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LABORATORY INVESTIGATION

Renal iron handling in the nephrotic syndrome

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Renal iron handling in the nephrotic syndrome. Renal iron handling was characterized in three experimental models of the nephrotic syndrome: puromycin aminonucleoside, adriamycin and nephrotoxic serum. In adriamycin-induced nephrotic syndrome, which has previously been shown to result from alterations in pore size of the filtration barrier, the transferrin leak was most severe with a fractional clearance of 25%, a value identical to albumin. In contrast, in puromycin nephrotic syndrome and nephrotoxic serum nephritis the fractional clearance of transferrin was never greater than 2% and consistently less than the fractional clearance of albumin. The fact that iron/transferrin ratios in urine and serum were frequently different, sometimes higher other times lower, documents that iron and transferrin can be dissociated in tubule fluid and handled differently in regards to tubule uptake. Kidney iron concentration is also increased in both immunological and non-immunological forms of nephrotic syndrome. In the proximal tubule iron is present largely on the luminal aspect of the cell. In contrast, the major deposition of iron occurs in the lysosomes of the distal tubule cells. Kidney iron concentration does not correlate with tubule fluid iron content but can be prevented from increasing by systemic iron and/or transferrin depletion. This suggests that iron enters the distal tubule cells with transferrin via its receptors from the basolateral side of the distal tubule cells. In association with the increase tubule fluid and kidney iron, there is a marked reduction in kidney selenium and copper content. It is concluded that urinary iron and transferrin losses can vary greatly in different types of experimental renal diseases, and that iron and transferrin can be dissociated in the tubule fluid. The increase iron in association with the reduction of copper and selenium, presumably with their respective enzymes, could place the kidney at risk for injury from free radicals.

Previously there has been little interest in characterizing the renal handling of iron in the nephrotic syndrome. Because of this there have been no experimental studies and only a few clinical studies directed at characterizing the renal handling of iron [1–6]. However, recently it has been suggested that the increased tubule fluid iron present in the nephrotic syndrome, as a result of the glomerular transferrin leak, may be responsible for the tubulo-interstitial disease resulting from glomerular disease [7]. Since iron is delivered to the tubule fluid with transferrin, it seems possible that the type of glomerular disease might be important in determining the amount of transferrin presented to the tubule fluid. This in turn would suggest that the amount of tubule fluid iron could also vary widely between different types of glomerular diseases. Although transferrin's molecular weight of 77,000 is not very different from albumin and it has been used for a marker of the renal handling of albumin [8], there are some major differences between these two proteins. In contrast to albumin, which is a spherical protein having a molecular radius of 36 angstroms, transferrin is globular in configuration with its molecular axes being 28 by 55 angstroms [9]. In addition it has less of a negative charge than albumin. This would suggest that its glomerular leak would be effected much more by alteration in pore size than charge.

Besides the increased tubule fluid iron found in the nephrotic syndrome recent studies have found that renal parenchymal iron is also increased, at least in some proteinuric states [7]. The reason for the increased kidney iron content is unknown in that it has been found not to correlate with the iron level in tubule fluid [7].

This study was undertaken to characterize renal iron metabolism in experimental models of the nephrotic syndrome in rats. Two of the models, adriamycin and puromycin aminonucleoside, were selected because the protein leak in the former has been shown to result from alteration in pore size; in the latter the leak results from alteration in charge [10, 11].

Methods

Uninephrectomized male Sprague-Dawley rats were used in all experimental models of the nephrotic syndrome. Puromycin nephrotic syndrome was induced by the administration of either 33 mg of puromycin aminonucleoside intraperitoneal (6 rats) or 5 mg/100 grams body weight i.v. (10 rats). Adriamycin nephrotic syndrome was induced by administering 7.5 mg/kg body wt of this compound intravenously through the tail vein to eight rats. Nephrotoxic serum nephritis (NSN) was produced in 38 rats by previously described techniques employing rabbit anti-rat glomerular basement membrane serum [12]. In 20 of these rats urine and blood were obtained at 48 days and then killed 59 days following injection of nephrotoxic serum. Six rats with NSN were killed 30 days and the remaining 12 150 days after receiving a less potent nephrotoxic serum for the measurement of kidney copper, iron and selenium levels. Six animals in this latter group were thyroparathyroidectomized (TPTX) prior to receiving nephrotoxic serum [13]. Iron deficiency was induced in one group of animals with nephrotoxic serum nephritis by periodic blood drawing prior to the induction of NSN and maintaining the animals throughout the study period on a low iron diet (AIN-76, ICN, Costa Mesa, California, USA) [7]. The puromycin and adriamycin groups were studied serially over a 42 day period. For urine collections all the animals were placed on a low iron rat chow while in Nalgene metabolic cages to reduce contamination of urine with dietary iron.

