Effects of Postural Changes upon Urinary Excretion of Protein in Renal Disease

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In chronic glomerulonephritis with hypercreatininemia (Group 1), there was a significant correlation between the changes in urinary protein excretion ($U_{prot} \cdot V$) and those in GFR during both quiet standing and walking. In chronic glomerulonephritis without hypercreatininemia (Group 2), there was a significant correlation between the changes in $U_{prot} \cdot V$ and those in GFR during quiet standing, but no correlation during walking. In nephrotic syndrome with definite evidence of chronic glomerulonephritis on renal biopsy (Group 3), there seemed to be a good correlation between the changes in $U_{prot} \cdot V$ and those in GFR during both quiet standing and walking, but in nephrotic syndrome with minimal changes on renal biopsy (Group 4), no correlation was found during both quiet standing and walking.

Relative clearances of urinary protein estimated by acrylamide gel electrophoresis and immunodiffusion method did not differ among supine bed rest, quiet standing and walking, although urinary protein excretion was either increased or decreased by postural changes.

Relative clearances of urinary protein showed that permeability of protein through the glomerular membrane was more selective in Group 2 than in Group 1 of chronic glomerulonephritis, and that also more selective in Group 4 than in Group 3 of nephrotic syndrome.

The changes in protein excretion by postural changes were explained by the concurrent decrease of GFR in Groups 1 and 2 of chronic glomerulonephritis, and also in Group 3 of nephrotic syndrome.

On the other hand, the alterations by postural changes in tubular reabsorptive capacity for protein might determine to some extent protein escape into urine in Group 4 of nephrotic syndrome.

The reports by King,¹ and Lathem and his associates² have been considered to be the representative studies on the effect of posture upon urinary protein excretion in renal diseases. But the results of their studies were not the same. King¹ reported that protein excretion increased on quiet standing in the majority of 27 patients with relatively mild renal diseases. Lathem and his associates² reported that the excretion of urinary protein decreased by 49% on the average in quiet standing and the fall in protein excretion was highly significant in 19 patients with acute and chronic renal diseases. This difference may be explained.

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by the fact that their studies were made on a small group of patients with various
renal diseases of variable severity.

The present study was undertaken to clarify further the mechanism of
changes in protein excretion by postural changes, and its clinical significance in
renal disease.

The effects of quiet standing and walking upon renal excretion of protein, and
analyses of urinary protein by acrylamide gel electrophoresis and immunodiffusion
method were made in 47 patients with proteinuria due to primary renal disease.

MATERIALS AND METHODS

The studies were carried out on 47 (33 males and 14 females) hospitalized
patients with proteinuria due to primary renal disease.

Table 1 summarizes the main clinical and laboratory findings.

There were 4 children, ranging in age from 9 to 14 years, and 43 adults,
ranging in age from 16 to 61 years. None was bedfast.

| Table 1. Clinical and laboratory findings of patients with proteinuria |

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cases</td>
<td>13 (8,5)</td>
<td>20 (15,5)</td>
<td>5 (3,2)</td>
<td>9 (7,2)</td>
</tr>
<tr>
<td>Urinary protein g/day</td>
<td>1-5</td>
<td>1-6</td>
<td>5-20</td>
<td>3-28</td>
</tr>
<tr>
<td>Plasmas protein g/100ml</td>
<td>6.0-7.2</td>
<td>5.5-8.6</td>
<td>4.2-5.0</td>
<td>4.0-6.0</td>
</tr>
<tr>
<td>plasma (mean)</td>
<td>6.3</td>
<td>6.7</td>
<td>4.7</td>
<td>6.5*</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>110-266</td>
<td>120-250</td>
<td>270-520</td>
<td>244-632</td>
</tr>
<tr>
<td>mg/100 ml (mean)</td>
<td>(185)</td>
<td>(190)</td>
<td>(423)</td>
<td>(434)</td>
</tr>
<tr>
<td>Edema</td>
<td>(-)-(+)</td>
<td>(-)-(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>GFR ml/min</td>
<td>6-44</td>
<td>57-136</td>
<td>69-108</td>
<td>65-144</td>
</tr>
<tr>
<td>(mean)</td>
<td>(22)</td>
<td>(83)</td>
<td>(70)</td>
<td>(87)</td>
</tr>
<tr>
<td>BUN mg/100 ml</td>
<td>36-72</td>
<td>10-27</td>
<td>8-24</td>
<td>7-16</td>
</tr>
<tr>
<td>(mean)</td>
<td>(52)</td>
<td>(15)</td>
<td>(17)</td>
<td>(13)</td>
</tr>
<tr>
<td>Plasma creatinine mg/100ml</td>
<td>2.0-11.0</td>
<td>0.8-1.6</td>
<td>0.7-1.6</td>
<td>0.9-1.6</td>
</tr>
<tr>
<td>(mean)</td>
<td>(4,7)</td>
<td>(1.1)</td>
<td>(1.2)</td>
<td>(1.0)</td>
</tr>
<tr>
<td>Kidney biopsy</td>
<td>Chronic glomerulonephritis</td>
<td>Chronic glomerulonephritis</td>
<td>Chronic glomerulonephritis</td>
<td>Minimal changes</td>
</tr>
</tbody>
</table>

