Differentiation of Glomerular, Tubular, and Normal Proteinuria: Determinations of Urinary Excretion of β_2-Microglobulin, Albumin, and Total Protein

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ABSTRACT A low molecular weight β_2-globulin (β_2-microglobulin), albumin, and total protein were measured in concentrated 24-hr urine specimens from 20 healthy subjects and 30 patients with clinical proteinuria of glomerular or tubular type. Classification of proteinuria was made on the basis of clinical diagnosis and size distribution of urinary proteins after gel chromatography. The molecular radii (Stokes' radii) of β_2-microglobulin and albumin, estimated by gel chromatography, were 15 A and 35 A.

The average 24-hr urinary excretion in healthy subjects was 0.12 mg for β_2-microglobulin, 10 mg for albumin, and 80 mg for total protein. The patients with renal glomerular disorders had normal or only somewhat increased excretion of β_2-microglobulin, despite considerably increased excretion of albumin and total protein. Most of the patients with tubular dysfunction excreted large amounts of β_2-microglobulin, although they excreted normal or only slightly increased amounts of albumin and only moderately increased quantities of total protein. Consequently, the ratio or urinary albumin/urinary β_2-microglobulin was high in glomerular proteinuria (1100:14,200), intermediate in normal proteinuria (33:163), and low in tubular proteinuria (1.0:13.3). Determinations of urinary clearances of β_2-microglobulin and albumin in four healthy subjects and 11 patients indicated that increased excretions of the two proteins were associated with increased clearances. The results suggest that quantitative determinations of urinary β_2-microglobulin and urinary albumin may be useful for detecting disorders of the renal handling of plasma proteins. The findings also seem to suggest a selective tubular reabsorption of the two proteins.

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Estimates on sera revealed a close correlation between serum levels of β_2-microglobulin and creatinine and also a greatly raised serum concentration of β_2-microglobulin after bilateral nephrectomy.

INTRODUCTION

Approximately 30 different plasma protein components have been identified in the urine of healthy individuals by means of qualitative and quantitative immunochemical methods (1–4). Plasma proteins of small molecular size seem to be preferentially excreted into normal urine (*cf.* references 1 and 2) although molecular size probably is not the only factor of importance for the renal handling of plasma proteins (4). Butler and Flynn (5) demonstrated a large difference between the composition of urinary proteins excreted in renal glomerular and in renal tubular disorders; they distinguished between glomerular proteinuria and tubular proteinuria. Creeth et al. (6) showed that the mean molecular size of the urinary proteins is considerably smaller in tubular than in glomerular proteinuria. Later studies (7–11) have suggested that most or all of the low molecular weight urinary proteins characteristic of tubular proteinuria originate from plasma.

An account is given in this paper of a quantitative study on the excretion of a recently described low molecular weight plasma protein, termed β_2-microglobulin (7, 12), and on the excretion of albumin and total protein in urine of healthy individuals and subjects with clinical proteinuria.

It appeared to be of special interest to compare the excretion of a very small plasma protein, like β_2-microglobulin (mol wt 11,800), with that of a relatively large protein, like albumin (mol wt 69,000), in different types

of clinical conditions affecting renal function. The results suggest that measurements of urinary β_2-microglobulin and urinary albumin may be useful for detecting disorders of the renal glomerular or tubular handling of plasma proteins. The findings also seem to indicate a selective tubular reabsorption of the two proteins.

METHODS

Urine and blood collection. 24-hr urine specimens were collected from 20 healthy male and female volunteers and from 30 patients with manifest or suspected clinical proteinuria. The females delivered urine between menstruations. Collections were carried out as described previously (12). Blood sera were obtained at the end of the urine collections from four normal subjects and 13 of the patients. Sera were also obtained from four patients with renal failure and from a patient whose kidneys were removed by operation after renal failure.

15 patients were classified as having glomerular proteinuria on the basis of gel chromatography of urinary proteins, as described below. These patients had the following diagnoses: nephrotic syndrome, glomerulonephritis, Alport's syndrome, and orthostatic proteinuria. The patients with the nephrotic syndrome had edema, decreased albumin, and total protein in plasma (*cf.* Table III), and elevated concentrations of blood cholesterol. Hematuria and somewhat raised blood pressure was observed in two patients with chronic glomerulonephritis (N. N. and L. L.). Hematuria was also noted in a third patient with glomerulonephritis (C. S.). Proteinuria in the erect position had been observed in the subjects with orthostatic proteinuria.

