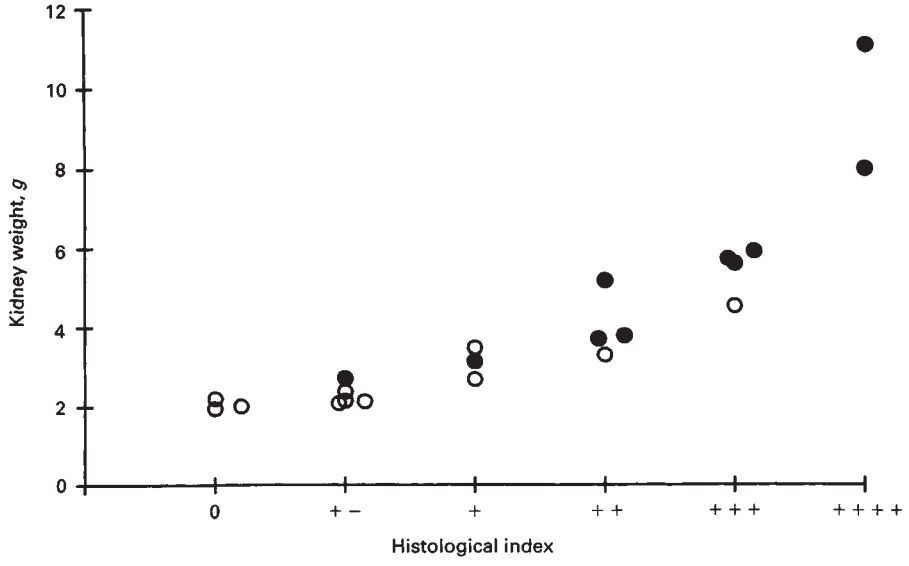

Fig. 6. *Correlation between kidney weight and histological index of tubulo-interstitial disease.* Open circles represent animals maintained on the low iron diet and closed circles animals maintained on a normal iron diet.

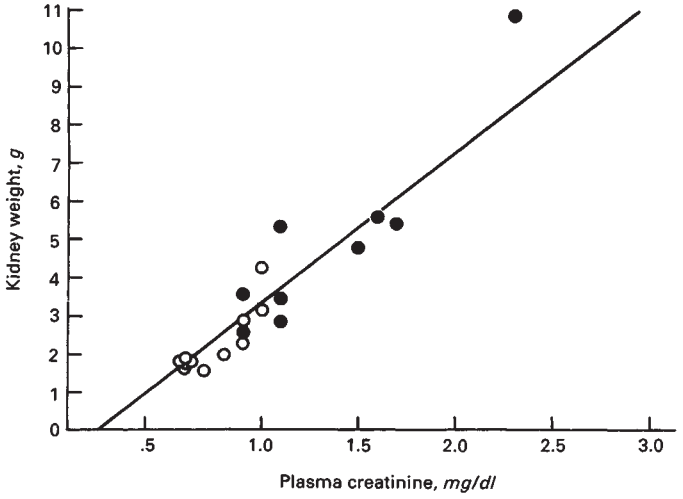

Fig. 7. *There was a significant correlation between kidney weight and plasma creatinine.* Open circles represent animals on a low iron diet and closed circles represent animals on a normal iron diet.

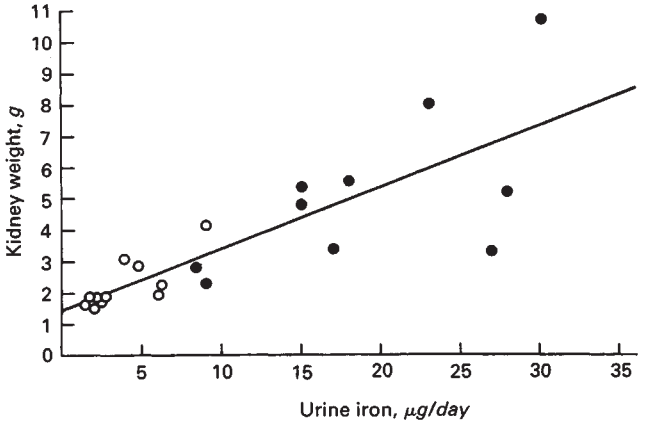

Fig. 8. *There was a significant correlation between urinary iron excretion and kidney weight.* Open circles represent animals on a low iron diet and closed circles represent animals on a regular iron diet (r = 0.815, $P < 0.001$).