* Chronic sclerosing glomerulonephritis in 3 cases, membranous glomerulonephritis in 1
case, and combined proliferative and membranous glomerulonephritis in 1 case.
† Prednisolone was administered immediately after the onset of heavy proteinuria,
edema and hypercholesterolemia.
Postural Changes and Urinary Protein Excretion

By a simplified clinical and histopathologic classification, the patients could be assigned to one of the following four groups:

Group 1: Chronic glomerulonephritis with hypercreatininemia.

Group 2: Chronic glomerulonephritis of variable severity, but without hypercreatininemia.

Group 3: Nephrotic syndrome, showing definite chronic glomerulonephritic changes on renal biopsy, and without hypercreatininemia.

Group 4: Nephrotic syndrome, showing minimal changes on renal biopsy, and without hypercreatininemia.

There were 13 patients in Group 1, 20 in Group 2, 5 in Group 3, and 9 in Group 4.

All patients in Groups 3 and 4 fulfilled the accepted criteria for the diagnosis of the nephrotic syndrome, namely, marked proteinuria, hypoproteinemia, edema and hypercholesterolemia. But none in Groups 1 and 2 presented the nephrotic syndrome.

Percutaneous renal biopsies, using a Franklin-Silverman biopsy needle, were performed in all patients but a few in Group 1.

The experiments were done in the morning on the fasting patients in supine bed rest since previous night. All patients were asked to take 300 ml of water at 7.30 a.m. and to empty their bladders at 8.00 a.m.

Then they drank 100 ml each of water 3 times for every consecutive 30 minutes, resting supine in bed for the first 30 minutes, quietly standing for the next 30 minutes, and then walking about for the last 30 minutes.

Urine specimens were collected every 30 minutes by voluntary voiding. Heparinized plasma was obtained just in the midtime of each urine collection. Patients who felt faint or developed orthostatic hypotension were excluded.

Endogenous creatinine clearance for the glomerular filtration rate (GFR), urinary excretion of protein (Uprot • V) and renal clearance of protein were estimated on each clearance period.

Creatinine concentration in plasma and urine was estimated by Brod and Sirota's method and concentration of protein in plasma and urine was determined by the biuret method. Urinary proteins were precipitated by 10% trichloroacetic acid and the precipitate was re-dissolved in 3% NaOH for protein estimation.

Electrophoretic analysis of plasma and urinary protein was carried out on acrylamide gel having molecular sieving effect, at 200 to 300 volts and 28 mA for a period of 6 hours. Tris-buffer of pH 8.9 was employed. After staining with amidoblack 10-B, the strips of acrylamide gel were scanned by Lumicon type 2-P densitometer at a wave length of 570 millimicrons. Urinary clearance of albumin expressed as percentage of creatinine clearance, and relative clearance of three proteins, namely, transferrin, alpha-1 globulin, and high molecular protein consisting largely of gammaglobulin, expressed as percentage of albumin clearance, were calculated.

Urinary clearances of IgG, IgA and IgM expressed as percentage of transferrin
clearance were assessed on samples of heparinized plasma and untimed urine by immunodiffusion method\textsuperscript{8} using a commercially prepared immunoplate (Human immunoplate, Hyland Laboratories, U.S.A.).

In order to increase the protein concentration of urine to levels sufficient to permit accurate evaluation by acrylamide gel electrophoresis and immunodiffusion method, collected urine was dialysed at a temperature of 4°C in Visking cellophane bags against 30% polyvinylpyrrolidone. Dialysis was continued until the protein concentration in the urine approximated 60 mg/ml.

**Results**

1) *Chronic glomerulonephritis (Groups 1 and 2)*

GFR: During quiet standing GFR diminished in 32 of 33 cases of chronic glomerulonephritis (in all of 13 in Group 1, and 19 of 20 in Group 2). On the average, GFR decreased by 23%, with a range of 3 to 52% of the values of supine bed rest.