15 patients had diseases affecting the renal tubules, i.e., chronic cadmium poisoning, Wilson's disease, cystinosis, lower urinary tract obstruction, oculo-cerebro-renal syndrome, renal tubular acidosis, tyrosinemia, and Laurence-Moon-Biedl syndrome with renal malfunction. Urinary proteins from these patients, with the exception of L. K., A. G., and A. W., were submitted to gel chromatography. All patients were classified in the category of tubular proteinuria on the basis of the observed size distribution of urinary proteins (see below). Slightly or moderately increased excretion of glucose was noted in all patients belonging to this group, except in K. L., P. Z., and A. G. Increased phosphaturia had been found in patients E. J., L. K., A. G., and A. W.; increased aminoaciduria had been observed in patients G. N., A. W., and I. W.

Glomerular filtration rate and serum creatinine (13) was determined in a number of the subjects (Table III and Fig. 2). Glomerular filtration rate was measured as endogenous creatinine clearance, and, in two patients, as inulin clearance (Table III). Values for glomerular filtration rate were also available for patients E. W. (106 ml/min), C. S. (73 ml/min), J. N. (98 ml/min), C. A. (55 ml/min), K. L. (100 ml/min), and S. R. (10 ml/min).

Concentration of proteins. The proteins in filtered urine specimens were concentrated by means of ultrafiltration (14), using 23/32 inch Visking dialysis tubing[1] as the ultrafiltration membrane. This tubing completely retains proteins down to the molecular weight range of 10,000–15,000 (15). The macromolecules in 24-hr urine specimens and in 1.0-liter portions of pooled urine were concentrated to a final concentration of total protein ranging from approximately 20 to 200 mg/ml.

[1] Union Carbide Corp., Chicago, Ill.

Gel chromatography and determination of molecular radii (r_s) of β_2-microglobulin and albumin. Samples of concentrated urinary proteins were chromatographed on a Sephadex[2] G 100 column (133.5 × 2.9 cm), equilibrated with 0.1 M Tris-HCl buffer, pH 8.0, containing 1.0 M NaCl. Protein in the eluates was recorded by reading the optical density at 280 nm. The void volume (V_o) of the column was obtained by measuring the elution volume of Blue Dextran[2] according to the instructions supplied by the manufacturer. The total elution volume (V_t) was determined by mixing 75 μCi ^3H_2O[3] with a sample of concentrated urinary proteins which was subjected to chromatography. Radioactivity in the effluent was estimated in a Tri-Carb 3003 well-type liquid scintillation counter.[4] V_t was obtained by locating the position of maximum radioactivity in the elution diagram. The elution volumes (V_e) of β_2-microglobulin and albumin were determined immunochemically by the single radial immunodiffusion method as described below. The mean molecular radii (Einstein-Stokes' radius, r_s) of β_2-microglobulin and albumin were calculated from the formula $K_{av} = e^{-\pi L(r_s+r_s)^2}$ given by Laurent and Killander (16). K_{av} was obtained from the formula $K_{av} = (V_e - V_o)/(V_t - V_o)$ (16). According to Laurent, Öbrink, Hellsing, and Wasteson, the constants L and r_r for Sephadex G100 with a dextran concentration of 0.1 g/ml are as follows: $L = 3.7 \times 10^{12}$ cm/cm^2 and $r_r = 7 \times 10^{-8}$ cm (17).

Preparation of antisera. Specific antisera against β_2-microglobulin and albumin were prepared by injecting a suspension containing equal parts of protein solution (4 mg/ml) and complete Freund's adjuvant[5] into the footpads of rabbits. The animals received 0.2 mg of antigen in each footpad on two occasions, at an interval of about 10 days. The rabbits were exsanguinated by heart puncture about 4 wk after the last injection.

Protein determinations. Total protein in concentrates of nonultrafilterable urinary macromolecules was determined by the Folin procedure of Lowry, Rosebrough, Farr, and Randall (18), with IgG as the reference substance for normal and tubular proteinuria. Albumin was chosen as the reference substance for glomerular proteinuria, as in all cases except one, albumin comprised more than 40% of the total protein. Albumin gave only about 60% of the absorbancy for an equal amount of IgG, β_2-microglobulin or transferrin with the Folin procedure. Total protein in sera was estimated by the Folin technique with a mixture of equal parts of albumin and IgG as the standard.

β_2-microglobulin and albumin quantities in urine concentrates and albumin contents in sera were measured by the single radial immunodiffusion method of Mancini, Carbonara, and Heremans (19). Highly purified β_2-microglobulin, prepared according to Berggård and Bearn (12), and albumin[6] were used as the standards. Samples and standards were applied in five different dilutions. The same amount of each dilution, usually 3 μl, was placed in wells of 2 mm diameter in the immunodiffusion plates by means of a microsyringe.[7] After incubation in a moist chamber at room temperature for at least 24 hr, the diameter of the precipitates was measured with a magnifier engraved in 0.1 mm intervals.[8] The concentrations of antigen were obtained by

[2] Pharmacia Fine Chemicals AB, Uppsala, Sweden.
[3] The Radiochemical Centre, Amersham, England.
[4] Packard Instrument Co., La Grange, Ill.
[5] Difco Laboratories, Detroit, Mich.
[6] Kindly supplied by AB KABI, Stockholm, Sweden.
[7] Hamilton Co., Inc., Whittier, Calif.
[8] Bausch & Lomb Incorporated, Rochester, N. Y.