Percent focal and global sclerosis, as determined by counting a minimum of 100 glomeruli in each kidney, was 56 ± 7 in the control animals versus 29 ± 5% in the iron deficient animals ($P < 0.01$). Histological grading of tubulo-interstitial disease was 2.45 ± 0.39 in the controls versus 0.77 ± 0.29 in the iron deficient group ($P < 0.01$).

There was a highly significant correlation between the histological index of severity of tubulo-interstitial disease and kidney weight (Fig. 6). There was also a very good correlation between kidney weight and renal function as determined by the final plasma creatinine (r = 0.94; Fig. 7). In contrast there was no correlation between percent sclerotic glomeruli and renal function (r = 0.34).

There was also a significant correlation between kidney weight and percent water content in both groups of animals (r = 0.85, $P < 0.001$). The kidney water content in the control group was 82.9 ± 0.9% as opposed to 79.5 ± 0.6% in the low iron group ($P < 0.001$). This represents a mean kidney water content of 4.26 ± 0.68 ml in the control group versus 1.76 ± 0.19 in the low iron group.

A highly significant correlation was found between urine iron excretion and kidney weight for both groups (r = 0.815, $P <$ 0.001) (Fig. 8). In the low iron group alone, there was also a good correlation between urine iron excretion and kidney weight (r = 0.789, $P < 0.01$; Fig. 9).

In contrast, there was no correlation between plasma iron, and the correlation between urine protein and kidney weight was much weaker than that found for urine iron (r = 0.565).

Following nephrotoxic serum, the kidney iron concentration increased in the control group (195 ± 9 vs. 486 ± 36 mg/kg, $P <$ 0.001) and correlated with kidney weight (r = 0.846, $P < 0.01$). In contrast in the low iron group, kidney iron concentration was higher at the beginning of the study (104 ± 3 mg/kg) than at its termination (84 ± 3 mg/kg, $P < 0.005$) and there was no

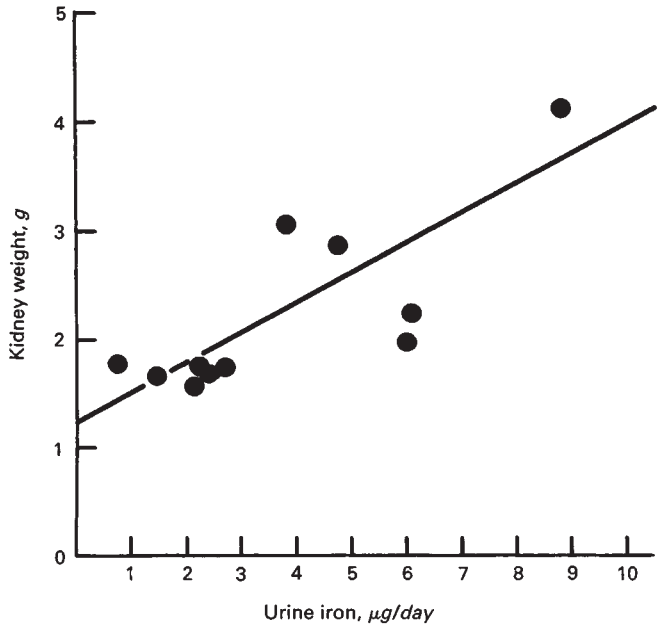

Fig. 9. *Low iron group evaluated alone also demonstrate a significant correlation between urinary iron excretion and kidney weight* ($r = 0.789$, $P < 0.01$).

correlation between kidney iron concentration and kidney weight.

Discussion

There recently has been increasing interest in the role of iron and free radicals in the causation of renal injury in various experimental models of renal disease [19, 20]. However, in regards to models of chronic renal disease, studies have been carried out early in the course and have been directed only at the glomerular disease [21–23]. Boyce and Holdsworth found that deferoxamine prevented the development of the heterologous phase proteinuria in nephrotoxic serum nephritis [23]. Similarly, Rehan et al found that catalase, presumably by accelerating H_2O_2 breakdown, decreased the heterologous phase proteinuria but had no effect on the autologous phase proteinuria [21, 22]. In the present study, iron depletion had no effect on either the heterologous phase or autologous phase proteinuria. In addition, the amount of proteinuria in the autologous phase of this disease was equal to or actually greater than most other investigators have reported, suggesting that iron depletion did not effect the glomerular disease resulting from nephrotoxic serum. However, iron depletion had a major effect on tubulo-interstitial disease.