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Table 1. Experimental glomerulonephritis

	NSN	Puromycin	Adriamycin
Plasma Cr *mg/dl*	0.97 ± 0.19	0.84 ± 0.19	0.90 ± 0.18
Total urine protein *mg/24 hr*	479 ± 206	558 ± 204	556 ± 124
Serum albumin *mg/dl*	1320 ± 400	1110 ± 250	370 ± 78[a]
FE albumin %	1.68 ± 0.32	1.98 ± 0.87	24 ± 10[a]
FE transferrin %	1.14 ± 0.25	0.62 ± 0.51	25 ± 11[a]

Abbreviation is: FE, fractional excretion.
[a] $P < 0.01$ between groups.

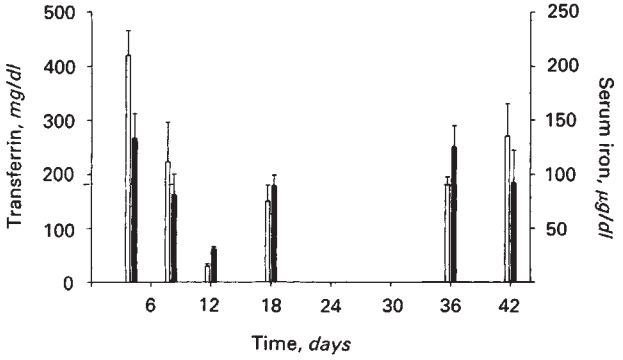


Fig. 1. *Serum iron (solid bars) and transferrin (open bars) in animals with nephrotic syndrome induced by puromycin aminonucleoside.*

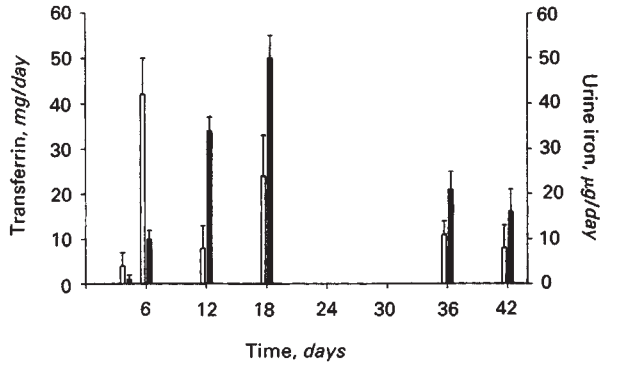


Fig. 2. *Urinary iron (solid bars) and transferrin (open bars) during the course of puromycin-induced nephrotic syndrome.*

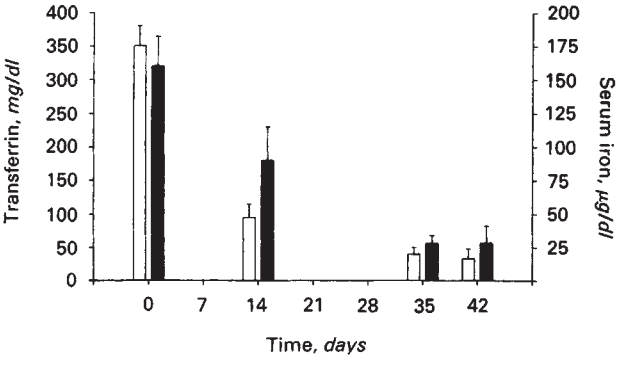


Fig. 3. *Serum iron (solid bars) and transferrin (open bars) during the course of adriamycin-induced nephrotic syndrome.*

Urinary and serum albumin and transferrin were measured by a nephelometric method employing antibodies obtained from Organon Teknika Inc. (Malvern, Pennsylvania, USA).