TABLE I

Recovery of Urinary β_2-Microglobulin, Albumin, and Total Protein after Concentration by Ultrafiltration

Experiment No.*	β_2-microglobulin recovered		Albumin recovered		Total protein recovered	
	mg	%	*mg*	%	*mg*	%
I	0.126	78	7.02	92	99.0	92
II	0.141	87	6.90	90	99.3	92
III	0.132	81	7.41	97	95.7	87
Average	0.133	82	7.11	93	98.0	90

* Portions of nonultrafilterable urinary macromolecules containing 0.162 mg of β_2-microglobulin, 7.65 mg of albumin, and 108.0 mg of total protein were added to each of three 1.0-liter portions of ultrafiltered urine. The urine samples were subsequently reconcentrated by ultrafiltration.

plotting the squares of the diameters on a standard curve. The results given are the mean values of figures for the five different dilutions of each sample.

The accuracy of the method was tested by performing 12 separate β_2-microglobulin determinations on the same 24-hr urine specimen. The mean value was 0.082 mg with a standard deviation of 0.002 mg.

The low concentrations of β_2-microglobulin in most sera did not allow precise measurements by the single radial immunodiffusion technique. Therefore, a more sensitive radioimmunosorbent method (20) was used for determinations of serum levels of this protein. The two methods gave similar results when used for assays of β_2-microglobulin in concentrates of urinary proteins.[9]

Recovery of protein after ultrafiltration and precision of quantitations of urinary components. The possible loss of significant quantities of β_2-microglobulin, albumin, and total protein during the ultrafiltration procedure was examined in two experiments. In a recovery experiment, 3.5 liters of pooled normal urine was subjected to ultrafiltration. Total protein, β_2-microglobulin, and albumin in the concentrate (7.2 ml) were estimated as described above. Equal volumes of this concentrate were added to each of 1.0-liter portions of the urinary ultrafiltrate, and new ultrafiltrations were carried out. The recoveries of total protein, β_2-microglobulin, and albumin in the concentrates (2.4–6.4 ml) obtained after the second ultrafiltration are shown in Table I. As can be seen from the table, there were only small losses of total protein and albumin. The losses of β_2-microglobulin were somewhat larger. Urine concentrates from tubular and glomerular proteinuria showed smaller losses, especially of β_2-microglobulin.

The possibility of passage of small amounts of β_2-microglobulin through the ultrafiltration membrane was examined in another experiment. A 24 hr urine specimen from patient A. W. with tyrosinemia was ultrafiltered. The concentrate contained 1.10 mg of β_2-microglobulin, which is about 10 times the amount of this protein found normally (see below). The ultrafiltrate was dialyzed at + 4°C in Visking 23/32 inch tubing against repeated changes of distilled water, and lyophilized. The lyophilized material was dissolved in 1 ml of 0.9% NaCl and assayed for β_2-microglobulin. β_2-microglobulin could not be detected, despite the high sensitivity of the single radial immunodiffusion method. Consequently, at most, 1 μg of this protein could have passed the ultrafiltration membrane.

[9] Peterson, P. A., P. E. Evrin, L. Wide, and I. Berggård. Unpublished experiments.

TABLE II

Contents of β_2-Microglobulin, Albumin, and Total Protein in Four 1.0-liter Portions from a Pool of Normal Urine

Urine sample No.	β_2-microglobulin	Albumin	Total protein
		mg	
I	0.11	22.6	88
II	0.11	20.2	84
III	0.10	18.9	87
IV	0.10	20.7	89
Average	0.11	20.6	87

The accuracy of the entire procedure for determination of urinary β_2-microglobulin, albumin, and total protein was

