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Renal tubular absorption of β2 microglobulin

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Renal tubular absorption of β2 microglobulin. ^{125}Iodinated human β2 microglobulin (β2m, 5 to 30 mg) was administered to anesthetized rats. Clearance studies showed a low threshold of excretion of injected β2m and a high Tm of 400 to 600 $\mu g \cdot min^{-1} \cdot kg^{-1}$. A glomerular sieving coefficient of 0.97 was calculated as the slope of the curve: β2m excretion rate = F (plasma β2m × glomerular filtration rate) for values above saturation. Electrophoresis analysis of proteinuria in agarose gel and sodium dodecyl sulfate polyacrylamide gel showed that injection of saturating doses of β2m induced the excretion of proteins of similar size but different charge and that of other proteins of different size. Among the latter, some were excreted transiently in association with β2m, whereas others had a delayed excretion suggesting existence of a complex mechanism of reabsorption whose steps remain to be elucidated.

Absorption tubulaire rénale de la β2 microglobuline. De 5 à 30 mg de β2 microglobuline (β2m) humaine marquée à l'Iode 125 ont été injectés à des rats anesthésiés. Des études de clairance ont montré un seuil d'excrétion bas et un Tm élevé de 400 à 600 $\mu g \cdot min^{-1} \cdot kg^{-1}$. Un coefficient de tamisage de 0,97 a été mesuré à partir de la pente de la courbe: excretion de β2m = F (concentration plasmatique de β2m × filtration glomérulaire) pour les points au-dessus de la saturation. L'analyse de la protéinurie par électrophorèse sur gel d'agarose et sur gel de polyacrylamide avec dodecyl-sulfate de sodium a montré que l'injection de doses saturantes de β2m provoque l'excretion de protéines de même taille mais de charge différente, ainsi que de protéines de taille différente. Parmi ces dernières, certaines sont excrétées de manière transitoire et en même temps que la β2m, tandis que d'autres ont une excrétion retardée suggèrant l'existence d'un mécanisme de réabsorption complexe dont les étapes restent à étudier.

β2 microglobulin (β2m) is a small protein with a molecular weight of 11,800 daltons [1], a Stokes-Einstein radius of 16 Å [2], and a negative charge [1]. It has been identified as a component of class I histocompatibility antigens [3]. When human β2m is administered to rats by injection, part of the protein binds to a carrier-protein of molecular weight 40 to 50,000 daltons [4]. Due to its size, complexed β2m does not cross the glomerular filter and it is catabolized in an extrarenal site. Conversely, free β2m is filtered by the kidney with a glomerular sieving coefficient (GSC) of 0.94 as calculated in studies utilizing the isolated perfused kidney [5]. The unique site of catabolism of free β2m is the kidney [4].

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The kidney is one of the major sites of catabolism of plasma low-molecular weight proteins; hence, it plays a role in regulating the metabolism of substances such as peptide hormones, enzymes, and immunoproteins [6]. However, the precise mechanism of uptake and degradation by the tubules has not yet been investigated completely. Filtered proteins are taken up by micropinocytosis [7, 8], and then degraded in the lysosomes. Owing to their nearly complete reabsorption, filtered proteins are thought to be bound to the brush border prior to absorptive transport into the cell. The questions then arise as to whether fixation to the brush border is specific or not, and what are the parameters of its specificity. Sumpio and Maack [5], and Cojocel et al [9] have shown that charge plays a role in the specificity of reabsorption since cationic cytochrome inhibits transport of cationic lysozyme but not that of anionic β2m and growth hormone.

We focused our present work on studying renal handling of β2m as a model of protein tubular reabsorption because its lack of biological activity or toxicity allows one to raise the plasma β2m concentration far above that necessary for tubular saturation. Tm and GSC were determined; the nature of proteinuria was also studied after saturating injections of β2m in order to obtain preliminary estimates of the size and charge of endogenous rat proteins which compete with β2m for tubular reabsorption.

