Tamm-Horsfall Protein Antibody In Patients with End-Stage Kidney Disease

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Circulating antibody to Tamm-Horsfall protein (THP) was measured using a radioimmunoassay in forty-five patients on maintenance hemodialysis and compared to levels of antibody titers measured in sera from ten healthy controls. The etiology of the end-stage kidney disease in the patient population was polycystic kidney disease in thirteen, glomerulonephritis in fourteen, diabetic nephropathy in nine, interstitial nephritis and chronic pyelonephritis in three each, multiple myeloma in two, and urinary tract obstruction in one. Four patients had significantly elevated titers of antibody to THP but shared no other unifying characteristics. The results also indicate that none of the groups studied had mean antibody titers significantly different from controls. Furthermore, no general trend was apparent between levels of antibody to THP and number of months on dialysis.

Observations made during the study revealed that heparinized samples of blood had lower titers of antibody to THP than did non-heparinized samples from the same patient. This finding was repeated when other anti-coagulants, i.e., ethylenediaminetetraacetate (EDTA) and sodium citrate, were used. Titers returned toward normal when CaCl2 was added back to samples anti-coagulated with EDTA and sodium citrate. This suggests that clotting factors, probably fibrinogen, interfered with the measurement of antibody titers. Therefore, only serum should be used in further investigations of THP antibody using this assay.

INTRODUCTION

Since its discovery and characterization by Tamm and Horsfall in 1950 [1], the importance of the 80,000 molecular weight urinary glycoprotein, known as Tamm-Horsfall protein (THP), remains unknown. Only the cells lining the ascending loop of Henle and distal convoluted tubule synthesize this protein, which is secreted into the tubular lumen [2,3]. No physiological role for the protein is known although THP is known to inhibit hemagglutination caused by influenza, mumps, and Newcastle disease virus [1], but has no effect on bacteria [4]. Approximately 50 mg of THP is excreted per day in human urine. In the presence of low pH or high salt concentration in the urine, the protein aggregates and forms casts [4–6].

Recent work suggests that THP may play a role in some pathological processes affecting the kidney including calculi formation [7] and other precipitates which may cause microscopic tubular obstruction [8]. Also THP may be involved in the pathogenesis of some interstitial diseases of the kidney. Intrarenal deposits of THP have been described in medullary cystic disease [9], chronic pyelonephritis, and hydronephrosis [9,10] as well as in end-stage reflux nephropathy. Furthermore, rats immunized with homologous THP develop an immune complex tubulo-interstitial...
nephritis characterized by mononuclear cell infiltrates and immune globulin deposition at the tubular location where THP is synthesized [11]. Nevertheless, the role of THP in the pathogenesis of these diseases has not been clearly defined.

Previous work by others in humans and experimental animals has shown that severe urinary tract obstruction and vesicoureteral reflux may cause reflux of urine into the venous and lymphatic circulation as well as into the renal parenchyma [12–14]. Consequently, the potential development of a circulating antibody response to substances found only in the urine, such as THP, might permit early non-invasive diagnosis of these conditions [12]. In fact, abnormal circulating antibodies to THP have been detected in experimental animals with vesicoureteral reflux [12], in schoolage girls with acute symptomatic upper urinary tract infection [15], and, by earlier work from this laboratory, in adults with acute urinary tract obstruction and infection [16]. Although the role of circulating antibodies to THP in the pathogenesis of these acute diseases remains unclear, severe vesicoureteral reflux and urinary tract obstruction can lead to silent renal destruction and end-stage renal disease in some patients [16–20]. Except for acute pyelonephritis, urinary tract obstruction, and vesicoureteral reflux, antibody to THP has not been determined previously in patients with other types of renal involvement, and in particular in patients with end-stage renal disease. Therefore, such a study seemed warranted in order to try to better understand the role, if any, of THP antibody in the pathogenesis of these diseases.