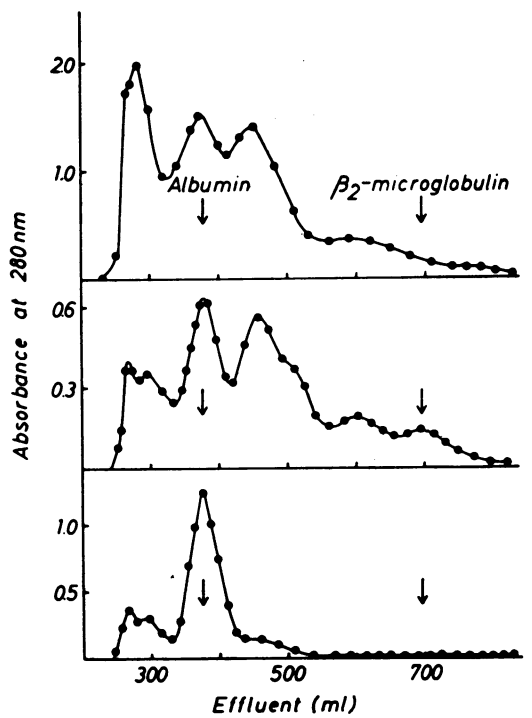

FIGURE 1 Chromatography on Sephadex G100 of concentrated proteins (329 mg) from pooled normal urine (top), of concentrated proteins (117 mg) from a patient with chronic cadmium poisoning (middle), and of concentrated proteins (146 mg) from a patient with glomerulonephritis (bottom). The column (2.9 × 133.5 cm) was equilibrated with 0.1 M Tris-HCl buffer, pH 8.0, containing 1.0 M NaCl. Fractions of 5 ml were collected at a flow rate of 15 ml/hr. The distribution in the effluent of albumin and β_2-microglobulin was determined by a single radial immunodiffusion technique. The position of the maximum concentrations of the two proteins are indicated by arrows.

TABLE III

Urinary Clearances of β_2-Microglobulin and Albumin in Normal Subjects and in Patients with Glomerular or Tubular Proteinuria

Subject	β_2-microglobulin		Albumin		Total protein		β_2-microglobulin clearance	Albumin clearance	β_2-microglobulin clearance/albumin clearance	Glomerular filtration rate*	Creatinine serum
	Serum	Urine	Serum	Urine	Serum	Urine					
	μg/ml	*mg/24 hr*	*mg/ml*	*mg/24 hr*	*mg/ml*	*mg/24 hr*	*μl/min*	*μl/min*		*ml/min*	*μg/ml*
Normal											
K. P.	1.5	0.11	40	9.6	82	64	51	0.17	300		8
R. N.	1.2	0.10	47	7.9	74	64	58	0.12	480		7
M. F.	1.8	0.07	48	8.1	78	43	27	0.12	230		9
B. B.	1.5	0.08	47	5.9	69	51	37	0.09	410		8
Nephrotic syndrome‡											
G. A.	2.8	0.66	20	9,390	45	13,700	160	330	0.50	130	8
B. O. I.	1.9	0.40	22	4,180	39	6,210	150	130	1.1	80	5
M. N.	1.0	1.02	19	1,120	47	2,580	710	40	17	130	4
Glomerulonephritis‡											
O. L.	2.5	0.11	46	440	89	800	31	6.7	4.6	100	10
L. L.	7.5	1.40	36	2,650	69	4,050	130	51	2.5	26	37
N. N.	3.1	0.08	29	216	79	310	18	5.2	3.5	41	17
Chronic cadmium poisoning§											
E. J.	6.5	105	48	578	87	1,570	11,200	8.4	1,300	60	19
T. J.	4.3	64.4	49	165	78	1,540	10,400	2.3	4,500	36	16
N. A.	3.1	13.7	34	13.8	76	153	3,100	0.25	12,000	51	15
Cystinosis§											
G. N.‖	5.6	42.0	36	72	82	565	5,200	1.4	3,700	5.9	16
Laurence-Moon-Biedl syndrome with renal malfunction§											
I. W.‖	6.2	74.2	42	73	68	550	8,300	1.2	6,900	12	11

* Measured as in ulin clearance in patients G. N. and I. W. and as endogenous creatinine clearance in the other patients. Glomerular filtration rates and protein clearances were determined at the same time (G. A., B. O. I., L. L., N. N., E. J., T. J., and N. A.) within 2 days (I. W.) or within a few days (M. N., O. L., and G. N.).

‡ Glomerular proteinurias.

§ Tubular proteinurias.

‖ Patients G. N. and I. W. were children aged 3 and 4 yr. Two children in the same age, without proteinuria, excreted similar amounts of urinary β_2-microglobulin as normal adults.

tested in the following experiment. A 4 liter pool of normal urine was divided into 1.0 liter portions, which were concentrated by ultrafiltration. The concentrates were assayed for β_2-microglobulin, albumin, and total protein. The results are given in Table II. The table shows that the values for the three constituents were similar in the four concentrates. The precision of the determinations can, accordingly, be considered as good.