Serum, urinary and kidney iron determinations were performed with a flamless atomic absorption technique utilizing a model 5000 Perkin-Elmer atomic absorption spectrophotometer equipped with an automatic sampler and Zeeman background correction. Urine and serum creatinine were measured with a Beckman creatinine analyzer. Selenium and copper were measured with an X-ray fluorescence method [14].

For statistical analyses comparison of multiple groups was evaluated by analysis of variance and intragroup comparisons with a paired *t*-test. All values are given as mean ± 1 SD.

Results

The plasma creatinine, urine protein excretion, serum albumin and fractional excretion of albumin and transferrin for the three models of glomerulonephritis are given in Table 1. Plasma creatinine was similar in the three models. In the puromycin and nephrotoxic serum nephritis models fractional excretion of albumin was less than 2% and fractional excretion of transferrin was consistently less than albumin. In contrast, in adriamycin-induced nephrotic syndrome the fractional excretion of transferrin and albumin were equal and greater than 20%. This large protein leak was further manifested by a marked reduction in serum albumin levels (normal 3213 ± 305 mg/dl) in the adriamycin group (Table 1). Urinary protein and albumin excretion was similar in all three groups of animals. Since urinary albumin excretion reflects the rate of albumin production, this suggests that the maximum production rate of albumin had been reached in all groups of animals. Thus the marked difference in plasma albumin levels resulted from the variation in albumin clearance.

The changes in serum and urine transferrin and iron during the course of puromycin induced nephrotic syndrome are shown in Figures 1 and 2. There was a rapid fall in serum iron and transferrin levels following the administration of puromycin. As the nephrotic syndrome abated the levels of transferrin and iron returned toward control values. During the early phase of the study urinary iron:transferrin ratio was approximately half the ratio found in blood (0.28 ± 0.07 vs. 0.47 ± 0.19 μg iron/mg transferrin, $P < 0.05$). In contrast, later in the study during the recovery phase, the iron:transferrin ratio was reversed, being up to 7 times greater in the urine than the blood (Fig. 2). Similarly in the autologous phase of nephrotoxic serum nephritis the urinary iron transferrin ratio was greater in urine than blood (0.97 ± 0.12 vs. 0.64 ± .07 μg iron/mg transferrin, $P < 0.01$).

The changes in serum and urine transferrin and iron during the course of adriamycin-induced nephrotic syndrome are shown in Figures 3 and 4. Serum iron and transferrin rapidly decreased with the induction of the nephrotic syndrome and these changes persisted throughout the study period. This was associated with a continuing urinary loss of iron and transferrin. However, unlike puromycin and nephrotoxic serum nephritis nephrotic syndromes, the iron transferrin losses were relatively constant throughout the study period with the ratio being similar to that found in serum.

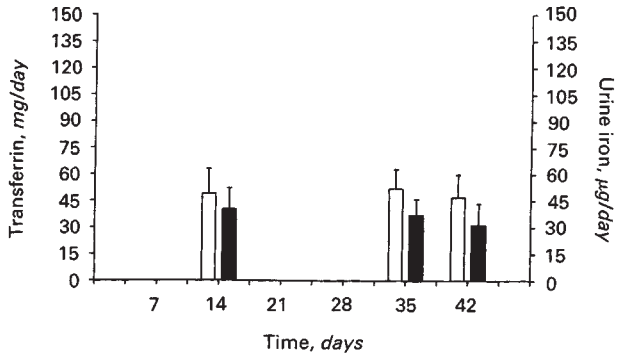


Fig. 4. *Urinary iron (solid bars) and transferrin (open bars) losses throughout the course of adriamycin-induced nephrotic syndrome.*

