Renal Reabsorptive Capacity for α2u-Globulin in the Adult Male Rat (43397)

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Abstract. **Adult male rats were maintained on 0, 5, 10, 15, and 20% casein diets to produce a series of animals having serum α2u-globulin levels varying linearly from a normal of 31 μg/ml to a minimum of 13 μg/ml. In this way, it was possible to titrate endogenously the renal reabsorption and urinary excretion of this low molecular weight protein. The average maximal reabsorption rate (T_m) was established to be 9.7 μg/min and was reached at a renal filtered load ($F_{\alpha 2u}$) of 13.6 μg/min. These data were expressed in terms of a T_m:$F_{\alpha 2u}$ ratio of 0.71. Below this value, the reabsorption declined from 70% to 50% of the $F_{\alpha 2u}$. Above 0.71, where $F_{\alpha 2u}$ is less than the T_m, the reabsorption increased to 80–90%. It was observed that the fractional renal uptake of the α2u-globulin varied linearly with the filtered load.** [P.S.E.B.M. 1992, Vol 200]

Kidneys play an essential role in the metabolism of plasma proteins (1, 2). This function includes the maintenance of plasma protein levels and the disposal of proteins reabsorbed by the kidneys. However, the factors regulating the reabsorptive capacity for different proteins are unclear. It is apparent from early studies that protein reabsorption is a saturable, energy-dependent, endocytotic process. Studies comparing the infusion of heterologous proteins into the rat kidney showed that the saturation point (T_m) for the reabsorption of egg white lysozyme (LZM) occurred at about 800 μg/min, whereas that for cytochrome *c* was only 6.5 μg/min (2–4). Although the reason for this difference is unknown, it suggests the potential existence of two processes, one of high capacity and one of low. The intravenous administration of increasing doses of LZM to rats indicated that its accumulation in the renal tissue was a constant fraction of the administered dose (1). Cojocel and Baumann (5), however, showed that about 99% of the endogenous filtered LZM load was reabsorbed when the plasma level was normal at 4.46 μg/ml and about 70% was reabsorbed when the plasma level was raised to 255 μg/ml by injecting egg white LZM (6). These studies showed that the fraction reabsorbed depended upon the filtered load (F_p). In general, it is accepted that the F_p of most proteins is well below the T_m for that protein and almost all of the protein is reabsorbed and little is excreted in the urine (2). An uncertainty regarding past studies is the fact that the proteins used were often (i) "foreign," (ii) of highly cationic net charge, or (iii) modified by radio-iodination.

One exception to the principle that proteins are largely reabsorbed and not excreted is the sex-dependent protein of the adult male rat called α2u-globulin (α2u) (7, 8). It is an anionic, low molecular weight protein (LMWP) (mol wt 18,000) normally excreted in the urine in large amounts. This endogenous protein might provide a unique model to establish the relationship between filtered load for α2u ($F_{\alpha 2u}$), T_m, and excretion. α2u-Globulin has already been employed for the study of the renal reabsorption process in adult male rats (9, 10). Normally, the blood serum level is about 30 μg/ml and the protein is reabsorbed by the kidneys to the extent of 40–60% of its $F_{\alpha 2u}$; the remainder is excreted in the urine. In other words, α2u is a major urinary protein in the adult male rat, amounting to 10–30 mg/24 hr and representing 25–30% of the total urinary protein (7, 8). Studies using intact male rats kept on a 0% casein diet showed that the serum level could be reduced to 14 μg/ml and the reabsorption increased to 90% (7, 8, 11). The hepatic synthesis of α2u is dependent upon the availability of dietary amino

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acids (11, 12). It is proposed that groups of rats having differing α2u serum levels and, hence, renal $F_{\alpha 2u}$ could be produced by varying the dietary intake of protein between 0% and 20%. Therefore, the purpose of the current study was to utilize variations in dietary protein intake as a means of endogenously titrating the renal reabsorptive process.

