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Dietary control of the renal reabsorption and excretion of α_{2u}-globulin

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Dietary protein supply is a factor in controlling the excretion of proteins in the urine. As early as 1926, Addis, Mackay, and Mackay observed that male rats on a 69% protein diet excreted more urinary proteins than did those on a 17% diet [1]. Protein deficiency had the opposite effect, resulting in a suppression of the proteinuria [2]. Of the total urinary proteins excreted by the adult male rat, approximately 30% is a sex-dependent globulin called α_{2u} [3, 4], which is synthesized by the liver [5] and controlled synergistically by androgens and glucocorticoids [6]. Dietary protein supply also had a profound influence on the excretion of α_{2u} [4]. On a 0% casein diet, the excretion was reduced to approximately 1 mg/24 hours compared with a normal of 10 to 15 mg. On a 50% casein diet, rats excreted 30 to 50 mg/24 hours, an increase of more than 100% above the normal [4].

Early studies also suggested that high protein diets exaggerated the leakage of plasma proteins caused by a spontaneous nephrotic syndrome observed in male rats [7, 8]. Rats previously castrated did not exhibit an increased excretion of urinary protein on a 50% casein diet, whereas supplementation with testosterone restored the augmented proteinuria [9]. This suggested that the elevated excretion of urinary protein was dependent on the presence of androgens. It is now known that a high-protein diet caused an increased excretion of α_{2u} without at the same time leading to a compensatory, stimulated hepatic biosynthesis. Conceivably, the increased excretion of α_{2u} was the consequence of an altered state of renal reabsorption [4]. The purpose of the present communication was to compare the degree of renal reabsorption under three different dietary conditions and to determine whether the kidneys controlled the urinary excretion of α_{2u} by altering its reabsorption.

Methods

Treatment of rats: Collection of urine. Adult, male rats (Sprague-Dawley strain, Sasco, Omaha, Nebraska), weighing approximately 250 g, were housed individually in stainless steel metabolism cages (Hoeltge, Inc., Cincinnati, Ohio) for the collection of 24-hour samples of urine. Samples were collected in flasks containing 0.5 ml of a solution containing 1.2% penicillin and 1.2% streptomycin and saturated with thymol. The original volumes of the 24-hour urine samples were measured. The cage funnels were rinsed with saline, and the wash was added to the urine samples; final volumes were made to a convenient level. Diluted urine samples were filtered and stored frozen. Urinary volumes were recorded before and after dilution. Each cage was provided with a urine-feces separator and tunnel feeder to minimize contamination with feces and food.

Diets. The normal or 20% protein diet consisted of 20 g of casein, 60 g of dextrin, 10 g of corn oil, 4 g of salt mixture (ICN-NBCo, Cleveland, Ohio) and 2.2 g of vitamin mixture (ICN-NBCo). The protein-free and high-protein diets were the same, except in the former the casein was replaced by additional dextrin and the latter contained 50 g of casein with an appropriate reduction in dextrin.

Immunologic assay for α_{2u}. The α_{2u} which was used for the development of the specific antiserum was prepared by fractionation on CM-cellulose equilibrated with 100 mM ammonium acetate (pH, 5.0) buffer. The column was eluted with a linear gra-

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dient between 100 and 500 mM ammonium acetate [10]. Three milligrams of α_{2u} were emulsified in complete Freund's adjuvant (Difco Laboratories, Detroit, Michigan). The emulsion was injected subcutaneously near the shoulder of a 45-kg goat; the injection was repeated after 3 weeks. The goat was bled from the jugular vein 5 days following the second injection. Blood plasma was brought to 50% of saturation with ammonium sulfate. After reprecipitation of the γ-globulin, the pellet was dissolved in 10 mM potassium phosphate (pH, 7.5), lyophilized, and stored at −20° C. The goat anti-α_{2u} antiserum was dissolved in water at a concentration of 50 mg/ml. For the quantitative radial-immunoassay [11], equal volumes of 3% agar (Noble Agar, Difco Laboratories, Detroit, Michigan) in 0.03 M dibasic potassium phosphate, 0.1 M sodium chloride buffer (pH, 8) and a solution of antiserum in buffer (1 to 1.4 mg/ml) were mixed at 56° C and poured onto calibrated plastic radial immunoassay diffusion plates (Miles Laboratories, Elkhart, Indiana). Thirty-six antigen wells (3 mm in diameter) were cut in the agar with a tubular cutter and a plastic template. The wells were filled with urine or serum samples (10 μg) and a serial dilution (15 to 100 μg/ml) of a standard α_{2u} solution. After developing the plates for 18 to 24 hours in a humid chamber, we measured ring diameters and converted them to α_{2u} concentration using the standard curve.

Determination of total urinary proteins. Total urinary protein was determined by the method of Lowry et al. [12] following a preliminary precipitation with phosphotungstic acid [4].

Preparation of blood serum and supernatant solutions from homogenates of kidneys. Blood, withdrawn from the posterior vena cava, was permitted to clot at room temperature. After allowing the clot to retract overnight at 4° C, we collected the serum by centrifugation at ×2000 g.

Kidneys were perfused with 0.9% saline via the hepatic portal vein (rats were anesthetized with ether). Both kidneys were minced in saline and blotted dry. The minced tissues were homogenized in two volumes of 0.1 M potassium phosphate buffer (pH, 8) and 0.15 M sodium chloride. Kidney homogenates were filtered through 110-mesh nylon cloth (Tetko, Inc., Elmsford, New Jersey) and centrifuged at ×105,000 g for 60 min at 5° C. The solutions were stored at −20° C.