It has been well established that extensive tubulo-interstitial disease develops in this model in association with renal functional deterioration and marked enlargement of the kidney [2, 16, 17]. This is again supported in the present study. Furthermore, there was no correlation with glomerular disease, either membranous glomerulonephritis which initially occurred and persisted in the protected animals or focal and global sclerosis which occurred late in the unprotected animals, and renal function.

Although there was an excellent correlation between the histological grading of tubulo-interstitial disease and kidney weight, we elected to correlate kidney weight with other features since its measurement is somewhat more precise. The majority of increase kidney weight with disease is the result of increase water representing interstitial edema and increase tubule fluid present in the dilated and distended tubules. In addition, as stated above there was also a highly significant correlation between kidney weight and renal function.

These studies strongly suggest that tubule fluid iron is responsible for the tubulo-interstitial disease in nephrotoxic serum nephritis. Not only was there a significant difference in functional and anatomical changes between the iron deficient and control group, there was also an excellent correlation between tubulo-interstitial disease, as determined by histological grading, kidney weight and renal function and urinary iron excretion. More importantly, this correlation was present when either the iron deficient group or control group was evaluated independently. In addition, besides the correlation between urine iron and kidney weight in these groups, when urine iron excretion overlapped between groups, kidney weight and renal function also overlapped. Although the diets were identical with the exception of iron, these latter findings exclude other variables which might have existed between groups. In further support of tubule fluid iron being important in production of disease rather than intrarenal or systemic iron is that disease occurred in the low iron group in association with low plasma iron levels and actually a fall in kidney iron concentration. This would further suggest that kidney iron concentration in the control group did not increase because of tubule fluid iron content but rather it resulted from other mechanisms. Irrespective of why kidney iron is increased in the control animals, such an increase in iron is obviously not required for the production of tubulo-interstitial disease and renal failure.

Although anemia has been shown to be protective in the remnant kidney model, at least, in regards to glomerular disease, this does not seem to be relevant in this model [6]. First, as stated above, anemic animals also developed tubulo-interstitial disease. Secondly, it was felt that anemia was protective in the remnant kidney model by reducing systemic blood pressure. In contrast, in this model, where blood pressure is not elevated, anemia had no effect on blood pressure. Finally, in contrast to the remnant kidney model where anemia prevented glomerular disease as determined by reduction of proteinuria, in this model, neither anemia nor iron deficiency-effected proteinuria.

Normally, iron enters the renal tubule cells from the basolateral side with transferrin, via its receptor, and is deposited in a non-toxic form with ferritin [24]. In contrast, because of the protein leak in glomerular disease, iron (in association with transferrin) enters the tubule lumen. Since it is unlikely that there are transferrin receptors on the luminal surface of the tubule cell, the iron transferrin complex could be taken up by endocytosis, as occurs for other proteins [25]. In addition, iron tends to dissociate from transferrin at a pH of less than 7 and is almost completely dissociated at a pH of less than 6.5 [26]. Proximal tubule pH is approximately 6.5 and bicarbonate content can be as low as 8 mmol, and pH and bicarbonate is even lower in other areas of the nephron [27]. This dissociation could be enhanced by the presence of chelators such as citrate in the tubule fluid. The finding that iron/transferrin ratio was twice as high in urine as serum is strong evidence that iron and

transferrin are indeed dissociated and handled differently by the renal tubule. The fact that in a number of urines the ratios were greater than 1.2 μg Fe/mg transferrin would mean there was not enough transferrin to bind all of the iron present even if both sites on transferrin were occupied. Although this finding does not prove the presence of free iron capable of catalyzing Haber-Wiess reaction it would be consistent with this possibility. Furthermore, these studies have not documented that lipid peroxidation was occurring.