RESULTS

Molecular size distribution of urinary proteins. Urinary proteins from normal and pathological urines were separated according to molecular size by gel chromatography on a Sephadex G100 column. Fig. 1 shows a chromatogram of concentrated proteins from pooled normal urine (top), a chromatogram of urinary proteins from a patient with tubular damage and proteinuria due to chronic cadmium poisoning (middle), and a chromatogram of urinary proteins from a patient with glomerulonephritis (bottom). The elution diagram of the urinary proteins from the patient with tubular damage is characterized by relatively large amounts of proteins of small molecular size, eluted late in the chromatogram. The protein curve shows a distinct peak at the β_2-microglobulin position. Urinary proteins from other patients with renal tubular disease gave gel chromatograms of similar type; in several instances the proteins of small size were more dominating than in the chromatogram illustrated in Fig. 1. Relatively large amounts of free light immunoglobulin chains were present in the protein peak eluted after albumin and an α_2-globulin of small size was mainly responsible for the peak preceding the peak at β_2-microglobulin position.[10] Both free light chains

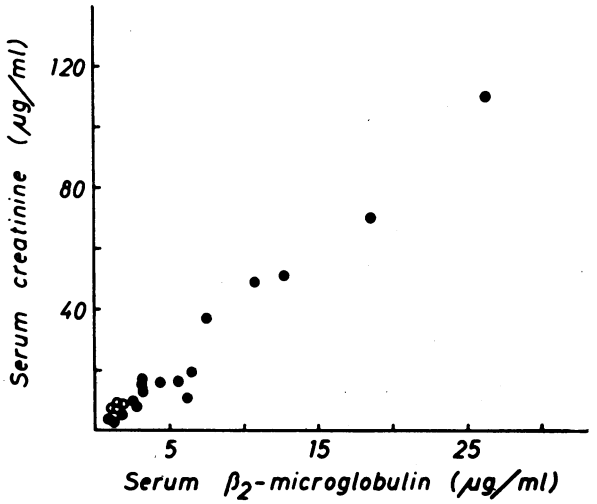

FIGURE 2 Relationship between serum concentrations of β_2-microglobulin and creatinine in four normal individuals (○) and 17 patients with renal disease (●). The normal individuals and 11 of the patients are listed in Table III. Four patients with renal failure and relatively high serum levels of creatinine (49–110 μg/ml) have been included in the diagram. The correlation coefficient was 0.98.

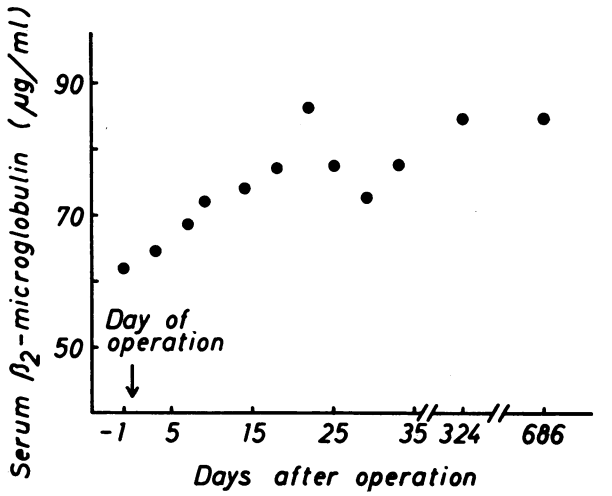

FIGURE 3 Serum concentration of β_2-microglobulin in a patient before and after bilateral nephrectomy. β_2-microglobulin was measured by a single radial immunodiffusion technique.

(21, 22) and this α_2-globulin[10] are present in low concentration in normal sera. Elution diagrams of urinary proteins from the patients with glomerulonephritis and from patients with the nephrotic syndrome, orthostatic proteinuria, and Alport's syndrome were similar, and showed a predominance of proteins of relatively large sizes with the dominating protein peak in the albumin position.

The molecular radii (r_s) of albumin and β_2-microglobulin were calculated from their elution volumes, as described above. The value obtained for albumin was 35 A, which agrees well with figures published earlier (*cf.* reference 15). The molecular radius of β_2-microglobulin was calculated to be approximately 15 A.

Clearance of β-microglobulin and albumin in healthy subjects and in patients with renal disease. Table III presents serum and urine levels of β_2-microglobulin and albumin, and urinary clearances of the two proteins for four normal individuals and 11 patients. Patients with the nephrotic syndrome had low contents of albumin and total protein in serum. The serum concentrations of β_2-microglobulin were similar in the four normal individuals listed in Table III, in three additional normal subjects, and in two patients with the nephrotic syndrome (1.0–1.9 μg/ml). One patient with the nephrotic syndrome, three patients with glomerulonephritis, and the patients with predominantly tubular disorders had higher serum levels of β_2-microglobulin (2.5–7.5 μg/ml).

[10] Peterson, P. A., and I. Berggård. Unpublished experiments.