The present report describes THP antibody in patients with end-stage renal disease on maintenance hemodialysis, with diagnoses of urinary tract obstruction, interstitial nephritis, and chronic pyelonephritis, and compares them with patients with other causes of end-stage renal disease including polycystic kidney disease, glomerulonephritis, and diabetic nephropathy, as well as healthy control subjects. Specifically, our purpose was to determine whether elevated titers of antibody to THP were also present in patients with chronic renal disease caused by different pathologic processes, as well as whether antibody titers correlated with specific types of renal injury.

MATERIALS AND METHODS

Patient Selection

A total of forty-five chronic hemodialysis patients at Yale–New Haven Hospital and the West Haven Veterans Administration Hospital participated in this study after giving informed consent. The diagnosis of renal failure in each patient was based on clinical, radiological, or histological evidence. In addition, information about urinary tract infection (UTI), current and past medications, and length of time on dialysis was obtained from the patient’s hospital record.

Patients were placed into groups based upon the etiology of their renal failure. These groups included: (1) thirteen patients with polycystic kidney disease (PKD) in whom the diagnosis was made by intravenous pyelography (IVP) or laparotomy; (2) nine patients with diabetic nephropathy (DN), in whom the diagnosis was made on clinical evidence of diabetes mellitus and sequellae including cataracts and retinopathy in eight, and in one a history of diabetes and a biopsy which showed interstitial nephritis; (3) fourteen patients with glomerulonephritis (GN), eleven of whom had biopsies, which showed membranoproliferative GN in three, membranous GN in two, chronic progressive hypocomplementemic GN in one, and chronic GN (CGN) in five, and in the remaining three patients without biopsy, the diagnosis of CGN was based on a history of acute GN which progressed to renal failure in one, a history of beta hemolytic streptococcal infection followed by hematuria, albuminuria, and progressive renal failure in another, and a history of skin infections leading to
progressive renal failure in the third; (4) six patients with the diagnosis of chronic pyelonephritis (CPN) or interstitial nephritis (IN), based on a history of phenacetin abuse in one, neomycin-induced deafness and renal failure in one, biopsy in two, and by renal scan and pyelography in the other two; (5) one patient with urinary tract obstruction (UTO) diagnosed by IVP; and (6) two patients with multiple myeloma (secretory type) who developed renal failure with progression of their disease.

**Blood Collection**

All patients received heparin during dialysis with an initial bolus of 5000 U followed by three hourly 1000 U doses until dialysis ended after four hours. Heparin antagonists were not given to any of the patients.

Blood was obtained from each patient for determination of anti-THP antibody titers. In order to evaluate the effect of dialysis on titers of anti-THP antibody, 5 ml samples of blood were obtained from participants pre- and post-dialysis. The samples from Yale–New Haven Hospital were collected in plain vacutainers and allowed to clot at room temperature for one hour before serum was removed. The samples from the West Haven Veterans Administration Hospital were collected in heparinized vacutainers (approximately 120U Hep) and the plasma removed immediately. Sodium azide was added to each specimen for bacteriostasis.

**Urine**

Clean catch urine specimens were obtained for culture from all patients who were not anuric. Quantitative urine cultures were performed by the standard pour plate technique. Bacteria recovered were identified by the scheme of Schaub and Foley [21].

**THP Antibody**

Serum antibody titers to THP were determined by a radioimmunoassay initially developed in this laboratory and described in detail in previous publications [16,22]. The assay depends on binding specific antibody to a solid phase antigen (THP adsorbed to a microtiter plate). The subsequent identification of bound antibody was achieved by adding $^{125}$I labeled protein A which binds to the Fc region of IgG [23].

Serial dilutions of rabbit sera containing known amounts of specific antibody to human THP were assayed simultaneously with the patient samples to obtain a standard curve for conversion of counts per minute to ng antibody/ml.

**Heparin Effect**

Preliminary results indicated that the heparinized samples consistently contained lower anti-THP titers than unheparinized samples. Further studies were done to evaluate the effect of heparin on the assay.