Methods

β2 microglobulin. Human β2m was purified from the pooled urine of several renal transplant patients and isolated according to the procedure described by Vincent and Revillard [10]. After preparation, salt-free β2m was lyophilized and kept at −40°C until the day before the experiment; it was then redissolved in distilled water and kept overnight at +4°C. β2m was labelled with ^{125}Iodine by the lactoperoxidase method of Thorell and Johansson [11]. Specific activity was about 0.75 μCi/μg protein (9.9×10^5 cpm/μg). More than 95% of the radioactivity was precipitable by a rabbit antiserum against human β2m and at least 99% was precipitable in 10% trichloracetic acid (TCA). After chromatography of a sample of labelled β2m on Sephadex S200, all the radioactivity was recovered as a single peak in the 12,000-dalton position, thus indicating the homogeneity of the preparation.

Animal experiments. Seven nonfasting male Wistar rats, weighing 180 to 300 g, were given potassium iodide (2 g/liter)

in their drinking water 24 hr before the experiment to prevent thyroid fixation of nonprecipitable iodine.

Early on the day of the experiment, each rat was weighed and anesthetized with the administration of sodium pentobarbital (Nembutal, Abbott Laboratories Hospital Products Division, North Chicago, Illinois, 5% w/v), 50 mg/kg i.p., then as often as necessary during the experiment. Each animal was placed on a heated board. The bladder was catheterized carefully with a funnel-shaped PE-50 polyethylene tubing for urine collection. After tracheotomy, the carotid artery was catheterized for blood sampling and 0.5 ml heparinized saline was infused via the carotid. Isotonic saline solution was infused into the jugular vein at a constant rate of 4.5 or 6 ml/hr according to body weight; surgical losses were replaced by a single intravenous injection of isotonic saline solution (2.0 ml).

A constant infusion of Polyfructosan (Inutest, Laevosan Gesellschaft, Linz, Austria) in 0.9% saline solution (0.03 g/ml) was administered and subsequent measurements of Polyfructosan clearance were used to measure glomerular filtration rate (GFR). Mean GFR was 10.6 ± 2.2 ml/min/kg (N = 44) in our experimental conditions and was not modified significantly during the course of the experiment, even after β2m injection. After a 60-min equilibration period, a control collection of urine was obtained for 20 min, and a blood sample was drawn at the mid-point of the urine collection period.

Labelled β2m (20 to 50 μg, 10^6 cpm/μg) and cold β2m (in doses ranging from 5,000 to 30,000 μg) were then injected through a lateral connection into the jugular vein at zero time, followed immediately by 0.1 ml saline. Blood samples were taken at 1, 3, and 10 min after β2m injection, then every 10 min, and liquid losses were replaced by saline. Urine was collected during consecutive 10-min periods, starting 5 min after β2m injection (urine collected 5 min after injection was not used for clearances determination). Urine pH was about 6, thus insuring no significant urinary degradation of β2m.

Analytical methods. Polyfructosan was determined by a fluorimetric method [12] using a dimedon reagent. Total and TCA precipitable radioactivity in blood and urine was determined on an autogamma scintillation spectrometer (Packard Instruments, Downers Grove, Illinois) as previously described [4].

Free versus total β2m ratio was determined by chromatography of 100 μl samples as described previously [4].

Urine electrophoresis was performed on agarose gel following the procedure of Jeppsson, Laurell, and Franzen [13] and on polyacrylamide gel in the presence of sodium dodecyl sulfate (SDS) as described by Laemmli [14]. Autoradiography of dessicated agarose plates was performed at −70°C with Kodak X-O MATR films and an intensifying screen Cronx lighting plus (Du Pont) [15].

Calculation of β2m clearance. The lag time between plasma and urine, as well as the nonsymetric distribution of β2m plasma content during each period of time had to be taken into account for β2m clearance determination. Since the lag time between filtration and the appearance of urine in the collection tubing depends on urine flow rate, six rats in the same experimental conditions as described above were injected with 0.1 ml lissamine green. The lag time between injection and dye appearance in the collection tubing was measured; lag time between injection and coloration of the kidney was negligible. The results are shown in Figure 1. This plot was used to determine lag time for each experimental period after β2m injection and thus to shift the limits of the urine period on the corresponding plasma kinetic curve. Mean plasma β2m concentration of each period was calculated from the area of the plasma kinetic curve divided by the time. Filtered load was calculated as plasma free β2m × GFR × GSC, with plasma free β2m calculated as described above.