During walking GFR diminished in 31 of 33 cases of chronic glomerulonephritis (in all of 13 in Group 1, and 18 of 20 in Group 2). On the average, GFR decreased

![Fig. 1. Relationship between the changes in $U_{prot}$ and those in GFR during quiet standing in chronic glomerulonephritis.](image)

- Chronic glomerulonephritis with hypercreatininemia (Group 1).
- Chronic glomerulonephritis without hypercreatininemia (Group 2).
Postural Changes and Urinary Protein Excretion

Fig. 2. Relationship between the changes in $U_{\text{prot}} \cdot V$ and those in GFR during walking in chronic glomerulonephritis.
- Chronic glomerulonephritis with hypercreatininemia (Group 1).
- Chronic glomerulonephritis without hypercreatininemia (Group 2).

by 21% with a range of 0.5 to 52% of the values of supine bed rest.
Both during quiet standing and during walking, the changes in GFR tended to be greater in patients with severe renal functional impairment than in those without it.

Protein excretion: During quiet standing the excretion of protein in the urine ($U_{\text{prot}} \cdot V$) decreased in 28 of 33 cases of chronic glomerulonephritis (in 12 of 13 in Group 1, and in 16 of 20 in Group 2).
On the average, $U_{\text{prot}} \cdot V$ decreased by 19% during quiet standing, a value corresponding closely to the change in GFR.

There was a highly significant correlation between the changes in $U_{\text{prot}} \cdot V$ and those in GFR (in Group 1, $r=0.723$, $p<0.005$; in Group 2, $r=0.583$, $p<0.01$) as illustrated in Fig. 1.

During walking $U_{\text{prot}} \cdot V$ decreased in 23 of 33 cases of chronic glomerulonephritis (in 12 of 13 in Group 1, and in 11 of 20 in Group 2).

There was no significant correlation between the changes in $U_{\text{prot}} \cdot V$ and those
Fig. 3. Relationship between the changes in $\text{U}_{\text{prot}} \cdot \text{V}$ and those in GFR during quiet standing in nephrotic syndrome.

- Nephrotic syndrome, showing definite chronic glomerulonephritis on renal biopsy (Group 3).
- Nephrotic syndrome, showing minimal changes on renal biopsy (Group 4).

In GRF ($r=0.383$, $p<0.05$), and urinary excretion of protein tended to increase despite of decreased GFR in Group 2.

On the other hand, in Group 1 there was a significant correlation between the changes in $\text{U}_{\text{prot}} \cdot \text{V}$ and those in GFR ($r=0.721$, $p<0.005$) as illustrated in Fig. 2.

2) Nephrotic syndrome (Groups 3 and 4)

In Group 4 of nephrotic syndrome there was no direct correlation between the changes in $\text{U}_{\text{prot}} \cdot \text{V}$ and those in GFR both during quiet standing and during walking as compared with supine bed rest, and $\text{U}_{\text{prot}} \cdot \text{V}$ increased despite of decreased GFR in the majority. But, in Group 3 of nephrotic syndrome the changes in $\text{U}_{\text{prot}} \cdot \text{V}$ seemed to be correlated with those in GFR both during quiet standing and during walking as illustrated in Figs. 3 and 4.

3) Acrylamide gel electrophoresis

Satisfactory acrylamide gel electrophoretic patterns of plasma protein and urinary protein were obtained in 21 patients (6 in Group 1, 7 in Group 2, 3 in Group
Postural Changes and Urinary Protein Excretion

Fig. 4. Relationship between the changes in $U_{prot}\cdot V$ and those in GFR during walking in nephrotic syndrome.

- Nephrotic syndrome, showing definite chronic glomerulonephritis on renal biopsy (Group 3).
- Nephrotic syndrome, showing minimal changes on renal biopsy (Group 4).

3, and 5 in Group 4).

The electrophoretic patterns of urinary protein did not differ among supine bed rest, quiet standing and walking, although urinary excretion of protein was either decreased or increased by postural changes. Clearance of albumin expressed as percentage of creatinine clearance and three relative protein clearances, namely, transferrin, alpha-1 globulin, and high molecular protein consisting largely of gammaglobulin, expressed as percentage of albumin clearance, showed no definite differences among supine bed rest, quiet standing and walking as illustrated in Figs. 5–9.