Heparinized and non-heparinized samples from medical student volunteers were obtained to evaluate the effect of heparin on freshly collected samples. The non-heparinized samples were allowed to clot for one hour and the serum was then removed. The heparinized samples were immediately centrifuged and plasma removed. All samples received sodium azide as a preservative.

To evaluate the effect heparin might have directly on antigen-antibody binding in the assay, heparin, in amounts approximately equal to 5, 10, 50, and 100 times the calculated concentration of heparin in patients’ serum immediately following dialysis, was added to serum from a rabbit immunized to human THP. The amount of heparin added was calculated by approximating the heparin T$^{1/2}$ in serum at 60
minutes [24] for the amounts used. Samples containing rabbit serum and heparin were then evaluated for anti-THP activity and compared to serum from the same rabbit to which no heparin was added.

In order to determine whether heparin exerted a specific effect, or if the inhibition observed was a result of some other plasma component, i.e., fibrinogen, blood samples anticoagulated with ethylenediaminetetraacetate (EDTA) and sodium citrate were compared to samples which were heparinized and to samples which were not anticoagulated. Four samples of blood were taken from each of ten volunteers; one sample without anticoagulant, one with heparin, one with EDTA, and one with sodium citrate. As a further study to evaluate the effect of fibrinogen on the assay, samples collected with EDTA and citrate were defibrinated by adding back CaCl₂. This was done by incubating samples and CaCl₂ (final concentration in samples 0.025M) for one hour at 37°C and then at 4°C for twelve hours [25]. The resulting clot was removed and the samples assayed.

Statistical Methods

Significantly elevated titers were defined as antibody titers greater than the mean level plus two standard deviations observed in healthy control subjects [16]. Independent groups were compared using T statistics for independent data, heparin vs. serum comparisons were made using the paired T test.

RESULTS

THP Antibody

The titer of Tamm-Horsfall antibody in the serum of patients with end-stage renal disease and in healthy volunteers is shown in Fig. 1 and is expressed in cpm as well as ng/ml of antibody. The mean anti-THP antibody titer in ten healthy subjects was 1050 ng/ml and the mean plus two standard deviations was 1920 ng/ml. Any titer
above this value was considered significantly elevated. The mean anti-THP antibody titer in sera from the thirteen patients with PCKD was \(721 \pm 341\) ng/ml and none was significantly elevated. The mean value of anti-THP antibody in sera from fourteen patients with GN was \(1348 \pm 1503\) ng/ml, which included two patients with significantly elevated titers of 5132 and 4437 ng/ml. The mean titer in sera from nine patients with DN was \(1328 \pm 1161\) ng/ml and included one patient with a titer of 4329 ng/ml. The mean antibody titer in sera from the three patients with CPN was \(1485 \pm 338\) ng/ml and none was significantly elevated. The mean titer in sera from the three patients with IN was \(1591 \pm 506\) ng/ml and included a significantly elevated titer of 2045 ng/ml in one patient. The mean titer in sera from the two patients with multiple myeloma (MM) was \(340 \pm 37\) ng/ml and was the lowest for any single diagnostic group. The serum from the one patient with UTO gave a titer of 1090 ng/ml.

The mean values for anti-THP antibody for each of the groups were not significantly different from the mean value for healthy subjects. The highest mean of 1591 ng/ml was seen in patients with IN, followed by 1485 ng/ml in patients with CPN, 1348 ng/ml in GN, 1328 ng/ml in DN, 1090 ng/ml in UTO, 721 ng/ml in PCKD, and 340 ng/ml in MM. Urine cultures were obtained from twenty-three patients, twelve of whom had significant bacteriuria. Urine cultures could not be obtained from twenty-two patients who were anuric. Sera from the four patients with *E. coli* bacteriuria had a mean titer of \(1640 \pm 1280\) ng/ml of anti-THP antibody. Sera from the eight other patients with infected urine had a mean anti-THP antibody titer of \(876 \pm 257\) ng/ml. Sera from the eleven patients with sterile urine had a mean titer of \(850 \pm 376\) ng/ml and the twenty-two patients who were anuric had a mean titer of \(1180 \pm 775\) ng/ml. Individual results appear in Fig. 2. None of the groups of patients was significantly different from the healthy subjects. Of the four patients with significantly elevated titers, one had an *E. coli* UTI and the other three were anuric.