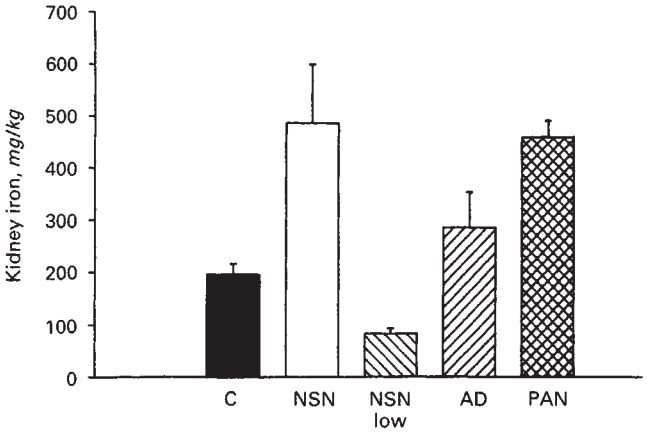


Fig. 5. *Kidney iron concentration in animals with NSN (nephrotoxic serum nephritis), NSN low (nephrotoxic serum nephritis, low iron), Ad (adriamycin), PAN (puromycin aminonucleoside) and C (control).*

The kidney iron concentrations found in the various groups are shown in Figure 5. Kidney iron concentration was markedly increased in the nephrotoxic serum nephritis and puromycin groups ($P < 0.01$). It was slightly increased above normal in the adriamycin group ($P < 0.05$), but this increase in iron was significantly less than that found in the NSN and puromycin groups ($P < 0.01$). In the iron deficient group of animals with NSN kidney, iron was significantly decreased from controls and did not change with induction of NSN. Using prussian blue stain iron was found to be most densely deposited in the distal tubules (Fig. 6) in NSN and puromycin induced nephrosis. In these tubules it was present largely in lysosomes and rarely in the mitochondria (Fig. 7 and 8). In contrast in the proximal tubule iron was present in the cytoplasm and along the brush border (Fig. 6).

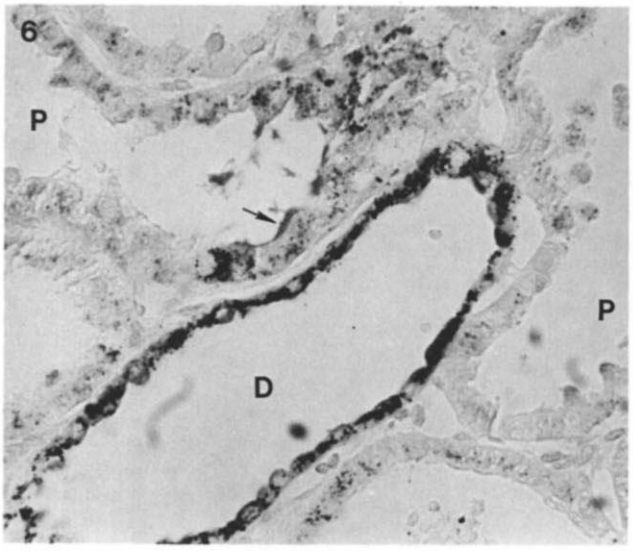
In association with the increase kidney concentration of iron there was also a marked decrease in kidney selenium and copper, as shown in an additional group of animals with nephrotoxic serum nephritis which were killed 30 days and 150 days after receiving nephrotoxic serum (Table 2). It can be further appreciated that kidney iron increased and selenium and copper decreased relatively early during the course of NSN. Thyroparathyroidectomy, a maneuver previously shown to be protective of renal functional deterioration and to reduce proteinuria in NSN also prevented iron accumulation and renal selenium and copper depletion (Table 2). This supports a relationship between the alteration of these three elements and histological injury.