The value of each relative protein clearance except for high molecular protein clearance did not differ between Groups 1 and 2 of chronic glomerulonephritis, and also did not differ between Groups 3 and 4 of nephrotic syndrome.

The values of clearance of high molecular protein consisting largely of gammaglobulin were higher in Group 1 than those in Group 2, and also higher in Group 3 than those in Group 4 as illustrated in Figs. 7 and 9.
Fig. 5. Acrylamide-gel electrophoresis of urine. Urine was obtained from a patient with chronic glomerulonephritis with hypercreatininemia. H, high molecular protein consisting largely of gamma-globulin. T, transferrin A, albumin

Fig. 6. Albumin clearance (% of creatinine clearance) in chronic glomerulonephritis.
- Chronic glomerulonephritis (Group 1).
- Chronic glomerulonephritis with hypercreatininemia (Group 2).

4) Immunodiffusion method

Analyses of urinary protein by the immunodiffusion method were obtained in 13 patients (3 in Group 1, 2 in Group 2, 5 in Group 3, and 3 in Group 4). The representative example of immunodiffusion is illustrated in Fig. 9. Relative IgG
Fig. 7. Relative globulin clearance (% of albumin clearance) in chronic glomerulonephritis. 
A, alpha-1 globulin clearance (% of albumin clearance). 
B, transferrin clearance (% of albumin clearance). 
C, high molecular protein clearance (% of albumin clearance). 
- Chronic glomerulonephritis with hypercreatininemia (Group 1). 
- Chronic glomerulonephritis without hypercreatininemia (Group 2).

Fig. 8. Albumin clearance (% of creatinine clearance) in nephrotic syndrome. 
- Nephrotic syndrome, showing definite chronic glomerulonephritis on renal biopsy (Group 3). 
- Nephrotic syndrome, showing minimal changes on renal biopsy (Group 4).
Fig. 9. Relative globulin clearance (% of albumin clearance) in nephrotic syndrome.
A, alpha-1 globulin clearance (% of albumin clearance).
B, transferrin clearance (% of albumin clearance).
B, high molecular protein clearance (% of albumin clearance).
- Nephrotic syndrome, showing definite chronic glomerulonephritis on renal biopsy (Group 3).
- Nephrotic syndrome, showing minimal changes on renal biopsy (Group 4).

Fig. 10. Radial diffusion of IgG (top), IgA (middle), IgM (bottom) of urine. Urine was obtained from a patient with chronic glomerulonephritis with hypercreatininemia.
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Fig. 11. Relative IgG and IgA clearance (% of transferrin clearance) in chronic glomerulonephritis.
- Chronic glomerulonephritis with hypercreatininemia (Group 1).
- Chronic glomerulonephritis without hypercreatininemia (Group 2).

Fig. 12. Relative IgG and IgA clearance (% of transferrin clearance) in nephrotic syndrome.
- Nephrotic syndrome, showing definite chronic glomerulonephritis on renal biopsy (Group 3).
- Nephrotic syndrome, showing minimal changes on renal biopsy (Group 4).
and IgA clearance expressed as percentage of transferrin clearance showed no
definite differences among three clearance periods, although urinary excretion of
protein was either increased or decreased by postural changes as illustrated in Figs.
11 and 12. The value of relative IgG clearance was about the same as that of
relative IgA clearance when assessed on the same urine of the same patient.

The values of relative IgG and IgA clearance did not differ between Groups 1
and 2, but IgM was found in the urine of some patients in Group 2 as illustrated
in Fig. 10.

In nephrotic syndrome, the values of relative IgG and IgA clearance were
demonstrably higher in Group 3 than those in Group 4 as illustrated in Fig. 12.
Furthermore, the values of relative IgG and IgA clearance were evidently higher in
Group 3 of nephrotic syndrome than those in Groups 1 and 2 of chronic glomerulo-
nephritis.

DISCUSSION

According to current views, the protein in urine originates from process of
glomerular filtration followed by non-selective tubular re-absorption.9,10 And an
increased glomerular permeability is generally considered to be the major deter-
minant of protein excretion in renal diseases.6,11

Brun12 reported that the upright position induced widespread vasoconstriction
involving the renal vasculature and led to decrease of GFR.

King1 reported that protein excretion increased in the majority of the patients
with relatively mild renal diseases on quiet standing and alterations in glomerular
pressure might be one of the significant determinants of protein excretion by the
kidney. On the other hand, Lathem and his associates2 reported that, on the
average, the excretion of urinary protein decreased by 49% on quiet standing, a
value corresponding closely to the average changes in inulin clearance.