![FIG. 2. Measurement of antibody to Tamm-Horsfall Protein (THP) in cpm in a 1:8 dilution of serum and calculated ng/ml of antibody in serum from patients with end-stage kidney disease who had significant bacteriuria (>\(10^5\) organism/ml), sterile urine, or anuria, as compared to abacteriuric controls. Left and right ordinates as in Fig. 1.](image-url)
The chemical data and antibody levels to Tamm-Horsfall protein in these forty-five patients with end-stage renal disease are shown in Table 1.

The relationship of each patient’s serum anti-THP antibody titer to the number of months on dialysis at the time of this study is shown in Fig. 3. Figures 4–7 show the same function for each diagnostic group, PCKD (Fig. 4); DN (Fig. 5); GN (Fig. 6); CPN (Fig. 7) and IN (Fig. 7). Significantly elevated titers were observed in 1/11 patients dialyzed for twenty months or less and in 3/14 patients dialyzed between twenty and forty months. None of the other patients dialyzed for forty months or longer had elevated titers.

**Effect of Heparin**

Pre-dialysis samples of blood obtained from patients at Yale–New Haven Hospital were considered free of heparin effect since blood was obtained 48 hours after previous dialysis and heparinization. Pre-dialysis samples (non-heparinized at Yale–New Haven Hospital, heparinized at the West Haven Veterans Administration Hospital) and post-dialysis samples (heparinized during dialysis at both hospitals) were compared by hospital. There is a suggestion of significant differences between means of pre- (1237 ng/ml) and post- (1081 ng/ml) samples at Yale–New Haven Hospital ($p = .03$) while there is no significant difference between pre- (637 ng/ml) and post- (666 ng/ml) samples at the West Haven Veterans Administration Hospital ($p = 0.64$).

Figure 8 presents results of serum and plasma anti-THP titers in ten volunteers expressed in cpm. Each volunteer gave two samples of blood, one heparinized and one not heparinized. The mean antibody titer for non-heparinized samples was 7530 cpm ± 4040 cpm, and the mean antibody titer in heparinized samples was 5590 cpm ± 2780 cpm (Table 2). These means are significantly different ($p = 0.003$).
TABLE 1
Clinical Data and Serum Levels of Antibody to Tamm-Horsfall Protein in 45 Patients with End-Stage Kidney Disease

