We measured daily excretion rates for urinary protein and the ratios of urinary protein to creatinine in 24-h urines and in untimed urines in 60 healthy adults, 30 patients with kidney disease, and 22 kidney-transplant recipients. The ratios for urinary protein/creatinine, mg/g, in untimed urines and in 24-h urines from the same subjects were closely correlated (r = 0.97) for rates of protein excretion ranging from normal (mean 44 mg/day) to nephrotic (maximum 19 300 mg/day). Because urinary protein/creatinine in healthy subjects never exceeded 100 mg/g, we propose that a ratio of < 100 mg/g in untimed urines, obtained in the absence of exercise, fever, or other evidence of urinary tract disease, is a criterion of normal kidney function. Among patients with nephrotic syndrome (urinary protein excretion rate ≥ 4000 mg/day), urinary protein/creatinine ratios always exceeded 2000 mg/g in both 24-h and untimed urines. Intermediate urinary protein/creatinine ratios (100 to 2000 mg/g) may reflect any type of kidney disease.

Additional Keyphrases: 24-h vs untimed urine collections • cut-off values • nephrotic syndrome • economics of laboratory operation • kidney disease

Measurements of daily urinary protein excretion, plus measurement of urinary creatinine to assure completeness of the urine collection, are commonly used in the diagnosis and follow-up of patients with kidney disease (1–3). Patients often find it inconvenient and difficult to collect accurate and complete 24-h urine collections; moreover, measurement of the urine volume is a relatively time-consuming laboratory operation. Previous studies suggest that the urinary protein/creatinine concentration ratio in random (untimed) urines accurately reflects 24-h urinary protein excretion (4–6). We wished to re-evaluate whether the use of the protein/creatinine concentration ratio in an untimed specimen would serve as well as a 24-h urine collection to distinguish pathological from normal proteinuria, and to differentiate nephrotic range proteinuria, reflecting glomerular disease, from less severe but still abnormal rates of protein excretion, which might reflect any type of kidney disease.

Materials and Methods

Patients

We studied the following groups of subjects:

- Sixty healthy subjects without kidney disease, 21 women and 39 men. Twenty-nine of these collected both a 24-h urine and an untimed urine on the following day; 14 collected only 24-h urines; 17 collected only untimed urines. Sixteen of the healthy subjects collected 24-h urines on four to 10 (average seven) occasions while maintaining their usual activities; they ate constant diets in the Medical

College of Wisconsin Clinical Research Center while participating in other metabolic studies.
- Thirty patients with kidney disease who collected 24-h urines and also provided an untimed urine specimen the following day. In these patients, concentrations of serum creatinine ranged from 7 to 115 mg/L. Twenty-four had primary glomerular diseases, documented by kidney biopsy in 18 of them (four with membranous nephropathy, three with minimal glomerular changes, four with focal glomerulosclerosis, four with proliferative glomerulonephritis, one with membranoproliferative glomerulonephritis, four with chronic glomerulonephritis of unknown type, one with amyloidosis, two with benign hematuria and erythrocyte casts, and one with systemic lupus erythematosus). One had vasculitis and glomerulitis. Two patients had vascular or tubulointerstitial kidney disease, and three had autosomal dominant polycystic kidney disease.
- Twenty-two patients who had received kidney grafts after having developed end-stage renal disease of various types. These patients collected 24-h urines and provided an untimed urine specimen the following day. Six of these patients had received kidney grafts from a related donor, and 16 had received cadaveric grafts. We studied these patients two months to seven years after kidney transplantation; their concentrations of serum creatinine ranged from 8 to 45 mg/L. Four of the patients had undergone bilateral nephrectomy before transplantation; the remainder retained their native diseased kidneys.

Materials

Coomassie Brilliant Blue G-250 was purchased from Sigma Chemical Co., St. Louis, MO 63178. We prepared the Coomassie Blue reagent according to Bradford (7), then stabilized it by adding 2 mL of 1% Triton X-100 surfactant per liter of reagent; the absorbance of this reagent at 465 nm remained unchanged for one year.