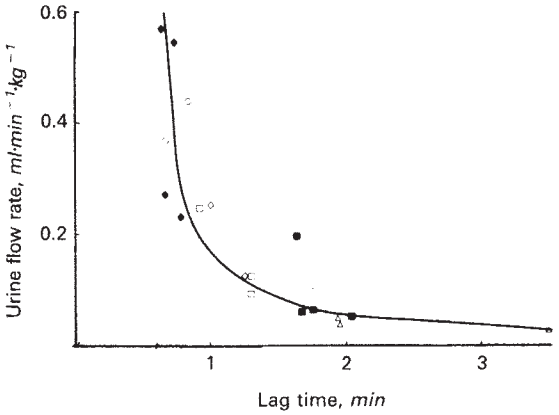

Fig. 1. *Lag time between injection and urinary output of lissamine green.* Each *symbol* represents experimental *points* from one animal.

Results

β2m clearance data: glomerular sieving coefficient Tm and kinetics of degradation. Injection of human β2m administered to rats in amounts up to 30 mg per animal allowed plasma content of total β2m to rise up to about 600 μg/ml, that is, about 150 times the normal value of rat plasma β2m [16]. Chromatography of each plasma sample and some urine samples showed that part of the injected β2m bound gradually to the plasma carrier protein, whereas complexed β2m was not found in urine. Free β2m was rapidly eliminated from plasma with a mean metabolic half-life of 30 min ± 7 (N = 7) which was very similar to that obtained when small doses of β2m were injected [4].

The relationship between plasma free β2m × GFR and urinary free β2m excretion, is presented in Figure 2. β2m appeared in urine as soon as the filtered load was increased by twofold. Then β2m excretion increased linearly as filtered load increased; the slope of 0.97 represents the glomerular sieving coefficient for β2m.

The titration curve could be drawn from the above values, taking 0.97 as GSC value for β2m (Fig. 3). That curve shows that reabsorption was saturated above 1,200 $\mu g \cdot min^{-1} \cdot kg^{-1}$ of filtered β2m. It gives a Tm value of about 400 to 600 $\mu g \cdot min^{-1} \cdot kg^{-1}$.

Figure 4 depicts the kinetics of events after β2m injection in four rats with the highest injected doses: Filtered load of free β2m decreased sharply after injection whereas reabsorbed amounts were grossly constant in the range of time considered. Degradation was estimated by the appearance of nonproteic radioactivity in urine. Urinary excretion of nonTCA-precipitable activity paralleled plasma content and clearance values averaged GFR (not shown), showing that the nonproteic degradation products of β2m are freely filtered. Urinary output of nonTCA-precipitable activity increased during the

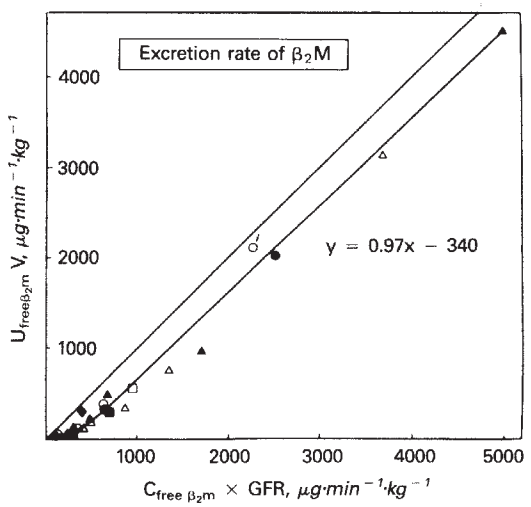

Fig. 2. *Urinary excretion rate of β2m.* Each *symbol* represents experimental *points* from one animal. Experimental *points* below 300 $\mu g \cdot min^{-1} \cdot kg^{-1}$ were excluded for calculation of the regression curve.