| Patient No. | (Age, Sex) | Dialysis | Urine Culture | Months on Dialysis | THP Antibody (Pre-Dialysis) ng/ml |
|-------------|------------|----------|---------------|--------------------|---------------------------------|
|             |            | Pre-cpm  | Post-cpm  | Δ% |                          |                                 |
|             |            |          |            |     |                          |                                 |
|             |            |           |            |     |                          |                                 |
| PCKD-YNHH: |            |           |            |     |                          |                                 |
| 1           | (63, F)    | 2,150    | 2,650     | +23| Enterobacter             | 39                              | 477                            |
| 2           | (54, M)    | 2,070    | 2,820     | +26| sterile                  | 34                              | 455                            |
| 3           | (60, M)    | 2,770    | 3,090     | +11| anuric                   | 48                              | 655                            |
| 4           | (49, M)    | 2,920    | 3,040     | +4 | anuric                   | 29                              | 700                            |
| 5           | (64, F)    | 3,030    | 2,460     | -19| anuric                   | 31                              | 733                            |
| 6           | (60, F)    | 2,390    | 2,830     | +19| anuric                   | 48                              | 544                            |
| 7           | (65, F)    | 5,720    | 4,380     | -24| diphtheroids             | 47                              | 1,625                          |
| 8           | (42, M)    | 3,080    | 1,850     | -40| anuric                   | 22                              | 748                            |
| 9           | (36, F)    | 3,890    | 3,280     | -16| diphtheroids             | 14                              | 1,002                          |
| 10          | (20, M)    | 2,260    | 3,020     | +33| anuric                   | 37                              | 508                            |
| WHVAAH:     |            |           |            |     |                          |                                 |                                |
| 11          | (59, M)    | 2,390    | 2,120     | -11| E. coli                 | 12                              | 544                            |
| 12          | (52, M)    | 4,030    | 3,300     | -18| anuric                   | 106                             | 1,048                          |
| 13          | (58, M)    | 1,630    | 2,270     | +39| anuric                   | 60                              | 337                            |
|             |            | mean = 2,950 | mean = 2,837 |  |                          |                                  | mean = 721                     |
|             |            | s.d. = 1,080 | s.d. = 642 |  |                          |                                  | s.d. = 341                     |
| DNVNH:      |            |           |            |     |                          |                                 |                                |
| 14          | (60, F)    | 12,500   | 9,730     | -22| anuric                   | 31                              | 4,329                          |
| 15          | (37, M)    | 3,930    | 3,250     | -17| sterile                  | 40                              | 1,015                          |
| 16          | (71, F)    | 4,400    | 4,330     | -2 | E. coli                 | 38                              | 1,170                          |
| 17          | (49, F)    | 4,210    | 3,540     | -16| anuric                   | 10                              | 1,107                          |
| 18          | (59, F)    | 3,010    | 2,890     | -4 | anuric                   | 96                              | 727                            |
| 19          | (57, F)    | 1,950    | 1,650     | -15| anuric                   | 43                              | 422                            |
| 20          | (49, F)    | 3,330    | 4,400     | +32| anuric                   | 19                              | 825                            |
| Patient No. (Age, Sex) | Dialysis | Urine Culture | Months on Dialysis | THP Antibody (Pre-Dialysis) ng/ml |
|------------------------|----------|---------------|--------------------|-----------------------------------|
|                        | Pre-cpm* | Post-cpm      | Δ%                 |                                   |
| **WHVAH:**             |          |               |                    |                                   |
| 21 (64, M)             | 5,140    | 5,330         | +4                 | anuric                            |
| 22 (61, M)             | 3,670    | 4,174         | +14                | diphtheroids                      |
| mean = 4,683           | mean = 4,370 |
| s.d. = 3,068           | s.d. = 2,270 |
| **GN-YNHH:**           |          |               |                    |                                   |
| 23 (28, M)             | 14,318   | 10,040        | -30                | anuric                            |
| 24 (28, M)             | 3,930    | 3,310         | +26                | sterile                           |
| 25 (32, M)             | 4,030    | 3,900         | -3                 | anuric                            |
| 26 (26, M)             | 2,460    | 4,650         | +90                | anuric                            |
| 27 (24, F)             | 3,250    | 3,620         | +11                | anuric                            |
| 28 (35, F)             | 5,360    | 5,470         | +2                 | anuric                            |
| 29 (47, M)             | 2,890    | 1,810         | -37                | sterile                           |
| 30 (19, F)             | 12,750   | 11,000        | -14                | E. coli                           |
| **GN-WHVAH:**          |          |               |                    |                                   |
| 31 (59, M)             | 1,810    | 2,640         | +45                | S. aureus                         |
| 32 (57, M)             | 1,900    | 1,410         | -26                | sterile                           |
| 33 (54, M)             | 4,400    | 3,690         | -16                | anuric                            |
| 34 (55, M)             | 1,860    | 1,750         | -6                 | sterile                           |
| 35 (53, M)             | 3,930    | 4,460         | +13                | anuric                            |
| 36 (43, M)             | 1,550    | 1,350         | -13                | E. coli                           |
| mean = 4,015           | mean = 4,170 |
| s.d. = 4,470           | s.d. = 2,980 |
**CPN-YNHH:**

| No | Age | CR    | CR Fluctuation | Count | S.D. |
|----|-----|-------|----------------|-------|------|
| 37 | 59  | 6,020 | -3             | sterile | 42   | 1,733 |
| 38 | 41  | 5,710 | -6             | sterile | 49   | 1,622 |
| 39 | 34  | 4,190 | -10            | anuric  | 48   | 1,100 |