We prepared standard solutions of protein by diluting a serum pool, the protein concentration of which had been established by the biuret method (8), with isotonic saline (NaCl 9 g/L) containing 0.5 g of NaNO2 per liter. To establish the long-term precision of the urinary protein assay, we used commercially prepared lyophilized controls.

The volume of the timed urine specimens was measured either directly or by weighing specimens that had been collected in tared containers, assuming a density of 1.010 kg/L.

Method

We used a Rotochem IIa centrifugal analyzer (American Instrument Co., Silver Spring, MD 20902) with 20 µL of sample and 500 µL of Coomassie Blue reagent. Five protein standard solutions (50, 150, 400, 800, and 1200 mg/L) were included in each run. The absorbance of the standards (measured at 600 nm) was corrected for the absorbance of the reagent blank, prepared by substituting saline for sample. Absorbance was measured 5 min after the reaction was initiated. Because the absorbance-concentration relationship is nonlinear, we used a five-parameter polynomial
for calculating the protein concentration in urine. This polynomial, made available to us by Dr. David Rhoads (9), is:

\[ \ln C = a + b (R - R_o) + c(R - R_o)^2 + d(R - R_o)^3 \]

where \( C \) is the concentration of the standard, \( R \) is the absorbance of the standard or sample, \( R_o \) is the absorbance of the reagent blank, and \( a, b, c, \) and \( d \) are parameters that define the model. We selected this model from log-log or exponential models because it provided the least mean squared error. (Note: for urinary protein concentrations <50 mg/g (below that of the lowest standard), results must be calculated manually, i.e., by use of the absolute absorbances of the urine specimen and the 50 mg/g standard.) Urinary and serum creatinine concentrations were measured either by the alkaline picrate method (AutoAnalyzer; Technicon Instruments Corp., Tarrytown, NY) or with the Beckman Astra analyzer (Beckman Instruments, Inc.).

Results were presented as group means ± SD. Linear regressions were calculated by least squares.

Results
Assay Precision

The day-to-day SDs for the two controls were 11 and 30 mg/L at urinary protein concentrations of 80 and 790 mg/L, respectively.

Urinary Protein Excretion in Healthy Adults

The distribution of values for urinary protein excretion in 24-h urines and in untimed urines is shown in Figure 1. Total urinary protein excretion in health averaged 44 (SD 27) mg/day (range 11–115). The mean excretion rates were similar in both sexes: 42 (SD 26) mg/day for the 20 women and 46 (SD 30) mg/day for the 23 men. Although daily protein excretion rates, 24-h protein/creatinine ratios, and protein/creatinine ratios for untimed urine specimens were not normally distributed (Figure 1), urinary protein in healthy subjects seldom (in only two of the 43 subjects) exceeded 100 mg/day. The mean ± SD values for protein/creatinine ratio in 24-h urines and in untimed urines were similar: 31 ± 21 (range 4–101) and 27 ± 18 (range 7–103, n = 46) mg/g, respectively.

Day-to-day variation. Among the subset of 16 healthy adults (eight women, eight men) for whom we measured protein excretion repetitively, urinary protein excretion averaged 47 (SD 21) mg/day (range 17–98), a value similar to that for all the healthy subjects. We consider these repetitive urine collections to be complete, the average individual CVs for daily creatinine excretion being only 5.0%. However, the intra-individual variation in protein excretion was as high as the inter-individual variation. Within individuals, CVs ranged from 4% to 107% (average 41%) similar to inter-subject CV of 60% (see above). For these 16 subjects, the protein/creatinine ratio in 24-h urines averaged 33 (SD 20) mg/g (range 7–66), which was also similar to the values for the entire group.

Protein/Creatinine Ratios in Untimed Daytime Urines and 24-h Urines

Figure 2, plotted on log-log scales because of the broad range of values, compares the urinary protein/creatinine ratios, mg/g, for each subject’s untimed urine with that for the 24-h urine from the same subject. Considering all ranges of urinary protein excretion, from normal to nephrotic, the correlation was remarkably close: ratio (random) = 1.04 × ratio (24-h) + 42 mg/g; \( r = 0.97 \).

