We describe a radioimmunoasay for \( \beta_2 \)-microglobulin (\( \beta_2 \mu \)) in serum and urine. We incubated aliquots of diluted samples at room temperature for 1 h with \(^{125}\text{I}\) labeled \( \beta_2 \mu \) and a rabbit antiserum monospecific for human \( \beta_2 \mu \), and separated the phases by the double-antibody technique. The logit-log transformed dose–response curve was linear in the range 2 to 64 ng, equivalent to 0.5 to 16 mg/L of serum and 0.5 to 320 mg/L of urine. Assay sensitivity was 2.4 ng of \( \beta_2 \mu \). Validation studies included tests of precision, accuracy, antibody specificity, and parallelism of the dose–response curves for standard and unknown. In a study of 25 normal individuals, serum and urine \( \beta_2 \mu \) ranged from 1.1 to 2.3 mg/L and 40 to 360 \( \mu \)g/24 h; the clearance of \( \beta_2 \mu \) was 8 to 130 \( \mu \)L/min. In 21 renal allograft recipients tested one to five weeks after transplantation, serum and urine \( \beta_2 \mu \) ranged from 3.9 to 15.6 mg/L and 7.2 to 611 mg/24 h; \( \beta_2 \mu \) clearance was 0.60 to 33.3 mL/min. Values for both serum and urine correlated well with severity of allograft rejection.

**Additional Keyphrases:** reference intervals • urine • renal allograft evaluation • renal glomerular and renal tubular function assessment • kidney transplantation • clearance

Traditionally, the diagnosis and management of proteinuria associated with renal transplantation has relied heavily on the measurement of total urinary protein, which, though useful, lacks the sensitivity and specificity needed to provide precise assessment of the nature of renal injury. Differential analysis of component proteins in the urine is proving to be a superior approach to monitoring kidney function in renal allograft recipients (1). Of the plasma proteins that are present in urine, albumin is the smallest that is almost totally excluded by the intact glomerular basement membrane (2, 3). Albuminuria thus serves as an index of renal glomerular dysfunction (4). Of the group of proteins with \( M_c \), < 40,000, \( \beta_2 \)-microglobulin (\( \beta_2 \mu \)) has for the past few years been the focus of interest as an indicator of renal tubular function (4, 5). With an \( M_c \) of 11 800 and a Stokes’ radius of 1.5 nm, \( \beta_2 \mu \) is freely filtered through the glomerular membrane ( sieving coefficient \( \approx 1 \), is almost entirely reabsorbed by the proximal tubule, and is subsequently catabolized in the kidney (4, 6, 7). The role of glomerular filtration in determining the concentration of \( \beta_2 \mu \) in serum explains the close correlation observed by several investigators between creatinine and \( \beta_2 \mu \) concentrations in serum (5, 8–10). As a corollary, the extensive tubular reabsorption of normally filtered \( \beta_2 \mu \) provides a physiological basis for quantitatively assessing renal tubular activity. Thus data on \( \beta_2 \mu \) concentrations in urine and serum provide reliable information on glomerular and tubular function.

Several techniques for measuring \( \beta_2 \mu \) have been described (4, 5, 11–13). Determinations by radial immunodiffusion (4) and by immunoprecipitin methods (11) generally lack sensitivity and require concentration of urine before the measurement. Subsequent immunoassay procedures involving immunosorbents (12) and enzyme-linked technique (5) either lack precision or are technically demanding. Although a sensitive radioimmunoasay (RIA) was reported recently (13), pertinent information with respect to reagent preparation, assay sensitivity, method specificity, normal range establishment, etc., has not been adequately presented. Based on its package inserts, the commercially available “Phadebas \( \beta_2 \)-Micro Test” kit (Pharmacia) utilizing solid-phase radioimmunoasay technique appears to have adequate precision and sensitivity. However, the incubation time is somewhat lengthy and the separation step is cumbersome. We describe a precise, sensitive, and specific RIA for the measurement of \( \beta_2 \mu \) in both serum and urine by the double-antibody technique. This procedure is the concluding sequel to two other recently developed RIA’s for the measurement of albumin (14) and immunoglobulin G (15). Combined, these assays are useful for detecting disorders in the renal handling of these proteins and may facilitate understanding of the rejection mechanisms and management of the renal allograft patients (16). We also report a preliminary study of \( \beta_2 \mu \) clearance, in which we used this assay to assess tubular dysfunction in patients undergoing renal allografts.