Mean = 5,300, S.D. = 976

**IN-YNHH:**

| No | Age | CR    | CR Fluctuation | Count | S.D. |
|----|-----|-------|----------------|-------|------|
| 40 | 43  | 6,870 | -22            | anuric | 31   | 2,045 |
| 41 | 59  | 5,880 | -27            | sterile | 77   | 1,682 |

Mean = 4,990, S.D. = 1,060

**Whvah:**

| No | Age | CR    | CR Fluctuation | Count | S.D. |
|----|-----|-------|----------------|-------|------|
| 42 | 50  | 4,020 | -24            | Enterococcus | 124 | 1,045 |

Mean = 5,590, S.D. = 1,450

**MM-YNHH:**

| No | Age | CR    | CR Fluctuation | Count | S.D. |
|----|-----|-------|----------------|-------|------|
| 43 | 49  | 1,740 | -43            | sterile | 21   | 366  |

**Whvah:**

| No | Age | CR    | CR Fluctuation | Count | S.D. |
|----|-----|-------|----------------|-------|------|
| 44 | 51  | 1,540 | -21            | Klebsiella | 24 | 314  |

Mean = 1,640, S.D. = 1,110

**UTO-YNHH:**

| No | Age | CR    | CR Fluctuation | Count | S.D. |
|----|-----|-------|----------------|-------|------|
| 45 | 21  | 4,170 | -11            | Proteus | 20   | 1,090 |

Mean = 340, S.D. = 37

*Counts per minute*
The effect of adding heparin to hyperimmune rabbit serum is shown in Fig. 9. The final concentration of added heparin was 5, 10, 50, and 100 times the calculated concentration of heparin in patients' serum at the end of dialysis. THP antibody levels remained the same in hyperimmune rabbit serum regardless of the concentration of added heparin, which suggests that the heparin molecule itself does not affect the method for measuring antibody.

*Effect of Other Anticoagulants*

The effect of other anticoagulants on the level of Tamm-Horsfall antibody in serum was studied in ten healthy subjects, each of whom contributed four freshly drawn samples of blood. Either heparin, sodium citrate, EDTA, or no anticoagulant (control) was added to one of the samples from each subject, after which the level of antibody was determined. Calcium chloride was then added to each sample and the antibody level was measured again. The results are presented in Table 3 and show a
significant difference in measured antibody titers between control serum (non-anticoagulated) and sodium citrated blood ($p = 0.003$), between control serum and blood anticoagulated with EDTA ($p = 0.004$), as well as between control serum and heparinized blood ($p = 0.01$). All three anticoagulants significantly reduced the titer of measured antibody. However, the addition of calcium chloride to blood anticoagulated with sodium citrate resulted in a return of measured antibody titer toward normal. Although the antibody titer in citrated blood to which calcium chloride had been added was lower than that observed in control serum with ($p = 0.01$) or without ($p = 0.02$) calcium chloride, it was significantly higher than that observed in citrated
FIG. 8. Measurement of antibody to Tamm-Horsfall Protein (THP) in cpm in a 1:8 dilution of serum in non-heparinized and heparinized samples from volunteers. Bar graphs represent one standard deviation.

TABLE 2
Levels of Antibody to Tamm-Horsfall Protein (THP) in Heparinized and Non-Heparinized Serum Samples Collected from Healthy Subjects

| Subject | Non-Heparinized | Heparinized | Δ% |
|---------|----------------|-------------|----|
| KB      | 5,140          | 3,270       | -36|
| GC      | 4,340          | 4,290       | -1 |
| BF      | 2,800          | 2,490       | -11|
| ML      | 11,090         | 6,430       | -42|
| BS      | 5,320          | 4,730       | -11|
| JW      | 5,180          | 4,930       | -5 |
| DB      | 9,970          | 7,400       | -25|
| JB      | 16,310         | 12,280      | -25|
| JK      | 6,510          | 3,980       | -39|
| BS      | 8,660          | 6,030       | -30|

|          | mean = 7,530   | mean = 5,590 |    |
|          | s.d. = 4,040   | s.d. = 2,780  |    |