Increased urinary excretions of β_2-microglobulin and albumin were associated with increased clearances of the two proteins. The values for urinary β_2-microglobulin clearance in the patients with glomerular proteinuria were in the normal range (O. L. and N. N.), or moderately higher. Urinary albumin clearances in these patients were much higher than in the normal subjects. In the patients with tubular proteinuria the β_2-microglobulin clearances were high and amounted to 6–88% of the glomerular filtration rates. The values for β_2-microglobulin clearance were more increased than the values for albumin clearance in these patients.

Fig. 2 shows that the serum concentrations of β_2-microglobulin were highly correlated to the serum concentrations of creatinine. The figure comprises determinations for all individuals presented in Table III and

TABLE IV

*Quantities of β_2-Microglobulin, Albumin, and Total Protein in Urine from Normal Individuals**

No. of subjects	Sex	β_2-microglobulin	Albumin	Total protein	Albumin/β_2-microglobulin
		mg/24 hr			
10	Male	0.13	8.8	90	72
10	Female	0.11	11.1	71	111
20	Mean	0.12	10.0	80	92
20	Range	0.06–0.21	3.9–24.2	43–127	33–163
20	SD	0.040	4.43	24.3	39.0

* The figures have been corrected for the average losses occurring during the concentration procedure (*cf.* Table I).

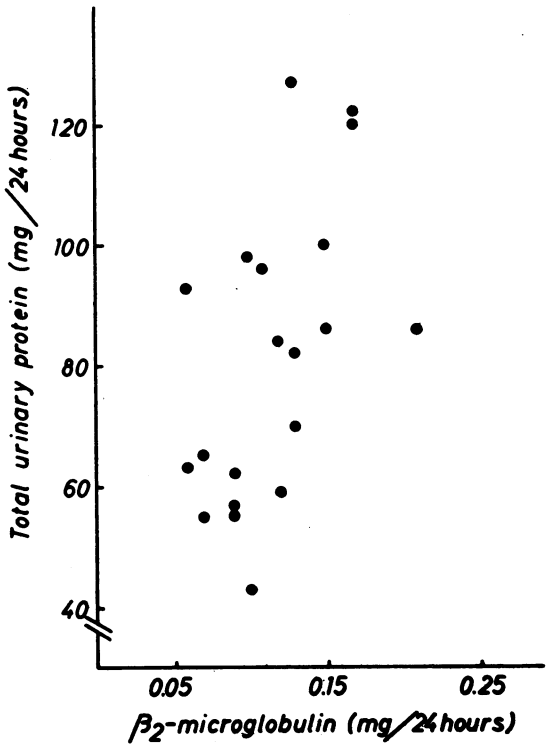

FIGURE 4 Correlation of β_2-microglobulin and total protein in concentrates of 24-hr urine specimens from 20 normal subjects. Correlation coefficient, 0.62.

values for two additional patients (C. O., Table V and A. N., Table VI). Determinations for four patients with renal failure and higher serum levels of both creatinine and β_2-microglobulin have also been included. The correlation coefficient was 0.98.

Serum β_2-microglobulin after bilateral nephrectomy. Serum β_2-microglobulin concentrations were estimated in a patient whose kidneys were removed by operation after renal failure. Serum was collected both before and after bilateral nephrectomy. Before operation, the serum level was about 40 times higher than the normal value of approximately 1.5 μg/ml. During the 1st 2–3 wk after operation, the serum concentration increased to approximately 50–60 times the normal value. Similar levels of β_2-microglobulin were found 10½ and 22½ months after the operation (Fig. 3).

Urinary excretion of β_2-microglobulin, albumin, and total protein in healthy individuals. The contents of β_2-microglobulin, albumin, and total protein in 24-hr urine specimens from 20 normal individuals are given in Table IV. It is evident that the excretion of albumin was, on the average, about 90 times higher than the β_2-microglobulin excretion, which was fairly small (0.06–0.21 mg/24 hr). The normal values for urinary albumin are similar to figures reported earlier (23, 4). The β_2-microglobulin values of each individual were plotted against the corresponding values for total protein, as shown in Fig. 4. The correlation suggested by Fig. 4 is noteworthy since β_2-microglobulin constitutes only 0.08–0.25% of the total protein. Correlation coefficients were calculated for the excretion of β_2-microglobulin and total protein, and for the excretion of albumin and total protein. β_2-microglobulin and total protein had a coefficient of 0.62, whereas the coefficient of albumin and total protein was 0.42.

Five different 24-hr urine specimens were obtained from one of the normal individuals. These specimens were collected at 1 wk intervals. The excretion of β_2-microglobulin (0.13–0.15 mg/24 hr) and total protein (80–88 mg/24 hr) was remarkably constant during the 4 wk period. The figures for albumin (7.0–9.1 mg/24 hr) showed a wider variation, although this variation was smaller than the variation between albumin quantities in different individuals.