Materials and Methods

Animals and Diets. Adult, male Sprague-Dawley rats (Sasco, Omaha, NE) weighing 225–300 g were fed *ad libitum* for 10 to 15 days on the diets described below. Rats were housed individually in stainless steel metabolism cages having tunnel feeders and urine feces separators. Twenty-four-hour urine samples were collected in flasks containing 1 ml of a solution consisting of streptomycin (1.2%) and penicillin (1.2%) saturated with thymol. Urine samples were filtered and stored frozen prior to use. Blood samples were collected under light ether anesthesia by infraorbital puncture. After allowing the blood to clot at room temperature, the clot was allowed to retract overnight at 4°C. The serum was removed after brief centrifugation.

The normal or 20% casein diet consisted of 20 g of casein, 60 g of dextrin, 10 g of corn oil, 4 g of salt mixture (ICN-NBCo, Cleveland, OH), and 2.2 g of vitamin mixture (ICN-NBCo). Diets of varying protein content were made in the same way, except that an appropriate amount of casein was replaced by additional dextrin (11).

Renal Loads. It was considered impractical at this point to measure the glomerular-filtration rate and the permeability ratio for α2u. Therefore, to estimate the $F_{\alpha 2u}$, its excretion was determined following the intraperitoneal injection of a solution of sodium maleate (MAL) (40 mg/100 g body wt) (8, 13). Urine samples were collected for a period of 24 hr following injection. It is clear that this provides only a reasonable estimate of the $F_{\alpha 2u}$ and is not a precise measurement.[2]

Analytical Methods. Immunologic assays for α2u were performed by radial immunodiffusion, as described previously (11). As indicated above, all urine samples were collected over a 24 hr period, thereby minimizing the complication produced by the cyclical excretion of α2u throughout the day (14). These data were then recalculated in terms of μg/min.

[2] Injection of MAL, *in vivo*, is believed to provide a realistic estimate of Fα2u, as indicated by earlier studies. For example, with *in vivo* infusion studies using LZM as the inhibitor of protein reabsorption, the α2u excretion rate became 18.1 ± 4.5 μg/min, with cytochrome *c* 21.9 ± 2.1 μg/min; with L-lysine 23.4 ± 4.1 μg/min, and with MAL 17.6 ± 7.1 μg/min (normals varied from 7.0 to 8.3 μg/min) (9, 10). Another approach used total nephrectomy; the accumulation of α2u in the serum above the normal yielded a rate of 17.6 μg/min for a 300-g rat. This would be equivalent to the amount handled by the kidneys per unit of time or the $F_{\alpha 2u}$ (7). The MAL-induced excretion rate reported in Table I (20% casein diet) of 19.4 ± 3.2 μg/min is comparable to the earlier data. Therefore, it is considered to be a reasonable estimate of $F_{\alpha 2u}$.

Results

The purpose of the following study was to titrate the renal reabsorption of protein using the endogenous, sex-dependent, LMWP α2u. A relationship was sought between $F_{\alpha 2u}$, the reabsorption of α2u, and its urinary excretion rate ($UV_{\alpha 2u}$).

Dietary Protein: Serum Levels and Urinary Excretion of α2u. Adult male rats were placed on a series of diets having protein contents varying from 20% to 0% casein. After 10 days, this reduction in protein intake resulted in stabilized blood serum levels and urinary excretion of α2u (11). The decline in these factors is the consequence of a reduced hepatic synthesis of the protein (11, 12). Figure 1A shows the decline in $UV_{\alpha 2u}$ from a normal range of 13–17 mg/24 hr to a minimum of 0.6–1.0 mg/24 hr. At the same time, the serum levels were reduced linearly from a normal average of 33 μg/ml to a minimum of 14 μg/ml (Fig. 1B). These values are comparable to those published previously (7, 11). As seen from Table I, the diminished intake of protein was also accompanied by a decline in $F_{\alpha 2u}$. The filtered load was estimated by injecting a bolus of MAL which, on the basis of previous work, blocked the reabsorptive process and provided an esti-