Discussion

These studies document that the renal handling of transferrin can be different in nephrotic syndromes of varying etiologies. In the nephrotic syndrome induced by the aminonucleoside of puromycin, where the defect is felt to result from loss of charge on the glomerular filtration barrier, the fractional excretion of albumin far exceeds that of transferrin [10]. The findings were similar in the autologous phase of nephrotoxic serum nephritis, a model of membranous nephropathy [7]. In adriamycin induced nephropathy the fractional excretion of albumin and transferrin are identical and much greater than the former two types of experimental renal disease. This later type of nephrotic syndrome has been shown to result from an alteration in pore size of the glomerular filtration barrier [11]. These studies also document that transferrin and iron are dissociated in the urine, at least in puromycin induced and NSN nephrotic syndromes. Early during the course of the nephrotic syndrome induced by puromycin, the iron:transferrin ratio in urine is only about 50% of that found in blood. However, later in the course of this disease this ratio in urine increases to up to seven times that found in blood. In fact, during this period the ratio was frequently greater than 1.4 μg iron/mg transferrin. This documents that iron was far in excess of the amount that could be bound by the amount of transferrin present in the urine. This suggests that early in the course of this disease iron is taken up by the tubule cells without transferrin. Later, this iron is released into the tubule fluid probably in association with cellular debris, as suggested by the histological findings in the proximal tubule cells. The most obvious reason for the dissociation of iron and transferrin in the tubule fluid is the reduced pH and bicarbonate content of the tubule fluid as compared to blood [15]. In contrast to NSN and puromycin induced disease, in adriamycin nephrotic syndrome urine and blood iron:transferrin ratios were identical, at least in the stage of the disease studied. It would seem that, unlike the former states, either iron and transferrin are taken up together by the tubules or that neither is taken up by the tubules. In view of the fact that the urine/blood ratio of transferrin and albumin were identical, this would imply that either fractional uptake of these two proteins was the same or more, likely, that neither were removed from tubule fluid. This could suggest that either adriamycin damages the proximal tubule cells or, because of the magnitude of the protein leak, that the capability for cellular uptake is saturated.

These studies also show that serum iron can be reduced in the nephrotic syndrome either as a result of iron deficiency or urinary loss of transferrin. It would appear in puromycin-induced nephrotic syndrome that the early fall in transferrin is a consequence of both the magnitude of protein loss and the inability to acutely increase transferrin production. In contrast, with adriamycin there is a continuing large urinary loss of transferrin throughout the period of study.

It is further shown in immunologically as well as non-immunologically induced experimental nephrotic syndrome that kidney iron concentration can be markedly increased. It



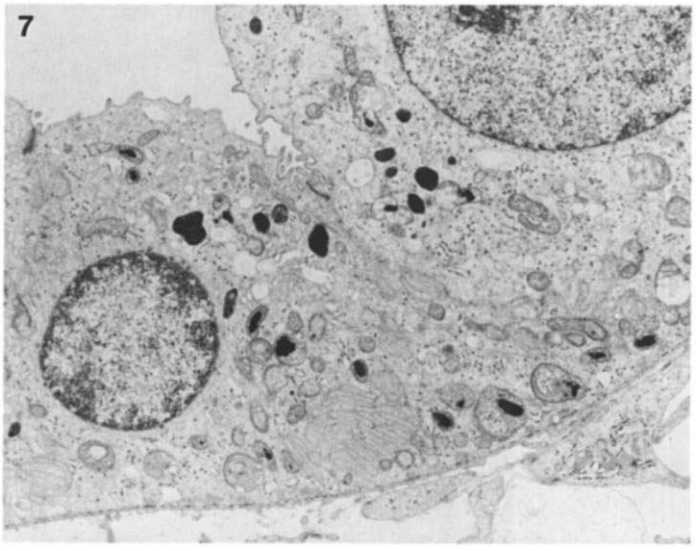


Fig. 6. *Heavy accumulation of ferric iron in the cells of a distal tubule (D).* Smaller amounts of iron are present in the cytoplasm of some of the proximal tubular cells (P) and focally along the brush borders (arrow). Prussian blue stain, ×256.

Fig. 7. *Dense aggregates of iron in lysosomes in distal tubular cells (×11,500).*