There is additional direct evidence for the renal tubule toxicity of intraluminal iron from studies using ferric nitrilotriacetate [28, 29]. Ferric nitrilotriacetate is a chelate of iron of low molecular weight which would be readily filtered by the glomerulus. This compound has repeatedly been shown to cause anatomic and functional changes in the proximal tubule which is thought to be the result of free radical formation and lipid peroxidation [28, 29]. Although these studies have been concerned with acute renal injury it seems possible with more prolonged exposure, chronic disease might also occur.

It is well recognized that not all forms of proteinuric renal disease develop extensive tubulo-interstitial disease and renal failure. This could result from variations in tubular fluid iron exposure, since these studies show that the rate of progression of renal failure is directly related to the amount and duration of tubule exposure to iron. Thus, the status of the body iron burden and size and duration of the glomerular protein leak might be major determinates of the rate of renal functional deterioration. In addition, because of difference in size and configuration of transferrin and albumin, the relative clearance of these protein could vary with different glomerular diseases [26]. Unfortunately, there have been but a limited number of studies evaluating urinary iron and transferrin excretion, and the results are somewhat conflicting [30–35].

In summary, these studies are consistent with the hypothesis that tubule fluid iron is responsible for the tubulo-interstitial disease present in nephrotoxic serum nephritis. Until now, all methods of preventing progression of renal failure have been directed at the glomerulus. However, these studies suggest that means may subsequently be found to prevent the tubulo-interstitial disease and functional deterioration that occurs as a consequence of the glomerular disease.

Acknowledgments

This work was supported by VA research funds. Dr. Froment was supported by a grant from the Foundation Canadienne du Rein (Kidney Foundation of Canada).

Reprint requests to Allen C. Alfrey, (111C), V.A. Medical Center, 1055 Clermont Street, Denver, Colorado 80220, USA.

References

1. Ibels LS, Alfrey AC, Haut L, Huffer WE: Preservation of function in experimental renal disease by dietary restriction of phosphate. *N Engl J Med* 298:122–126, 1978
2. Karlinsky ML, Haut L, Buddington B, Schrier NA, Alfrey AC: Preservation of renal function in experimental glomerulonephritis. *Kidney Int* 17:293–302, 1980
3. Hostetter TH, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM: Hyperfiltration in remnant nephrons: A potentially adverse response to renal ablation. *Am J Physiol* 241:F85–F93, 1981
4. Garcia DL, Rennke HG, Brenner BM, Anderson S: Chronic glucocorticoid therapy amplified glomerular injury in rats with renal ablation. *J Clin Invest* 80:867–874, 1987
5. Anderson S, Meyer TW, Rennke HG, Brenner BM: Control of glomerular hypertension limits glomerular injury in rats with reduced renal mass. *J Clin Invest* 76:612–619, 1985
6. Garcia DL, Anderson S, Rennke HG, Brenner BM: Anemia lessens and its prevention with recombinant human erythropoietin worsens glomerular injury and hypertension in rats with reduced renal mass. *Proc Natl Acad Sci USA* 85:6142–6146, 1988
7. Meyer TW, Rennke HG: Progressive glomerular injury after limited renal infarction in the rat. *Am J Physiol* 254:F856–F862, 1988
8. Neugarten J, Feiner HD, Schacht RG, Gallo GR, Baldwin DS: Aggravation of experimental glomerulonephritis by superimposed clip hypertension. *Kidney Int* 22:257–263, 1982
9. Motoki K, Kusuyama Y, Ueno Y, Miyamoto Y, Nishio I, Masuyama Y: Chronic nephrotoxic nephritis in spontaneously hypertensive rats. *Jap Heart J* 19:662–664, 1978
10. Nishimori I: The production of hypertension and cardiovascular lesions by nephrotoxic nephritis combined with adrenal regeneration in rats. *Acta Pathol Jap* 10:409–417, 1960
11. Risdon RA, Lond MB, Sloper JC, Cantab MD, DeWardener HE: Relationship between renal function and histological changes found in renal-biopsy specimens from patients with persistent glomerular nephritis. *Lancet* 2:363–366, 1968
12. Mackensen-Haen S, Bader R, Grund KE, Bohle A: Correlations between renal cortical interstitial fibrosis, atrophy of the proximal tubules and impairment of the glomerular filtration rate. *Clin Nephrol* 15:167–171, 1981
13. Paigen K, Peterson J: Coordinancy of lysosomal enzyme excretion in human urine. *J Clin Invest* 61:751–762, 1978
14. Hoyer JR, Seiler MW: Pathophysiology of Tamm-Horsfall protein. *Kidney Int* 16:279–289, 1979
15. Halliwell B, Gutteridge MC: Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem J* 219:1–14, 1984
16. Tomford RC, Karlinsky ML, Buddington B, Alfrey AC: Effect of thyroparathyroidectomy and parathyroidectomy on renal function and the nephrotic syndrome in rat nephrotoxic serum nephritis. *J Clin Invest* 68:655–664, 1981
17. Crosson JT, Lubowitz H, Mazumdar DC, Weisser F, Lang S, Rolf D, Germuth FG: The production of experimental glomerulonephritis in the rat. *Arch Pathol* 98:344–350, 1974
18. Unanue ER, Dixon FJ: Experimental glomerulonephritis: Immunological events and pathogenetic mechanisms. *Adv Immunol* 6:1–9, 1967
19. Paller MS, Hedlund BE: Role of iron in postischemic renal injury in the rat. *Kidney Int* 34:474–480, 1988
20. Thakur V, Walker PD, Shah SV: Evidence suggesting a role of hydroxyl radical in puromycin aminonucleoside-induced proteinuria. *Kidney Int* 34:494–499, 1988
21. Johnson KJ, Rehan A, Ward PA: The role of oxygen radicals in kidney disease, in *Oxygen Radicals and Tissue Injury*, edited by Halliwell B, Proceedings of a Brook Lodge Symposium, Augusta, MI, April 27–29, 1987, pp. 115–121
22. Rehan A, Johnson KJ, Wiggins RC, Knukel RG, Ward PA: Evidence for the role of oxygen radicals in acute nephrotoxic nephritis. *Lab Invest* 51:396–403, 1984
23. Boyce NW, Holdsworth SR: Hydroxyl radical mediation of immune renal injury by desferrioxamine. *Kidney Int* 30:813–817, 1986
24. Fuller SD, Simons K: Transferrin receptor polarity and recycling accuracy in "tight" and "leaky" strains of Madin-Darby canine kidney cells. *J Cell Biol* 103:1767–1779, 1986
25. Carone FA, Peterson DR, Oparil S, Pullman TN: Renal tubular transport and catabolism of proteins and peptides. *Kidney Int* 16:271–278, 1979
26. Morgan EH: Transferrin, biochemistry, physiology and clinical significance. *Molec Aspects Med* 4:1–123, 1981
27. Koeppen B, Giebisch G, Malnic G: Mechanism and regulation of renal tubular acidification, in *The Kidney Physiology and Pathophysiology* (vol 2), edited by Seldin D, Giebisch G, New York, Raven Press, 1985, pp. 1491–1525