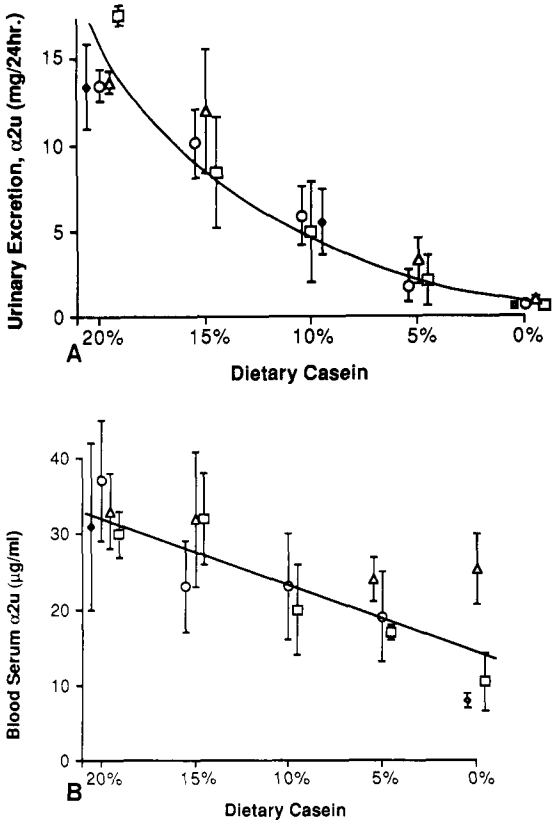

Figure 1. (A) Relationship between dietary protein intake and the urinary excretion of α2u. From three to four independent experiments (○, △, ◆, □) were performed, involving a total of 12–18 adult male rats. (B) Variation of serum α2u levels with dietary protein intake. Experimental data same as for Figure 1A.

Table I. Excretion of α2u-Globulin by Adult Male Rats Maintained on Diets of Varying Protein Contents[a]

Diet casein[b] (%)	$UV_{\alpha 2u}$[c] (μg/min)	$F_{\alpha 2u}$[c] (μg/min)	$P_{\alpha 2u}$ (μg/ml)	Renal reabsorption (μg/min)	Renal reabsorption (%)	$T_m:F_p$[c,d]
20	10.1 ± 1.6 (15)	19.4 ± 3.2 (14)	31.0 ± 8.0	9.5 ± 3.8 (14)	49.0	0.50
15	6.9 ± 1.8 (14)	16.7 ± 4.0 (12)	28.0 ± 7.0	9.9 ± 4.4 (12)	59.3	0.58
10	3.8 ± 1.3 (16)	13.6 ± 5.2 (13)	21.0 ± 6.0	9.6 ± 5.3 (13)	70.6	0.71
5	1.7 ± 0.8 (16)	8.2 ± 3.8 (12)	22.0 ± 8.0	6.5 ± 3.5 (14)	79.3	1.18
0	0.6 ± 0.2 (18)	4.7 ± 1.4 (15)	13.0 ± 7.0	4.2 ± 1.4 (15)	89.4	2.06

[a] Data are provided as means ± SD for three to four independent experiments. Total number of rats used is given in parentheses.
[b] Rats were maintained on experimental diets for a minimum of 10 days prior to collection of samples.
[c] All urine samples were collected in 24-hr periods and were recalculated as μg/min; UV, normal 24-hr urinary excretion; $F_{\alpha 2u}$, 24-hr excretion following the injection of MAL (40 mg/100 g).
[d] T_m, 9.7 μg/min; average of the three maximum values for reabsorption.