β_2-microglobulin, albumin, and total protein in urine from patients with pathological proteinuria. The urinary excretion of β_2-microglobulin, albumin, and total protein in 30 individuals with manifest or suspected clinical proteinuria is presented in Tables V and VI.

Table V shows that the patients with glomerular proteinuria excreted considerably increased amounts of total protein and albumin. The albumin quantities amounted to more than 40% of the total protein in all patients except P. H. (27%). The amounts of β_2-microglobulin were normal in five of the patients with glomerulonephritis, and in the patients with orthostatic proteinuria and Alport's syndrome. One patient with glomerulonephritis and all patients with the nephrotic syndrome had somewhat raised values.

Table V shows the excretion of β_2-microglobulin, albumin, and total protein in 15 patients with tubular proteinuria. These patients had a considerably elevated excretion of β_2-microglobulin, but usually only a moderately increased or normal excretion of albumin. Patients S. A., N. A., and I. W. excreted similar amounts of β_2-microglobulin and albumin.

Table VI summarizes the results in normal subjects and patients with pathological proteinuria. It can be seen that the ratio of albumin/β_2-microglobulin is high in glomerular proteinuria, intermediate in normal subjects, and low in tubular proteinuria, without overlap between the three groups.

DISCUSSION

The results of a large number of studies indicate that the predominant part of plasma proteins in urine arises from a process of glomerular filtration followed by tubular reabsorption (*cf.* reference 1). The glomerular permeability of various substances is greatly dependent

TABLE V

Quantities of β_2-Microglobulin, Albumin, and Total Protein in Urine from Patients with Glomerular Proteinuria

Patients	Sex*	β_2-microglobulin	Albumin	Total protein	Albumin/β_2-microglobulin
			mg/24 hr		
Nephrotic syndrome					
G. A.	M	0.66	9,390	13,700	14,200
E. W.	F	1.10	2,820	6,270	2,560
B. O. II.	M	4.70	48,800	54,100	10,400
C. O.	F	0.90	1,940	4,090	2,160
M. N.	M	1.02	1,120	2,580	1,100
Glomerulonephritis					
O. L.	M	0.11	441	800	4,010
P. O.	M	0.06	370	934	6,170
C. S.	F	0.06	206	483	3,430
I. J.	F	0.07	361	864	5,160
L. L.	M	1.40	2,650	4,050	1,900
N. N.	M	0.08	216	310	2,700
Orthostatic proteinuria					
P. H.	M	0.08	88.0	322	1,100
K. E.	F	0.10	218	523	2,180
J. N.	F	0.14	446	1,100	3,190
Alport's syndrome					
C. A.	F	0.09	375	658	4,170

* M = male; F = female.

on their size. Experiments in normal subjects have shown that dextran fractions with a molecular weight below 10,000–15,000 pass across the glomerular membrane at the same rate as water, whereas larger dextran molecules are retained in increasing degree with increasing molecular weight (24, 25). Dextran of molecular weight 50,000 is practically excluded from the glomerular filtrate (25). Dextran with a molecular weight of 10,000–15,000 has a molecular radius (r_s) of about 20–25 A (*cf.* reference 17). In the present study, it was found that β_2-microglobulin of molecular weight 11,800 has a molecular radius (r_s) of 15 A. β_2-microglobulin should accordingly pass across the normal glomerular membrane at 100% of the glomerular filtration rate. This might, however, not be true, since dextran is an uncharged molecule, whereas proteins have charged groups. A charged molecule may interact with the glomerular structure, and make the glomerular passage more difficult.

A high normal glomerular permeability for β_2-microglobulin and a low glomerular permeability for albumin is supported by the present findings. Patients with glomerular proteinuria, i.e., increased glomerular permeability to relatively large plasma proteins, had a normal or only somewhat increased excretion of β_2-microglobulin, despite a highly increased excretion of albumin and total protein. Further support for this conclusion is given by the observation of a close positive correlation between serum levels of β_2-microglobulin and creatinine. Bernier, Cohen, and Conrad (26) have found a similar correlation in patients with chronic renal failure. These findings may suggest that β_2-microglobulin is handled by the glomerular membrane in a similar way as creatinine.

The increased excretions of the two proteins in tubular or glomerular proteinuria apparently were associated with increased clearances. There is no good evidence for tubular secretion of plasma proteins (*cf.* reference 1) and the findings of increased serum concentrations of β_2-microglobulin after bilateral nephrectomy do not support a tubular origin for this protein. Therefore, it seems most probable that an increased excretion of β_2-microglobulin in renal disorders signifies an impaired tubular reabsorption, particularly since the normal glomerular permeability of β_2-microglobulin seems to be high.