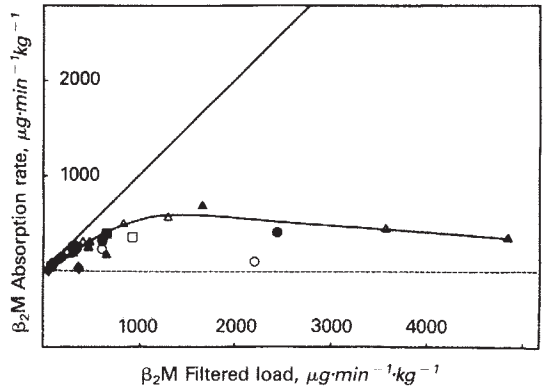

Fig. 3. *Titration curve of free β2m.* Reabsorption rate: filtered load-urinary excretion rate. Filtered load: plasma β2m × GFR × GSC. The *symbols* are the same as in Figure 2. The *lower line* was drawn from the data for one rat (▲).

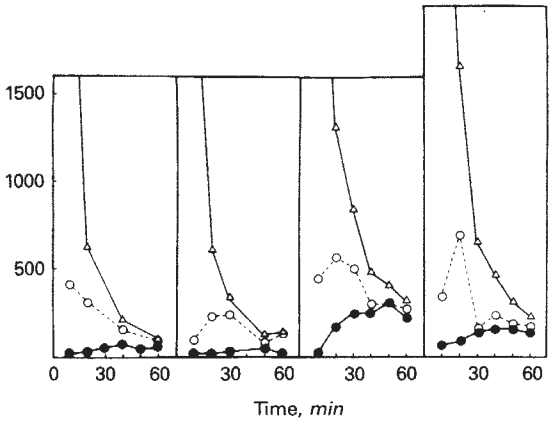

Fig. 4. *Kinetics of renal handling of injected β2m (highest doses); β2m filtered load (△-△), plasma free β2m × GFR × GSC; β2m absorption rate (○--○), filtered load—excretion rate; degraded β2m (●-●), equivalent amount of β2m estimated from urinary excretion rate of nonproteic radioactivity.* All forms of β2m are expressed as $\mu g \cdot min^{-1} \cdot kg^{-1}$.