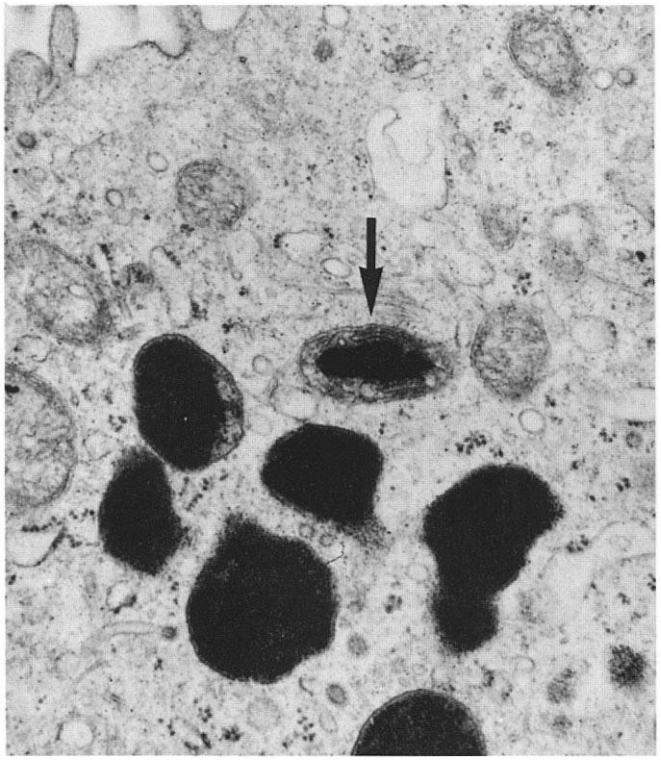


Fig. 8. *Rarely, iron is demonstrated in mitochondria (arrow) as well as in lysosomes in distal tubular cells (×50,750).*

was also found by using prussian blue staining that the largest accumulation of iron occurred in the distal tubule cell. Electron micrographs further demonstrated that iron deposits in the distal nephron were largely present in lysosomes and rarely in the mitochondria. Iron was found to be present in the proximal tubule, but in much less concentration than was found in the distal tubule. Its location also was different, being present mainly in the cytoplasm and at the brush border. In contrast to puromycin and nephrotoxic serum nephritis, kidney iron was only modestly increased in the kidneys obtained from animals with adriamycin induced nephropathy.

Table 2. Kidney elemental alterations

	Controls	NSN 30d	NSN 150d	NSN 150d (TPTX)
Plasma Cr *mg/dl*	0.65 ± 0.2	0.98 ± 0.2[a]	2.33 ± 1.2[a]	0.7 ± 0.2[b]
Urine protein *mg/24 hr*	10 ± 5	255 ± 75[a]	497 ± 137[a]	134 ± 85[a,b]
Iron *mg/kg*	181 ± 36	260 ± 79[a]	488 ± 229[a]	159 ± 39[b]
Selenium *mg/kg*	8.6 ± 0.7	5.6 ± 1.5[a]	3.8 ± 0.8[a]	7.9 ± 1.1[b]
Copper *mg/kg*	37 ± 8	14 ± 4[a]	12 ± 3[a]	25 ± 7[a,b]

Abbreviation is: TPTX, thyroparathyroidectomy.
[a] $P < 0.01$ between controls.
[b] $P < 0.01$ between NSN 150d.

It would appear unlikely that the increased tubule fluid iron is responsible for most of the increased kidney iron content. It has previously been shown in iron deficient animals with nephrotoxic serum nephritis that urine and kidney iron levels can be dissociated [7]. This is further suggested in the adriamycin group of animals, where urine iron was higher and the duration of iron leak more protracted than the other groups, and yet kidney iron was significantly less. Another factor which makes tubule fluid iron an unlikely cause for most of the increased kidney iron is that the majority of the excess iron is deposited in the distal tubule cells. It has been well documented that endocytosis of tubule fluid protein is largely restricted to the proximal tubule, which makes it unlikely that iron was taken up in association with transferrin by the distal tubule cells [16]. Since there are transferrin receptors on the basolateral aspect of the distal tubule cells [17], it seems more likely that the excess iron resulted from transferrin transporting iron intracellularly

via its receptors. The two factors associated with a reduction of kidney iron concentration in the nephrotic syndrome are decreased serum iron levels, as found in iron deficiency [7] and low transferrin levels in association with low iron levels, as found in adriamycin induced nephropathy. Both of these factors would limit the availability of iron to be transported intracellularly. It is unclear at this time why distal tubule iron is increased in the nephrotic syndrome. However, it is possible that this represents a defence system in a diseased kidney, much as that which occurs with infection, where the organism attempts to immobilize iron [18].

It is of additional interest that in association with the increased kidney iron content, there is a marked reduction in kidney selenium and copper levels. In support of the fact that these three elemental alterations are related is that thyroparathyroidectomy, in association with prevention of renal damage in NSN, also prevents renal iron accumulation and copper and selenium depletion. These latter two elements are essential for glutathione peroxidase [19] and supraoxide dismutase activity [20]. Since iron is able to catalyze the Haber-Weiss reaction, the increase in renal and tubule fluid iron in association with a reduction in these elements and their respective enzymes, could markedly predispose the kidney to injury from hydroxyl radicals [21]. This is supported by the fact that tissue concentration of selenium has been shown to correlate closely with glutathione peroxidase activity [19, 22, 23].