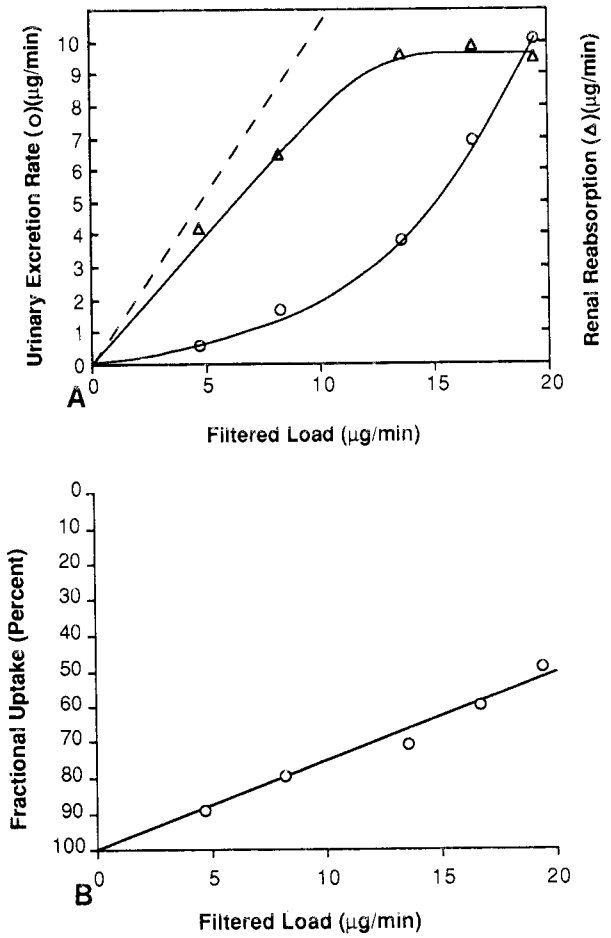

Figure 2. (A) Relationship between $F_{\alpha 2u}$, urinary excretion rate (-○-), and renal reabsorption (-△-). Values used are means ± SD (Table I) for a total of 12–18 rats in three to four independent experiments. Dashed line represents hypothetical excretion rates in the absence of reabsorption. (B) Variation of percentage reabsorption of α2u with $F_{\alpha 2u}$.

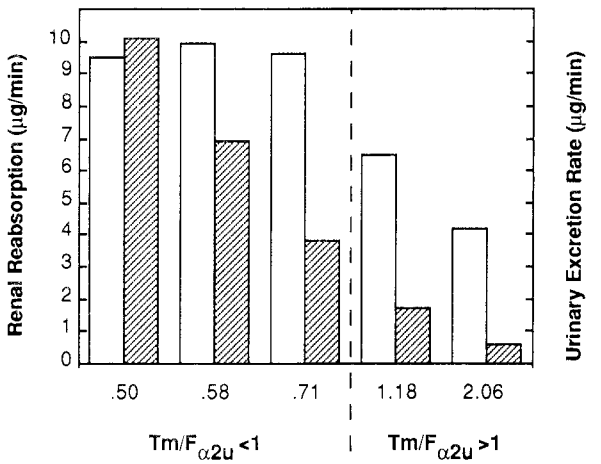

Figure 3. Histogram showing α2u reabsorption (open bars) and urinary excretion (hatched bars) at various values of ($T_m/F_{\alpha 2u}$). Vertical dashed line represents a hypothetical situation in which the ratio is 1.00.

mate of the $F_{\alpha 2u}$ (8, 14). Urine samples for each casein diet, control, and MAL-treated rat, were collected at 24-hr intervals; data were recalculated as μg/min and reported in Table I as means ± SD.

α2u-Globulin Reabsorption Versus Fα2u. Figure 2A compares the $UV_{\alpha 2u}$ with $F_{\alpha 2u}$; the dashed line represents the expected excretion in the absence of any reabsorption (1, 3). It is apparent from Figure 2 that as the amount presented to the kidneys was increased above a saturation level, the excretion curve paralleled the hypothetical (dashed line) and the amount excreted became proportional to $F_{\alpha 2u}$. The shape of this curve is comparable to that reported for LZM, cytochrome *c*, and β2-microglobulin (3, 15). When the data were plotted in terms of the total amount reabsorbed by both kidneys ($F_{\alpha 2u} - UV_{\alpha 2u}$; Fig. 2A), then the absorption rate reached an average maximum value (T_m) of 9.7 μg/min. Figure 2B compares the fractional uptake ($F_{\alpha 2u} - UV_{\alpha 2u}:F_{\alpha 2u} \times 100$) with $F_{\alpha 2u}$ and shows this relationship to be linear. It is apparent from Table I and Figure 2A that the absolute amount of protein reabsorbed had an average value of 9.7 μg/min (9.5–9.9), beginning with a minimal $F_{\alpha 2u}$ value of 13.6 μg/min. Figure 2B shows that the renal α2u reabsorption was 70% of this minimum load. Increases above 13.6 to the physiological level of 20 μg/min resulted in a 50% reabsorption,

whereas a decline to 4.7 μg/min increased the reabsorption to 90%.