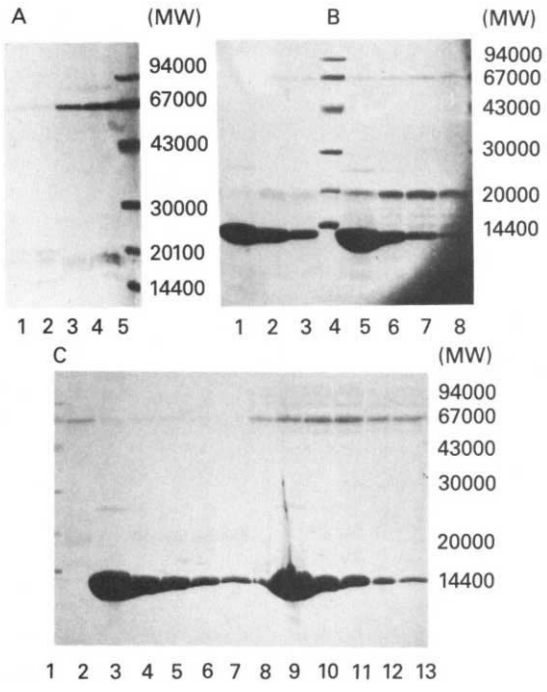

Fig. 5. *Gel electrophoresis on polyacrylamide + SDS.* Calibration mixture (A5, B4, C1) was obtained from a low molecular weight calibration kit (Pharmacia Diagnostics, Uppsala, Sweden). Molecular weights are indicated on the *right side* of each photograph. **A** *Electrophoresis on 15% gel of control urine from two rats (1, 2, and 3, 4, respectively) with 15 μl (1,3) and 100 μl (2,4) inclusion.* **B** *Electrophoresis on 20% gel of urine from two rats (1-3 and 5-7, respectively) after β2m injection.* Inclusions of 15 μl were ordered from the *left* to the *right* in the chronologic order, always beginning by the first period following β2m injection. **C** *Electrophoresis on 20% gel of urine from two rats (2-7 and 8-13, respectively).* Inclusion volume was 30 μl for every inclusion of the first rat, 15 μl for every inclusion of the second rat. Inclusions were ordered from the *left* to the *right* in chronologic order, always beginning by a control period (2 and 8), then the first period following injection (3 and 9), and so on. The lower band of 8 *(control)* is an artefact due to side diffusion of the very intense band 9.

course of the experiment and, after 1 hr, was close to the reabsorbed amounts. There was a delay between the bulk of reabsorption of filtered β2m and the appearance of degradation products.

Analysis of proteinuria after β2m injection. SDS polyacrylamide gel electrophoresis of urine before and after β2m injection are shown in Figure 5. Control samples of urine from rats just before an injection was administered (Fig. 5 **A** and **C**) were found to contain mainly albumin (upper band) and a number of thin bands between the 12,000- and the 20,000-dalton positions. One rat (Fig. 5**C**, right) had a glomerular proteinuria during the control period, that was slightly increased by β2m injection and remained after 1 hr. After β2m injection (Fig. 5 **B** and **C**) urine contained so much β2m that the electrophoresis plate was overloaded in the 12,000-dalton zone, thus preventing identification of additional proteins in that zone. β2m band intensity decreased during the course of the experiment but never returned to the control pattern within the range of time chosen. The albumin band was not inforced by the β2m injection. Besides, the excretion pattern of smaller proteins was markedly modified by the β2m injection: One band appeared in

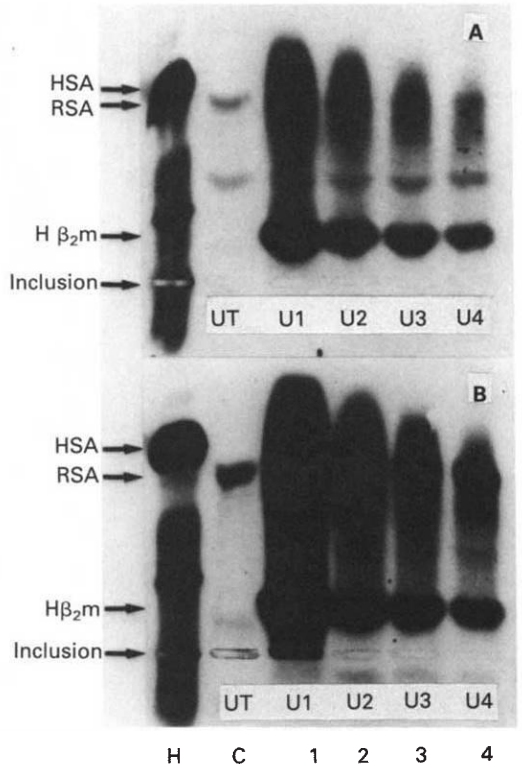

Fig. 6. *Electrophoresis on agarose gel at pH 8.6 of urine from two rats (A and B, respectively).* From the left to the right, abbreviations are: H, human serum; C, control rat urine; 1, 2, 3, 4, the first four urine samples from the same rat after β2m injection; HSA, human serum albumin; RSA, rat serum albumin; Hβ2m, human β2 microglobulin. Each sample was collected during 10 min.

each rat in a 27,000-dalton zone and decreased in parallel with β2m. In the 17,000 to 20,000-dalton zone, some bands appeared or were inforced after the β2m injection, but their intensity did not decrease. Some of these bands seemed to appear after a delay of one period or to increase during the second or third period.

The observed changes in the proteinuria pattern after β2m injection were quantitatively but not qualitatively different from one animal to another.