28. HAMAZAKI S, OKADA S, EVINA Y, FUJIOKA M, MIDORIKAWA S: Nephrotoxicity of ferric nitrilotriacetate: An electron-microscope and metabolic study. *Am J Pathol* 123:343–350, 1986
29. HAMAZAKI S, OKADA S, EBINA Y, MIDORIKAWA S: Acute renal failure and glucosuria induced by ferric nitrilotriacetate in rats. *Toxicol Appl Pharmacol* 77:267–274, 1985
30. WILTINK WF, VAN EUK HG, BOBECK-RUTASERT MM, GERBRANDY J, LEUNSE B: Urinary iron excretion in the nephrotic syndrome. *Acta Haemat* 47:269–276, 1972
31. RIFKIND D, KRAVETZ HM, KNIGHT V, SCHADE AL: Urinary excretion of iron binding protein in the nephrotic syndrome. *N Engl J Med* 265:115–118, 1961
32. ELLIS D: Anemia in the course of the nephrotic syndrome secondary to transferrin depletion. *J Pediatr* 90:953–955, 1977
33. HANCOCK DE, ONSTAD JW, WOLF PL: Transferrin loss into the urine with hypochromic, microcytic anemia. *AJCP* 65:73–78, 1975
34. BROWN EA, SAMPSON B, MULLER BR, CURTIS JR: Urinary loss in the nephrotic syndrome—an unusual case of iron deficiency with a note on urinary copper losses. *Postgrad Med J* 60:125–128, 1984
35. HAGG JH, SMITH JA, GOLDBERG A: Urinary excretion of iron. *Clin Sci* 30:495–503, 1966