**Materials and Methods**

**Reagents and Solutions**

- **Phosphate-buffered saline,** 10 mmol/L. Ten millimoles of Na\(_2\)HPO\(_4\), 150 mmol of NaCl, and 1.5 mmol of Na\(_3\)(4.1 g, 8.8 g, and 0.1 g, respectively) were dissolved in 900 mL of reagent-grade water. The pH was adjusted to 7.0, and the solution was diluted to 1 liter.
- **Assay buffer.** Normal rabbit serum, 7.5 mL, was mixed with 500 mL of phosphate-buffered saline.
- **Human \( \beta_2 \mu \) antisem, rabbit.** This material was kindly provided by Ralph Riesfeld, M.D., Scripps Clinic and Foundation, La Jolla, CA. We determined the working titer of this antisem by performing an antibody dilution study according to the procedure to be described later. An antisem dilution of 1:50-fold yielded an optimal initial binding \( (B/T)_0 \) of 50%. The phosphate-buffered saline was used as the antisem diluent.
- **Human \( \beta_2 \mu \).** This was isolated from urine of a patient with Fancconi’s syndrome, as follows: Fresh urine was dialyzed against tap-water for 18 h, then lyophilized. After reconstitution in phosphate-buffered saline, 15 mmol/L, it was applied to a 100 cm × 2.5 cm column packed with superfine Sephadex G-75 (Pharmacia Fine Chemicals, Piscataway, NJ 08854); the same buffer was used as eluent. \( \beta_2 \mu \) was eluted as a discrete peak contaminated principally with lysozyme. The \( \beta_2 \mu \)-rich fractions were dialyzed against a 10 g/L solution of glycine for two days and lyophilized before preparative isoelectric focusing in a pH range of 3.5–10 in a flat bed of Sephadex. \( \beta_2 \mu \) was located as a discrete peak at a pH range of 4.4–5.8. The fractions containing \( \beta_2 \mu \) were combined and concentrated before testing with rabbit anti-\( \beta_2 \mu \) serum, which gave a single precipitin line. This protein fraction also formed a single band on polyacrylamide gel electrophoresis in the presence of 10 g of sodium dodecyl sulfate per liter, and its position was appropriate to a relative molecular mass range of 12 000 as referenced with low-molecular-mass proteins such as retinol.

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2. Also symbolized \( \beta_{\text{M}} \) and \( \beta_{\text{M}} \) in various publications.—Ed.
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binding globulin. The $\beta_{2u}$ content in the concentrated eluent was determined spectrophotometrically at 260 and 280 nm (17). Working standard solutions were prepared freshly before each assay by diluting the concentrated elute with assay buffer to yield solutions containing 64, 32, 16, 8, 4, and 2 ng/100 $\mu$L.