Figure 6 shows agarose gel electrophoresis of rat urine before and after the β2m injection. The main band was β2m which decreased during the course of time. The bands detectable in control urine, albumin (Fig. 6 **A** and **B**), and a component of lower mobility (Fig. 6**A**), were also detectable after β2m injection and did not decrease during the course of time. Two additional bulks of proteins appeared in the albumin zone and the region of middle mobility: These bulks were very intense and represent several intricated bands. Their intensity decreased in the same way as did the β2m band. A very pale band appeared after β2m injection on the anodic migration side of the plate. Autoradiography of the agarose plates (not shown) showed that only the band in β2m position was radioactive. In addition some precipitated material in the inclusion well was also radioactive in the case of Figure 6**B**. From agarose gel electrophoresis of serially diluted (1:2 to 1:640) urine samples, it was estimated that the amount of β2m was at least 30 times greater than that of the bulk of proteins migrating in the albumin position.

Discussion

Renal clearance of β2m. Our results show a low threshold of β2m urinary excretion, which is a common feature for small proteins [6]. From determinations of urine and serum β2m in patients with normal or altered GFR, Wibell [17] suggested a β2m plasma threshold of 4.5 μg/ml (normal serum levels in humans are 1.7 μg/ml), above which urinary excretion of β2m was increased. However, it is, to our knowledge, not possible to estimate the extent of tubular alterations in patients with decreased GFR.

Due to the large amount of purified material needed for each experiment, little is known about the Tm of proteins in the kidney tubules. The generally low threshold of excretion of filtered proteins, added to the increasing-type relationship between filtered load and excretion, led to suppose that their reabsorption was saturated for filtered loads close to normal values [18]. Further studies [6, 19] on lysozyme and albumin showed that when filtered load increases above the threshold of excretion, there occurs a "splay" region [5] where part of the filtered load is increasingly reabsorbed whereas the remaining is excreted. Plasma protein level may have to be very largely increased before the real Tm is reached, since Tm for lysozyme is 50 to 100 times larger than the normal filtered load. The absolute value of Tm by itself may be very different from a protein species to another. For instance Sumpio and Maack [5] showed a Tm of 800 μg/min per rat kidney for lysozyme and in in the same conditions, a Tm of 6.5 μg/min per rat kidney for cytochrome C. The rate of reabsorption depends on the charge and configuration of the molecule [20]. These findings support the view that protein tubular absorption is a complex phenomenon of low affinity, high capacity, and low specificity.

Our results provide further evidence for this conception: There is a "splay" region beyond the threshold of excretion where β2m reabsorption is not saturated. The Tm value is not very high (four times the normal filtered load) when compared to the lysozyme Tm value. However, one should keep in mind that elevated plasma β2m levels are observed in many common pathologic situations such as inflammatory disorders [21, 22], viral infections [23], and hepatitis [24]; in such cases plasma β2m level is generally included in the splay region. Therefore, increased urinary excretion which may be encountered in such situations does not provide an estimate of the increased biosynthesis of β2m. The glomerular sieving coefficient was drawn from the curve $UV = f(P \times GFR)$ considering only filtered loads far beyond Tm. The calculation of Tm is usually drawn from clearance measurements performed in steady-state conditions by using continuous intravenous infusion. Such a method could not be used because it would require much greater amounts of highly purified β2m than those available for the present study. Therefore, the single injection technique was chosen to achieve very high plasma levels at least during a short period. Errors in GFR determination by this technique depend mainly on the nonequilibrium of the test substance among fluid compartments [25]. In the present study no assumption on the diffusion of β2m was required because for each period the plasma concentrations of β2m were graphically derived from the measured plasma levels. Kinetics of re-