The tubular reabsorption of protein has generally been assumed to be a nonselective process. This view has largely been based on the finding that intravenous infusions of albumin in nephrotic patients produce an increase in the clearance of globulin fractions in proportion to the increase in the clearance of albumin (27). The

TABLE VI

Quantities of β_2-Microglobulin, Albumin, and Total Protein in Urine from Patients with Tubular Proteinuria

Patients	Sex*	β_2-microglobulin	Albumin	Total protein	Albumin/β_2-microglobulin
			mg/24 hr		
Chronic cadmium poisoning					
E. J.	M	105	578	1,570	5.5
T. J.	M	64.4	165	1,540	2.6
F. J.	M	69.2	102	808	1.4
A. N.	M	74.4	114	780	1.5
N. A.	M	13.7	13.8	153	1.0
K. L.	M	7.51	19.6	412	2.6
Wilson's disease					
P. Z.	F	5.12	32.0	154	6.3
B. Z.	M	19.3	65.7	798	1.1
V. A.	M	14.6	38.8	173	2.7
Cystinosis					
G. N.	M	42.0	71.9	565	1.7
Lower urinary tract obstruction					
S. R.	M	9.18	62.1	282	6.8
Oculo-cerebrorenal syndrome					
L. K.	M	14.5	156	612	10.8
Renal tubular acidosis					
A. G.	F	22.1	35.1	303	1.6
Tyrosinemia					
A. W.	M	1.10	14.6	129	13.3
Laurence-Moon-Biedl syndrome with renal malfunction					
I. W.	F	74.2	72.6	550	1.0

* M = male; F = female.

concept of nonselective tubular reabsorption has been questioned recently (28, 2, 4, 11). A consequence of a mechanism of this type should be similar relative amounts of different plasma proteins in normal urine and in urine from patients with impaired tubular reabsorption of protein. The results of the present study do not seem to fit with a nonselective tubular reabsorption of β_2-microglobulin and albumin, since the urinary ratios of the two proteins were much lower in tubular proteinuria than in normal proteinuria. Furthermore, some patients with tubular proteinuria had a considerably increased excretion of β_2-microglobulin despite a normal excretion of albumin. These findings suggest that the tubular reabsorption of the two proteins is selective in nor-

TABLE VII

Ranges of Urinary Excretion of β_2-Microglobulin, Albumin, and Total Protein in Normal, Glomerular, and Tubular Proteinuria

No. of subjects	Type of proteinuria	β_2-microglobulin	Albumin	Total protein	Albumin/β_2-microglobulin
			mg/24 hr		
20	Normal	0.06–0.21	3.9–24.4	43–127	33–163
15	Glomerular	0.06–4.70	88.0–48,800	310–54,100	1,100–14,200
15	Tubular	1.10–105	13.8–578	129–1,570	1.0–13.3

mal and tubular proteinuria, and that the reabsorption of β_2-microglobulin is more affected by tubular dysfunction than is the reabsorption of albumin.

Free immunoglobulin light chains and some other plasma proteins of small molecular size were found to behave similarly to β_2-microglobulin in normal, tubular, and glomerular proteinuria, whereas larger proteins, like transferrin and immunoglobulins A and G, behaved more like albumin.[11] Evidence of an increased excretion of low molecular weight plasma proteins in tubular proteinuria was also given by Piscator (8), Harrison and Blainey (29), Walravens, Laterre, and Heremans (10), and Flynn and Platt (11). Factors other than size are, however, probably also of importance for the renal handling of plasma proteins, as suggested by a study on clearances of various plasma proteins in normal individuals (4).

Greatly raised serum levels of β_2-microglobulin were found by Bernier et al. (26) and by us in patients with renal failure and in two patients with both kidneys removed by operation after renal failure. The serum concentration of β_2-microglobulin in one of the latter patients, observed by us, rose after bilateral nephrectomy. These observations seem to exclude the possibility that the renal tubules are a major source of β_2-microglobulin, and suggest that this protein is normally catabolized in the kidney. The kidney is apparently the most important site for catabolism of Bence Jones proteins (30, 31) and of insulin (32). It seems likely that the main mechanism for catabolism of β_2-microglobulin and possibly of other small plasma proteins is rapid glomerular filtration, followed by reabsorption and digestion in the cells lining the proximal renal tubules.

The results of the present study suggest that quantitative determinations of urinary β_2-microglobulin and urinary albumin may be useful for detecting disorders of the renal handling of plasma protein. The patients in this investigation were selected in order to facilitate a classification into glomerular and tubular proteinuria, on the basis of clinical diagnoses and size distribution of urinary proteins after gel chromatography. Such criteria of classification of proteinurias have also recently been adopted by Davis, Flynn, and Platt (33). We have observed proteinuria of apparently mixed glomerular-tubular types mainly in patients with renal failure, and in patients with chronic pyelonephritis. In such cases, estimates of the β_2-microglobulinuria and albuminuria may give information as to the extent of one or the other type of proteinuria.

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