Discussion

Renal reabsorption of protein from tubular fluid is considered to occur via a "selective constraint model" that includes (i) binding of positively charged regions on the proteins with negatively charged membranal receptors, (ii) migration of the complex to the endocytotic site at the base of the microvilli, and (iii) endocytosis itself (1, 2). This process is known to be saturable and can be described in kinetic terms. The renal accumulation of LZM is of first order under conditions of continuous infusion of the protein into the whole rat (16). Hypothetically, the point of saturation of protein uptake (T_m) might be reached at an equal filtered load, so that the ratio of T_m:F_p theoretically should be 1.0. It is generally recognized, however, that the F_p for a given protein must exceed the T_m itself in order to reach the saturation point (T_m). Thus, studies using perfused rat kidneys have shown that the uptake of egg white LZM reached a saturation point of 800 μg/min only after the filtered load exceeded a level of 1200 μg/min (2). In other words, the ratio of T_m:F_{LZM} was less than 1.0 (0.44–0.67).[3] Similarly, cytochrome *c* reached a T_m of 6.5 μg/min, at filtered load of cytochrome *c* of 10 μg/min or a ratio of 0.65. Comparable data for β2-microglobulin (15) yielded a range of 0.33–0.50. In each case, the ratio was less than 1.0. To obtain these data, it was necessary to inject or infuse increasing amounts of the protein studied. Under normal, physiological conditions, however, this appears not to be the case because the T_m:F_p ratio is generally greater than 1.0. In other words, in the intact rat, the F_p is much less than the T_m. For example, for LZM, the filtered load was shown to be 4.44 μg/min compared with the T_m of 800 μg/min; the ratio of T_m:F_{LZM} far exceeded 1.0. Under these conditions 99.5% of the filtered load was reabsorbed; only 0.5% was excreted in the urine (5). In general, it has been concluded that normally there is a high absorptive capacity in relation to the filtered loads and that the excretion of any protein is negligible, with over 90% being absorbed by the kidneys (1).

This, however, cannot be true for a LMWP normally having an F_p in excess of the T_m. Such is the case for α2u, which is excreted in the urine of adult male rats in large quantities (10–30 mg/day) (17). Although competitive studies have shown that α2u shares a general reabsorptive process with other LMWP, including LZM (10), it appears that normally the $F_{\alpha 2u}$ exceeds the T_m and that the T_m has been reached at physiological F_p levels. Based on current data (Table I and Fig. 3), the average T_m of 9.7 μg/min had been reached when the $F_{\alpha 2u}$ level was 13.6 μg/min or when the T_m:$F_{\alpha 2u}$ ratio was 0.71. As seen from Figure 3, when the ratio became 0.71 or less, the unabsorbed α2u was excreted. The increasing excretion rate then paralleled a hypothetical curve representing the excretion rate in the absence of absorption (Fig. 2A). At the normal $F_{\alpha 2u}$, i.e., 20 μg/min, 50% of the α2u was reabsorbed (Fig. 2B) and the remainder was excreted in the urine. When the ratio of T_m:$F_{\alpha 2u}$ exceeded 1, then both the absolute amount reabsorbed and that excreted declined. At this point, Figure 2A exhibits a so-called "splay" region comparable to that observed for other proteins (15). It is apparent from Figure 2B that the relationship between $F_{\alpha 2u}$ and the fractional absorption is linear. These data show that at low $F_{\alpha 2u}$, e.g., 4.5 μg/min, the fractional absorption was 80–90% of the filtered load. It is under these conditions that the urinary excretion of α2u also becomes negligible.

It is concluded that the kinetics for α2u reabsorption are qualitatively comparable to, but differ quantitatively, from those of other LMWP. Despite the fact that α2u must share a common reabsorptive process with other LMWP, there must be a selectivity in the overall mechanism. In accord with Maack et al.'s (1) suggestions, this selectivity cannot be defined in exclusive terms, but rather as differential rates of saturation and excretion among varying filtered loads, namely, the T_m to F_p ratio. This ratio for most LMWP studied is normally greater than 1 and only a small proportion of the filtered load is excreted. For α2u-globulin, however, the ratio under physiological conditions is less than 1 and approximately 50% of the $F_{\alpha 2u}$ is excreted. Two important points are derived from the present study: (i) although the absolute T_m may vary widely from protein to protein, the ratio of T_m to F_p, where F_p is the minimum at which T_m is reached, may be within a constant range less than 1.0; and (ii) the relationship between fractional reabsorption and $F_{\alpha 2u}$ is linear.

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[3] All values for the ratio of T_m to F_p were calculated by the author based on published data.

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