The experiments reported here showed that urinary excretion of protein
decreased to about the same extent as GFR on quiet standing in chronic glomerulo-
nephritis of variable severity (Groups 1 and 2).

Furthermore, acrylamide gel electrophoresis and immunodiffusion method failed
to detect any definite changes in urinary protein patterns and relative protein
clearances between supine bed rest and quiet standing. These results suggest
that the permeability of glomerular basement membrane remains at approximately
constant levels both during supine bed rest and during upright position. Hence,
inha-renal vasoconstriction appeared to affect protein out-put by reducing GFR
rather than by changing glomerular permeability in chronic glomerulonephritis.

The previous reports of electrophoretic patterns of urinary protein seemed to
bear no relation to the stage or severity of chronic glomerulonephritis2,13,14 Further-
more, the studies of glomerular permeability in chronic glomerulonephritis by
immunochemical methods have been reported probably only for chronic glomerulo-
nephritic patients with nephrotic syndrome.8,15,16

The author observed that acrylamide gel electrophoresis showed a clear
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difference in relative clearance of high molecular protein between chronic glomerulonephritis with hypercreatininemia and chronic glomerulonephritis without hypercreatininemia; namely, high value of high molecular protein clearance was observed in the former.

Furthermore, IgM, a very high molecular protein which was considered to be absent in urine, was found by immunodiffusion method in some cases with hypercreatininemia. These observations give good evidence of high porosity and poor selectivity of protein in glomerular basement membrane in advanced chronic glomerulonephritis.

In nephrotic syndrome, protein clearance calculated by acrylamide gel electrophoresis and immunodiffusion method failed to show any definite difference in each patient among three clearance periods, namely, supine bed rest, quiet standing and walking. But relative clearance of high molecular protein by acrylamide gel electrophoresis and relative IgG and IgA clearance by immunodiffusion method were demonstrably higher in Group 3 than those in Group 4 of nephrotic syndrome. This fact fits well the recent results by the specific immunochemical technique, which demonstrate the presence of selective permeability of protein in nephrotic syndrome with minimal changes.

In Group 4 of nephrotic syndrome, protein clearance calculated by acrylamide gel electrophoresis and immunodiffusion method failed to show any definite difference in each patient among three clearance periods, namely, supine bed rest, quiet standing and walking. But relative clearance of high molecular protein by acrylamide gel electrophoresis and relative IgG and IgA clearance by immunodiffusion method were demonstrably higher in Group 3 than those in Group 4 of nephrotic syndrome. This fact fits well the recent results by the specific immunochemical technique, which demonstrate the presence of selective permeability of protein in nephrotic syndrome with minimal changes.

In Group 4 of nephrotic syndrome, there was no correlation between the changes in $U_{prot} \cdot V$ and those in GFR. On the other hand, there was a good correlation between the changes in $U_{prot} \cdot V$ and those in GFR in Group 3 of nephrotic syndrome. Therefore, the changes in urinary excretion of protein in Group 3 can be explained by the decrease in GFR as well as in Groups 1 and 2 of chronic glomerulonephritis.

The fact that values of relative IgG and IgA clearance were demonstrably higher in Group 3 than those in Groups 1 and 2 is very interesting and may give some approach to the mechanism of nephrotic syndrome due to chronic glomerulonephritis.

This study was not designed to solve the question of the relative contribution of glomerular and tubular impairments to proteinuria in the renal diseases. Furthermore, the true mechanism of proteinuria remains still obscure because of the lack of technique for estimating clinically the changes in tubular reabsorptive capacity by postural changes during a short clearance period.

But the following results were obtained in nephrotic syndrome with minimal anatomical changes (Group 4): firstly, no correlation between the changes in $U_{prot} \cdot V$ and those in GFR, secondly, increased urinary excretion of protein despite of decreased GFR during postural changes in the majority, and thirdly, no definite changes in protein clearances during postural changes. These observations suggest that factors other than changes in GFR, for example, the alterations in tubular reabsorptive capacity by postural changes may determine to some extent protein escape into urine in nephrotic syndrome with minimal anatomical changes.

Similar findings were also obtained in some of Group 2 and in orthostatic proteinuria (unpublished data), where slight changes were found in renal function
and histology.\textsuperscript{18,19} This may be explained by the same theory mentioned above.

\textbf{Acknowledgment}

I wish to express my thanks to Prof. T. Torikai, Dr. T. Furuyama and Dr. C. Suzuki for their guidance in performing this study.

\textbf{References}