absorption (Fig. 4) would suggest that reabsorption is not saturated by the initially high filtered β2m load, but that maximum of the transport capacity is achieved after a delay of sometimes more than 5 min. If β2m itself could trigger an increase in its reabsorption, then the slope in Figure 2, that is, the GSC, would be slightly overestimated. Calculation of GSC was therefore performed with one rat in which saturation was with certitude achieved 5 min after injection (Fig. 4, left). The calculated GSC value was of 0.91 in this case. Whatever are the potential pitfalls of the single injection technique, the values obtained (0.97 for the seven rats or 0.91 for the latter) are close to those (0.94) obtained by Sumpio and Maack [5] in the isolated rat kidney in presence of iodoacetate, an inhibitor of tubular reabsorption. The similarity of the values provides a certain confidence to them and indicates that free β2m is almost not hindered by the glomerules. This may be explained by the small size of the molecule (16 Å) since Brenner, Hostetter, and Humes [26] have shown that sieving of macromolecules appears beyond a 20-Å radius. The negative charge of β2m does not seem to interfere very much, although negatively charged small molecules are significantly hindered by the glomerulus.

The kinetics data agree with previous findings in humans [28] and rat [29] showing that there is a lag time between β2m reabsorption and appearence of the degradation products. Autoradiography of urine electrophoresis showed that after β2m injection, the migration properties of β2m excreted in urine remained identical to that of intact β2m. This suggests that brushborder proteases which were shown to degrade small peptidic hormones [30] do not affect the β2m molecules that are not reabsorbed.

Competition experiments. Electrophoresis of urine on SDS polyacrylamide gel shows that no competition occurs between β2m and albumin, although the small filtered fraction of albumin is extensively reabsorbed by the kidney [19]. Two groups of small proteins appeared in urine after β2m injection. Some appeared and disappeared at the same time as β2m whereas some appeared after a delay and were still excreted after 1 hr. This result could be explained by the hypothesis that the first group may share the same site of fixation or transport on the cell wall and the same degradation pathway whereas the second group quantitatively more important only shared the same degradation pathway.

Electrophoresis in agarose gel showed a very intense bulk of protein in addition to the β2m band. Because in SDS polyacrylamide gel electrophoresis no such important band of protein was seen outside the β2m zone, the bulk of protein in agarose gel must be proteins of the same molecular weight as β2m but of a different charge. Autoradiography demonstrated that these proteins are not degradation products of β2m. Thus, β2m must be reabsorbed in competition with several proteins of similar molecular weight, which represents a major part of the competition between proteins. Agarose gel electrophoresis pointed out a possible competition between proteins of a different charge at a very high dose of β2m, although at a more physiological concentration no competition could be seen between cationic and anionic proteins [5, 9, 31]. In conclusion, the survey of proteinuria after injection of a single high dose of β2m allowed the discrimination between several groups of proteins, suggesting the existence of a complex mechanism of reabsorption whose steps remain to be elucidated.