Procedures

Radioiodination of human $\beta_{2u}$. Human $\beta_{2u}$ was iodinated by the Chloramine T procedure of Hunter and Greenwood (18). Human $\beta_{2u}$, prepared as previously described, 2.5 $\mu$g in 10 $\mu$L of phosphate buffer (0.4 mol/L, PH 7.4) and 5 $\mu$L (500 $\mu$Ci) of carrier-free Na$^{125}$I (Amersham Searle, Arlington Heights, IL 60005) were mixed gently in a 500-$\mu$L plastic AutoAnalyzer cup. Twenty microliters of freshly prepared Chloramine T solution (1.5 g/L) was added and the mixtures were allowed to react for 1 min. The reaction was terminated by adding 100 $\mu$L of sodium metabisulfite solution (0.5 g/L). Before we purified the labeled $\beta_{2u}$, 5-10 $\mu$L of iodination mixture were reacted with 0.5 mL of a 150 g/L trichloroacetic acid solution to precipitate the protein fraction containing the iodinated $\beta_{2u}$, and the specific activity was assessed from the $125^{I}$ content in this fraction. The iodinated preparation was purified by gel-filtration chromatography on a 0.9 cm $\times$ 30 cm column packed with Sephadex G-50 (Pharmacia). We eluted the column with the phosphate-buffered saline, and collected 500-$\mu$L fractions in 16 $\times$ 100 mm tubes, each of which had previously been coated with 50 $\mu$L of bovine serum albumin solution. Fractions containing the iodinated $\beta_{2u}$ (tubes 19-22) were combined. We added a calculated amount of unlabeled human $\beta_{2u}$ to the purified iodination preparation to achieve a final specific activity of 10 Ci/g. Just before assay, we diluted an aliquot of this iodinated preparation with assay buffer to give a concentration of 1.5 ng/100 $\mu$L (about 20 000 cpm/100 $\mu$L).

Radioimmunoassay. Assay-standard incubation mixtures in 12 $\times$ 75 mm disposable glass tubes were diluted to a total volume of 1000 $\mu$L with (a) 200 $\mu$L of $\beta_{2u}$ antiserum (1/1500 dilution), (b) 100 $\mu$L of working $\beta_{2u}$ standard solution (2-64 ng), (c) 100 $\mu$L of $^{125}$I-labeled $\beta_{2u}$ containing a radioactivity of 20 000 cpm, and (d) appropriate amount of assay buffer. All serum (25-fold) and urine (25-fold and 500-fold) samples were diluted with phosphate-buffered saline before assay, with the use of an Autodiluter (LKB Instruments, Inc., Rockville, MD 20852). Sample incubation mixtures were prepared similarly, except that b was substituted with equal volume of the diluted urine or serum sample. To correct for nonspecific binding of the labeled $\beta_{2u}$, we prepared incubation tubes with antisera diluted replacing a and with phosphate-buffered saline replacing b. All standards and samples were assayed in duplicate. The contents of these tubes were well mixed and allowed to incubate for 1 h at room temperature. One hundred microliters of precipitin antibody, goat antiserum to rabbit gamma-globulin, was added to each tube, and incubation was continued for 1 h at room temperature. Antibody-bound and free $\beta_{2u}$ were separated by centrifugation for 15 min at 2000 $\times$ g in a refrigerated centrifuge. The supernatant fluid was drained from each tube, and the precipitate, which contained the bound fraction, was counted for 1 min in a gamma scintillation spectrometer (Packard Instrument Co., Downers Grove, IL 60515; Model 5230).

Radioimmunoassay data obtained from the bound fraction was processed with a Hewlett-Packard 9845A programmable calculator equipped with graphics capability. The dose-response variables were subjected to logit-log transformation and iterative regression analysis to yield a linear slope, an 80% intercept, and a 50% intercept, all of which serve as quality control data for assay monitoring. In urine sample calculation, an averaged $\beta_{2u}$ value obtained from both urine dilutions was converted to the unit reported: mg/24 h. Where the binding for one of the dilutions fell beyond the acceptable limits of precision, i.e., 85% $< B/B_0 < 15\%$, the final calculation was performed on one dilution only.

Specimens

Timed 24-h urines were collected from 25 ostensibly healthy laboratory subjects known to be taking no drugs. Serum was sampled from the same individuals during the course of their 24-h urine collection. Timed 12-h urines were collected between 5 a.m. and 5 p.m. from 21 renal allograft recipients one to five weeks after transplantation; their serum samples were collected at 5 a.m.