*cpm = counts per minute
TABLE 3
The Effect of Three Anticoagulants With and Without Calcium Chloride on Tamm-Horsfall Protein Antibody Levels in Healthy Subjects

| Subject | Control | Citrate | EDTA | Heparin | Control +CaCl₂ | Citrate +CaCl₂ | EDTA +CaCl₂ | Heparin +CaCl₂ |
|---------|---------|---------|------|---------|----------------|----------------|--------------|----------------|
| AM      | 3570*   | 2500    | 2280 | 2230    | 2380           | 2100           | 2340         | 1860           |
| SD      | 2630    | 2230    | 2610 | 2400    | 2950           | 2135           | 2890         | 2120           |
| TM      | 2710    | 2334    | 2400 | 2250    | 3150           | 2820           | 3070         | 2590           |
| BC      | 3620    | 2590    | 2820 | 3090    | 3200           | 3190           | 3170         | 2820           |
| RD      | 1830    | 1430    | 1510 | 1780    | 2130           | 1970           | 1990         | 1560           |
| JS      | 2950    | 2300    | 2330 | 2660    | 3020           | 2560           | 2680         | 2410           |
| SD      | 5120    | 3670    | 4300 | 3770    | 3770           | 3370           | 4130         | 4070           |
| JW      | 3180    | 2060    | 2380 | 3090    | 3290           | 3360           | 4050         | 3070           |
| SS      | 6290    | 4070    | 5310 | 4180    | 5200           | 4530           | 6480         | 5310           |
| DB      | 7020    | 4040    | 4560 | 4230    | 5390           | 5410           | 4270         | 4120           |

MEAN = 3890
S.D. = 1690

*Counts per minute of I¹²⁵

blood without calcium chloride ($p = 0.05$). Similar results were observed when calcium chloride was added to blood anticoagulated with EDTA. In fact, there was no significant difference in antibody titer between control serum with ($p = 0.78$) or without ($p = 0.27$) calcium chloride and EDTA anticoagulated blood to which calcium chloride had been added, whereas there was a significantly higher antibody titer in EDTA plus calcium chloride treated blood as compared with blood anticoagulated with EDTA alone ($p = 0.04$). In contrast, the addition of calcium chloride to blood anticoagulated with heparin did not change the measured antibody titer ($p = 0.87$). Specifically, antibody titers remained significantly lower in heparinized blood to which calcium chloride was added as compared with control blood with ($p = 0.01$) or without ($p = 0.01$) calcium chloride (Table 3).

FIG. 9. Measurement of antibody to Tamm-Horsfall Protein (THP) in cpm in a 1:8 dilution of immune rabbit serum with heparin added to make final concentrations 1, 5, 10, 50, and 100 times the concentration of heparin in the serum of patients at completion of dialysis.
DISCUSSION

Previous attempts to identify antibody to kidney tissue in patients with pyelonephritis and chronic nephritis using complement fixation [26], latex agglutination [27], and hemagglutination [28] have been unsuccessful. However, those assays were less sensitive than the present one and could not identify circulating antibody in normal controls. Using the present assay, Marier et al. [16] demonstrated small amounts of antibody to THP in normal subjects and significantly elevated titers in patients with acute urinary tract obstruction and infection. Hanson et al. [15] also observed elevated titers of antibody to THP in 9/10 school-age girls with acute symptomatic upper urinary tract infection using an enzyme linked immuno-absorbent assay.