As both Figures show, none of the healthy subjects exhibited protein/creatinine ratios >100 mg/g in either 24-h urines or untimed urines. The mean + 2 SD for protein/creatinine ratios was 73 mg/g in 24-h urines, 63 mg/g in the untimed urines. Such ratios correspond to urine protein concentrations of 100 mg/L or less when creatinine concentrations in urine are 1000 mg/L (about 100-fold greater than the creatinine concentrations in serum in health).

Eleven of the patients with kidney disease and one of the kidney-transplant patients, who had developed recurrent membranous nephropathy in the graft (proven by biopsy), met the generally accepted clinical criteria for proteinuria in the nephrotic range, namely, a daily urinary protein excretion rate ≥4 g or 3.5 g/1.73 m² or a theoretical albumin clearance >150 mL/day (assumed that all of the urinary protein was albumin and that no albumin that was filtered across the glomeruli was reabsorbed by the renal tubules). These 12 patients averaged urinary protein excretion rates of 7.5 g/day and protein/creatinine ratios of 6800 and 7500 mg/g in 24-h and untimed urines, respectively. In all 12 of these patients, the protein/creatinine ratios exceeded 2000 mg/g. An additional four patients with biopsy-proven glomerular diseases and urinary protein excretion averaging 3.0 g/day also exhibited protein/creatinine ratios ≥2000 mg/g (average, 2500 mg/g) for their 24-h urines; in three of these four patients the protein/creatinine ratios for untimed urine exceeded 2000 mg/g (average, 2850 mg/g).

Discussion

On the basis of many previous studies, it is generally accepted that urinary protein excretion in healthy adults does not exceed 150 to 200 mg/day in the absence of exercise or intercurrent acute febrile illness (1). Our observation that
urinary protein excretion did not exceed 125 mg/day (Figure 1) in 43 healthy men and women is in agreement with this.

The present studies also extend and confirm observations showing that the ratio of protein to creatinine in an untimed urine, obtained during the day when a healthy subject is upright and active (10), can also be used to assist in the laboratory definition of normal kidney function. In the earlier study Shaw et al. (5) reported the mean + 2 SD for the protein/creatinine ratio for untimed urines from 10 healthy subjects was 106 mg/g; the mean + 2 SD for the ratio in 32 hospitalized patients without kidney disease was 112 mg/g. The highest individual ratio in those two groups was 120 mg/g. Similarly, among the 30 normal subjects studied by Ginsberg et al. (6), the mean + 2 SD for the protein/creatinine ratio in untimed urines was 77 mg/g; the greatest ratio being 170 mg/g. In our group, the mean + 2 SD for the protein/creatinine ratio in untimed urine from 49 healthy subjects was 63 mg/g (greatest ratio, 103 mg/g). Collectively, these data support the view that a urinary protein/creatinine ratio of 100 mg/g in an untimed urine is an acceptable quantitative upper limit of normal. Lower urinary protein/creatinine ratios for untimed urines are thus evidence against the presence of significant kidney disease.

Our finding that the urinary protein/creatinine ratio in untimed urines is closely correlated to the urine protein/creatinine ratio in 24-h urines confirms reports by Shaw et al. (5) and Ginsberg et al. (6). The latter observed that the protein/creatinine ratio in untimed urines was correlated to

24-h urinary protein excretion per 1.73 m², i.e., after correcting for surface area, which also corrects for body mass and thus for creatinine excretion. Collectively, therefore, these data suggest that laboratory evidence of significant kidney disease can be regarded as a protein/creatinine ratio >100 mg/g in an untimed urine. This avoids the need for having patients collect 24-h urines (unless other urinary constituents require measurement) and for measuring 24-h urine volumes in the laboratory. Furthermore, for sequential follow-up of patients with established kidney disease, measurement of the urinary protein/creatinine ratio in an untimed urine can detect decreasing or increasing proteinuria as an indication of improving or worsening kidney disease.

Finally, the observation that patients with biopsy-proven glomerular diseases and nephrotic-range proteinuria ≥4 g/day always exhibited urine protein/creatinine ratios ≥2000 mg/g in both untimed and 24-h urines leads us to suggest that a protein/creatinine ratio of 2000 mg/g for untimed urine can be used as an alternative definition of nephrotic-range proteinuria in adults.

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