Clearance of $\beta_{2u}$ ($C_{\beta_{2u}}$) was calculated for both normal subjects and transplant recipients as follows:

$$C_{\beta_{2u}} = (U_{\beta_{2u}} \times V)/(S_{\beta_{2u}} \times t)$$

where $U_{\beta_{2u}}$ = urinary $\beta_{2u}$ excretion, in mg/L; $S_{\beta_{2u}}$ = serum $\beta_{2u}$ concentration, in mg/L; $V$ = volume of timed urine, in mL; and $t$ = duration of urine collection, in min.

Results

Iodinated $\beta_{2u}$. The specific activity of the iodinated $\beta_{2u}$ as determined after trichloroacetic acid precipitation was usually 50-60 Ci/g. After adjusting to specific activity of 10 Ci/g and diluting to its working concentration, each iodination mixture was stable for at least 30 days as frozen (−20 °C) aliquots.

Standard curve. Figure 1 shows a representative dose-response curve after logit-log transformation and iterative weighted regression analysis. The relationship was linear over an absolute range of 2-64 ng of $\beta_{2u}$ per assay tube, equivalent to 0.5-16 mg/L of serum at a sample dilution of 25-fold and 0.5-320 mg/L of urine at sample dilutions of 25-fold and 500-fold. The assay sensitivity, as defined by $B/B_0$ of 85%, was 2.4 ng (0.6 mg/L of serum or urine both at 25-fold dilution). The upper limit of precision, as defined by $B/B_0$ of 15%, was 50 ng (12.5 mg/L of serum at 25-fold dilution, or 250 mg/L of urine at 500-fold dilution).

Precision. To assess the between-run precision, we assayed 23 serum specimens with $\beta_{2u}$ concentrations ranging from 1.7 to 10.6 mg/L, on each of two days. The CV was 7.8%. The be

![Fig. 1. Dose-response curve for human $\beta_{2u}$ after logit-log transformation](image-url)
 tween-run precision (CV), based on 20 urine samples with values from 6.0–51.3 mg/L, was 11.3%. Another precision study in which two commercially available lyophilized serum controls (Hyland, Costa Mesa, CA 92626) were assayed in 22 individual runs resulted in a CV of 6.8% at 0.76 mg/L and 6.2% at 1.3 mg/L.

Accuracy. Known amounts of purified human β2μ standard (8.1–64.5 ng/tube) were added to aliquots of both serum and urine from a normal individual. These aliquots were then assayed for β2μ. Analytical recovery of β2μ ranged from 90.5 to 96.9% in serum and 85.5 to 96.4% in urine.

Specificity. The cross reactivity of this antiserum to other plasma proteins such as albumin, immunoglobulins G, A, and M, retinol-binding globulin, and κ light-chain fragments was individually evaluated by reacting each protein with the assay system for measuring β2μ. As shown in Figure 2, this antiserum cross reacted by 0.62% with retinol-binding globulin, but was virtually nonreactive towards the other proteins tested.

Assay validation. Figure 3 shows the logit-log transformed standard curves obtained with β2μ as the standard, serial twofold diluted serum and urine specimens both from a renal allograft patient. The urine dilution curve covering a concentration range of 3.4–50 ng/assay tube (equivalent to 0.85–12.5 mg/L for a 25-fold diluted urine sample, and 17–250 mg/L for a 500-fold diluted urine sample) yielded a slope of −1.186, and the serum dilution curve covering a concentration range of 2.8–50 ng/assay tube (equivalent to 0.7–12.5 mg/L for a 25-fold diluted serum sample) showed a slope of −1.085. Both values are about 1 SD from the slope of −1.071 we obtained with β2μ as the standard.