In summary, these studies document that the renal handling of transferrin and albumin can vary markedly in different types of experimental glomerular diseases. They further show that iron and transferrin can be dissociated in urine. Although the present studies would be consistent with recent studies suggesting that tubular fluid iron may be important in the pathogenesis of the tubulo-interstitial disease which accompanies glomerular diseases [7], additional studies are required to confirm and extend these observations.

Acknowledgments

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References

1. WILTINK WF, VAN EUK HG, BOBECK-PUTASERT MM, GERBRANDY J, LEUNSE B: Urinary iron excretion in the nephrotic syndrome. *Acta Haemat* 47:269–276, 1972
2. 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
3. ELLIS D: Anemia in the course of the nephrotic syndrome secondary to transferrin depletion. *J Pediatr* 90:953–955, 1977
4. HANCOCK DE, ONSTAD JW, WOLF PL: Transferrin loss into the urine with hypochromic, microcytic anemia. *AJCP* 65L:73–78, 1975
5. 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
6. HAGG JH, SMITH JA, GOLDBERG A: Urinary excretion of iron. *Clin Sci* 30:495–503, 1966
7. ALFREY AC, FROMENT DH, HAMMOND WS: The role of iron in the tubulo-interstitial injury and functional deterioration in nephrotoxic serum nephritis. *Kidney Int* 36:753–759, 1989
8. CAMERON JS, BLANDFORD G: The simple assessment of selectivity in heavy proteinuria. *Lancet* 2:242–247, 1966
9. MORGAN EH: Transferrin, biochemistry, physiology and clinical significance. *Molec Aspects Med* 4:1–123, 1981
10. OLSON JL, RENNKE HG, VENKATACHALAM MA: Alteration in the charge and size selectivity barrier of the glomerular filter in aminonucleoside nephrosis in rats. *Lab Invest* 44:271–279, 1981
11. WEENING JJ, RENNKE HG: Glomerular permeability and polyanion in adriamycin nephrosis in the rat. *Kidney Int* 24:152–159, 1983
12. KARLINSKY ML, HAUT L, BUDDINGTON B, SCHRIER NA, ALFREY AC: Preservation of renal function in experimental glomerulonephritis. *Kidney Int* 17:293–302, 1980
13. 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
14. ALFREY AC, NUNNELLEY LL, RUDOLPH H, SMYTHE WR: Medical applications of a small sample x-ray fluorescence system. *Adv X-ray Anal* 19:497–510, 1976
15. KOEPPEN B, GIEBISCH B, 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
16. CARONE FA, PETERSON DR, OPARIL S, PULLMAN TN: Renal tubular transport and catabolism of proteins and peptides. *Kidney Int* 16:271–278, 1979
17. FULLER SD, SIMONS K: Transferrin receptor polarity and recycling accuracy in "tight" and "leaky" strains of Madin-Darby canine kidney cells. *J Cell Biology* 103:1767–1769, 1986
18. WEINBERG ED: Infection and iron metabolism. *Am J Clin Nutr* 30:1485–1490, 1977
19. BEHNE D, WOLTERS W: Distribution of selenium and glutathione peroxidase in the rat. *J Nutr* 113:456–461, 1983
20. GOLDSTEIN S, CZAPSKI G: The role and mechanism of metal ions and their complexes in enhancing damage in biological systems or in protecting these systems from the toxicity of O_2^-. *J Free Radicals Biol Med* 2:3–11, 1986
21. HALLIWELL B, GUTTERIDGE MC: Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem J* 219:1–14, 1984
22. PLEBAN PA, KERKAY J, PEARSON KH: Cadmium, copper, lead, manganese and selenium levels and glutathione peroxidase activity in human kidney cortex. *Anal Lett* 14:1089–1109, 1981
23. HAFEMAN DG, SUNDE RA, HOEKSTRA WG: Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. *J Nutr* 104:580–587, 1974