The objective, in the present study, was to determine, using our new assay, whether elevated titers of antibody to THP were present in patients with various forms of end-stage kidney disease. The results of the present study indicate that four of the forty-five patients with chronic renal disease had significantly elevated titers of antibody to THP, but they did not share any other unifying characteristic. Two patients with glomerulonephritis dialyzed for three and twenty-six months, respectively, had elevated titers, one of whom also had an E. coli urinary tract infection, though the other was anuric. One patient with diabetic nephropathy dialyzed for thirty-one months, had elevated titers and was anuric, and another anuric patient with elevated titers and also dialyzed for thirty-one months, had kidney failure based on long-standing interstitial nephritis. The present work indicates that none of the groups studied had mean antibody titers significantly different from controls. Furthermore, in all groups of patients studied, no general trend was apparent between levels of antibody to THP and number of months on dialysis.

The present data do not exclude the possibility of elevated antibody titers at some point in the progression of various types of kidney disease, but this study indicates that titers of antibody to THP are not useful in diagnosing the cause or presence of end-stage kidney disease. The patients reported in the present study have end-stage renal disease and have been on dialysis for periods of one month to ten years. The failure to detect elevated antibody titers in many of these patients might have been expected since these patients were not likely to have active or progressive renal disease at the time of study, and elevated antibody titers may be dependent on active kidney disease. If this concept is true, then our observations would suggest that further study to identify patients with elevated titers of antibody should involve patients with active kidney disease. Such studies should include sequential determinations to better define the time course of this antibody response.

During the course of these studies we observed a significant difference in antibody titers between pre- and post-dialysis samples of blood obtained from patients at the Yale–New Haven Hospital but not in samples obtained from patients at the West Haven Veterans Administration Hospital. The pre-dialysis samples (serum) from patients at Yale–New Haven Hospital were not anti-coagulated, while the samples (plasma) from patients at the West Haven Veterans Administration Hospital were anti-coagulated with heparin. Post-dialysis samples (plasma) at both hospitals were collected identically and both were heparinized. These results suggest two important findings: first, heparin interferes in some way with the quantitative measurement of circulating antibody to THP, which explains the difference noted between pre (serum) and post (plasma) dialysis samples obtained from patients at Yale–New Haven Hospital; and second, dialysis has no effect on the quantity of circulating antibody since pre- and post-dialysis samples (plasma) obtained from patients at the West Haven Veterans Administration Hospital were the same. Further studies were
performed to attempt to clarify the effect of heparin on the assay. Specifically, heparin was added directly to hyperimmune serum obtained from rabbits immunized with human THP and no changes in antibody titers were noted. These results suggested that the heparin molecule, which exists as a heterogenous assortment of polymeric chains [29], did not directly interfere with the quantification of antibody to THP when that antibody was in serum obtained from clotted blood. That is, heparin did not appear to interfere directly with antigen-antibody binding but instead seemed to act through or because of some substance present in plasma and not present in serum, such as fibrinogen or heparin activated anti-thrombin III. Additional experiments with other anti-coagulants, i.e., EDTA and sodium citrate, further showed that the inhibition on lowered levels of measured antibody to THP observed in heparinized samples (plasma) also occurred when other anti-coagulants were used to collect the blood and the assay was performed with the plasma obtained from these samples. Antibody levels in samples of blood collected with these two chelators of calcium returned near to control values when fibrinogen was removed by the addition of CaCl$_2$ to the samples, but did not return toward normal when CaCl$_2$ was added to heparinized samples. These results suggested that anti-coagulants interfere indirectly with the quantification of circulating antibody to THP and that clotting factors are probably the source of this interference. Since calcium chelators halt the clotting cascade by preventing conversion of prothrombin to thrombin (a calcium-dependent step), which also prevents conversion of fibrinogen to fibrin, and heparin also stops the clotting cascade by inhibiting certain activated factors: XIa [30], IXa [31], Xa [32], and thrombin [33], and since the defect could be corrected by the addition of CaCl$_2$ to EDTA and citrated but not heparinized samples, the protein most likely to interfere with this assay appears to be fibrinogen. Fibrinogen is the most plentiful of the coagulation proteins and is preserved when all the above anti-coagulants are used. These results indicate that anti-coagulated blood should be avoided in future use of this assay to determine THP antibody levels, in order to prevent obtaining spuriously low levels of THP antibody.

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