Quality-control parameters. Radioimmunoassay functions that are useful in quality-control monitoring are: total count, %B/T0, % nonspecific, slope of the logit-log transformed standard curve, the 80% intercept, and the 50% intercept (19). Between-run variations in these are graphically presented in Figure 4. The mean (and standard deviations) for each function for 22 runs are: total count = 18 800 (1500), % nonspecific = 2.4 (0.9), %B/T0 = 51 (7), slope = −1.14 (0.05), 80% intercept = 2.95 (0.09), and 50% intercept = 9.7 (1.5).

Normal range and data on patients. Based on results for 25 apparently healthy laboratory individuals, the normal

Fig. 2. Specificity of antiserum for human β2μ

The antiserum cross reacted 0.62% with retinol-binding globulin, and it was nonreactive with albumin, immunoglobulins G, A, and M, and κ light-chain fragments.
ranges for \( \beta_2 \mu \) in serum and urine were 1.1–2.3 mg/L and 40–360 \( \mu \)g/24 h, respectively; the corresponding \( \beta_2 \mu \) clearance was 8–113 \( \mu \)L/min. Serum and urine \( \beta_2 \mu \) for 21 patients after renal allograft ranged from 3.9 to 15.6 mg/L and 5.2 to 438 mg/24 h, respectively, and the corresponding \( \beta_2 \mu \) clearance was 0.6–33.3 mL/min. These values are graphically presented in Figure 5.

**Discussion**

The range of concentrations covered in this assay, 0.5 to 16 mg/L of serum and 0.5 to 320 mg/L of urine, is broad enough to include individuals with normal renal function, renal allograft patients experiencing episodes of allograft rejection. As compared with the immunosorbent assay reported by Evrin and Wibell (12), and the commercially available Phadebas \( \beta_2 \)-Micro Test (Pharmacia) in which Sephadex particles are used as the solid-phase support, the method that we describe excels in its simplicity in reagent preparation and in assay set-up. The precision of our assay, with a CV of 7.8% for serum and 11.3% for urine, is superior to that of Evrin and Wibell, who reported 1.5 (SD 0.4) mg/L for serum and 0.073 (SD 0.033) mg/24 h for urine (12), and is comparable to the precision data stated on the Phadebas kit. Our assay sensitivity (2.4 ng, or 0.6 mg/L of serum or urine, both at 25-fold dilution) is more than adequate because both urine and serum samples require ample dilution before assay. The normal range for serum \( \beta_2 \mu \) we obtained by this assay (1.1–2.3 mg/L) agrees well with that reported by suppliers of the Phadebas kit (1.1–2.4 mg/L), but is somewhat narrower than that reported by Evrin and Wibell (0.9–3.0 mg/L). Their slightly wider range may be attributed to the effect of age, which is known to affect serum \( \beta_2 \mu \) concentration (20). The 25 normal subjects in our study ranged in age from 22 to 44 years; in Evrin and Wibell’s study the normal individuals varied from 17 to 76 years. The normal values for \( \beta_2 \mu \) excretion obtained by our assay ranged from 40 to 360 \( \mu \)g/24 h, which agree well with those reported by suppliers of the Phadebas kit (30–370 \( \mu \)g/24 h), but are higher than those reported by Evrin and Wibell (males: 75 ± 52 \( \mu \)g/24 h, and females: 78 ± 59 \( \mu \)g/24 h). We believe this discrepancy can be attributed to differences in methodology.

The results of our study indicate that both serum \( \beta_2 \mu \) concentration and its clearance were significantly increased in all 21 patients. It is worth noting that the lowest \( U_{\beta_2 \mu} \) in the patient group (5.2 mg/24 h) was from a transplant recipient of a kidney from a living donor. On the other hand, the highest such value (438 mg/24 h) was from a patient during an episode of renal allograft rejection. We now are measuring clearances of albumin, creatinine, and \( \beta_2 \mu \) on serial samples from renal allograft recipients four to five weeks after transplantation, in the hope that monitoring of both albumin and \( \beta_2 \mu \) metabolism will help to identify impaired tubular and glomerular function.

**References**