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References

1. Berggard I, Bearn AG: Isolation and properties of a low molecular weight β2 globulin occurring in human biological fluids. *J Biol Chem* 243:4095–4103, 1968
2. Karlsson AF: Physical chemical properties of β2 microglobulin. *Immunochem* 11:111–114, 1974
3. Rask L, Lindblom JB, Peterson PA: Subunit structure of H-2 alloantigens. *Nature* 249:833–835, 1974
4. Nguyen-Simonnet H, Vincent C, Gauthier C, Revillard JP, Pellet MV: Turnover studies of human β2 microglobulin in the rat: evidence for a β2 microglobulin binding plasma protein. *Clin Sci* 62:403–410, 1982
5. Sumpio BE, Maack T: Kinetics, competition and selectivity of tubular absorption of proteins. *Am J Physiol* 243:F379–F392, 1982
6. Maack T, Johnson V, Kau S, Figueiredo J, Siguelm D: Renal filtration, transport and metabolism of low molecular weight proteins. A review. *Kidney Int* 16:251–270, 1979
7. Bariety J, Druet P, Laliberte F, Sapin C, Belair MF, Paing M: Ultrastructural evidence, by immunoperoxydase technique, for a tubular reabsorption of endogenous albumin in normal rat. *Lab Invest* 38:175–180, 1978
8. Straus W: Occurrence of phagosomes and phagolysosomes on different segments of the nephron in relation to the reabsorption, transport, digestion and extrusion of intravenously injected horseradish peroxidase. *J Cell Biol* 21:295–308, 1964
9. Cojocel C, Franzen-Sieveking M, Beckman G, Bauman K: Inhibition of renal accumulation of lysozyme (basic low molecular weight protein) by basic proteins and other basic substances. *Pflügers Arch* 390:211–215, 1981
10. Vincent C, Revillard JP: Comparison of radioimmunoassay and lymphotoxicity inhibition techniques for the determination of β2 microglobulin. *J Immunol Methods* 10:253–259, 1976
11. Thorell JI, Johansson BG: Enzymatic iodination of polypeptids with ^{125}I to high specific activity. *Biochem Biophys Acta* 251:363–369, 1971
12. Vurek GE, Pegram SE: Fluorimetric method for the determination of nanogram quantities of inulin. *Anal Biochim* 16:409–419, 1966
13. Jeppson JO, Laurell LB, Franzen B: Agarose gel electrophoresis. *Clin Chem* 25:629–638, 1979
14. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685, 1970
15. Laskey RA, Mills D: Enhanced autoradiographic detection of ^{32}P and ^{125}I using intensifying screens and hypersensitized film. *FEBS Lett* 82:314–316, 1977
16. Logdberg L, Ostergren PO, Berggard I: Rat β2 microglobulin. Isolation properties and relation to β2 microglobulins from other species. *Mol Immunol* 16:577–587, 1979
17. Wibell LB: Studies on β2 microglobulin in patients and normal subjects. *Acta Clin Belg* 31:14–26, 1976
18. Lambert PP, Gassee JP, Askenasi R: Physiological basis of protein excretion, in *Proteins in Normal and Pathological Urines,* edited by Manuel Y, Revillard JP, Bétuel H, Karger, Basel, New York, 1970, pp 67–82
19. Galaske RG, Baldamus CA, Stolte H: Plasma protein handling in the rat kidney: micropuncture experiments in the acute heterologous phase of anti GBM nephritis. *Pflügers Arch* 375:269–277, 1978
20. Christensen EI, Rennke HG, Carone FA: Renal tubular uptake of protein: effect of molecular charge. *Am J Physiol* 244:F436–F441, 1983
21. Karlsson FA, Wibell L, Evrin PE: β2 microglobulin in clinical medicine. *Scand J Clin Lab Invest* 40:27–37, 1980

22. REVILLARD JP, VINCENT C, CLOT J, SANY J: β2 microglobulin and β2 microglobulin-binding proteins in inflammatory diseases. *Eur J Rheumat Inflam* 5:398–405, 1982
23. LAMELIN JP, VINCENT C, FONTAINE-LEGRAND C, REVILLARD JP: Elevation of serum β2 microglobulin levels during infectious mononucleosis. *Clin Immunol Immunopathol* 24:55–62, 1982
24. REVILLARD JP: Significance of β2 microglobulin in liver diseases. *Vox Sang* 339–342, 1980
25. LEVINSKY NG, LEVY M: Clearance techniques, in *Handbook of Physiology, Section 8,* edited by ORLOFF J, BERLINER RW, Washington, D.C., American Physiological Society, 1973, p 103
26. BRENNER BM, HOSTETTER TH, HUMES HD: Glomerular permselectivity: barrier function based on discrimination of molecular size and charge. *Am J Physiol* 234:F455–F460, 1978
27. VINCENT C, POZET N, REVILLARD JP: Plasma β2 microglobulin turnover in renal insufficiency. *Acta Med Belg* 35:2–13, 1980
28. MAUNSBACH AB, CHRISTENSEN EI: Lysosomal accumulation and digestion of β2 microglobulin in rat kidney tubules. *Int Rev Cytol [Suppl] (Res Biochem Biophys Mem Cell Biol Kidney Urin Sys)* 2:1737, 1974
29. CARONE FA, PETERSON DR, OPARIL S, PULLMAN TN: Renal tubular transport and catabolism of proteins and peptides. *Kidney Int* 16:271–278, 1979
30. HALL PW, VACCA C, CROWLEY A: The effects of β2 microglobulin loading on the renal extraction of low molecular weight proteins in the dog *(abstract). Kidney Int* 16:853, 1979