Determination of DNA in kidney homogenates. DNA was determined in 0.3-ml aliquots of kidney homogenates with the method of Burton [13]. Standard curves were prepared with calf thymus DNA (Sigma Chemical Co., St. Louis, Missouri).

Results

Effects of dietary protein on α_{2u} concentrations. The relative accumulations of serum α_{2u} in totally nephrectomized rats maintained on 0% and 50% casein diets indicated that the protein deficiency reduced the biosynthesis of α_{2u}, and the high protein diet had no apparent effect [4]. As seen from Table 1, rats kept on a protein-free diet for 10 days excreted only 0.2 mg/24 hours, compared with a normal of 11.3 ± 8.4 mg/24 hours, a reduction of 98%. Simultaneously, the serum levels were reduced by approximately 60% (from 3.0 ± 0.7 to 1.4 ± 0.2 mg/100 ml), and the concentration in the kidneys was reduced by nearly 86% (69.8 ± 7.5 vs. 489 ± 231 μg/mg DNA). The reduction in serum α_{2u} concentration is consistent with a suppression in hepatic biosynthesis [4].

On a 50% casein diet, the urinary excretion was increased by nearly 80% (19.9 ± 3.9 vs. 11.3 ± 8.4 mg/24 hours), whereas the serum level remained in the normal range, 2.3 ± 0.1 mg/100 ml (Table 1). The accumulation of serum α_{2u} in nephrectomized rats was repeated with 2 to 3 rats maintained on 20% and 50% casein diets. As reported previously, the accumulation in 4 hours was identical for both groups of animals, indicating a normal rate of α_{2u} biosynthesis on the two diets [4]. Thus, the in-

Table 1. Changes in tissue content of α_{2u} with diet[a]

Tissue	Casein in diet		
	0%	20%	50%
Serum, *mg/100 ml*	1.4 ± 0.2 (4) ↓57%	3.0 ± 0.7 (25)	2.3 ± 0.1 (4)
Kidneys, *μg α_{2u}/mg DNA*	69.8 ± 7.5 (5) ↓86%	489 ± 231 (23)	140 ± 8 (4) ↓71%
Urine, *mg/day*	0.2 ± 0.1 (4) ↓98%	11.3 ± 8.4 (25)	19.9 ± 3.9 (4) ↑76%

[a] Rats were kept on the appropriate diet for 10 days prior to sacrifice. All data are averages ± SD. Number of rats are indicated in parentheses.

creased urinary loss of α_{2u} could not be ascribed to a compensatory stimulation in hepatic output. If the elevated, daily loss of 8.6 mg were not restored by synthesis, then the protein must have derived from another source, for example, by a decline in renal reabsorption. In support of this hypothesis, the data of Table 1 show that the renal concentration of α_{2u} declined by 70% (140 ± 8 μg/mg DNA compared with the normal of 489 ± 231 μg/mg DNA).

Effect of dietary protein on the renal reabsorption of α_{2u}. To prove that the increased excretion of α_{2u} on the high-protein diet was accompanied by a compensatory decline in renal reabsorption, it was necessary to estimate the α_{2u} removal rate and to compare this value to that actually excreted. Royce [10] compared the hourly accumulation of sex-dependent protein in the serum of bilaterally nephrectomized rats with its urinary excretion in controls. In the unoperated male, the serum level was found to be 14 μg/ml compared with 76 μg/ml in the first hour after nephrectomy; the change in serum concentration was 62 μg/ml. The normal plasma volume was assumed to be 3.9 mg/100 g body wt, and, therefore, the α_{2u} removal rate was 238 μg/hr/100 g body wt. Because the actual urinary excretion rate was 122 μg/hr/100 g body wt, Royce concluded that 51% of the protein filtered was reabsorbed by the kidneys [10].

Rats kept on 20% and 50% diets were nephrectomized, and the accumulation of serum α_{2u} was measured for a total of 4 hours. As suggested by Royce [10], the renal removal rate should be equivalent to the hepatic input of α_{2u} over a specified period of time. During the first hour after nephrectomy, the increase in serum α_{2u} of rats on the 20% casein diet was 90 μg/ml (Table 2). This represented a renal removal rate of 351 μg/hr/100 g (average body wt, 300 g) or a reabsorption of 57% of the filtered α_{2u}, which compared favorably with 51% obtained by Royce [10]. Rats on a high-protein diet had normal α_{2u} levels of 36 μg/ml; 1 hour after nephrectomy these increased by 97 μg/ml, thereby yielding a normal renal removal rate of 378 μg/hr/100 g body wt. As seen in Table 2, the actual urinary excretion, however, was elevated considerably, 278 μg/hr/100 g compared with the normal of 150. Thus, renal reabsorption was only 26%. This reduction in reabsorption (26% vs. 57%) may account for the increased excretion of 128 μg/hr/100 g, which, as mentioned previously, represented an increase of more than 80%. It is suggested, therefore, that renal reabsorption may be the factor controlling the urinary excretion of α_{2u} in rats on a high-protein diet.

The renal removal rate of α_{2u} in rats on a 0% casein diet was calculated using data published previously [4]. The serum α_{2u} concentration of the unoperated rat 10 days after substitution of the diet was 28 μg/ml, but the level 1 hour after bilateral nephrectomy was 46 μg/ml. The renal removal rate was calculated to be 70 μg/hr/100 g body wt (Table 2). The actual urinary loss was 2 μg/hr/100 g body wt or a reabsorption of 97% of the α_{2u} filtered (Table 2).

Renal control of α_{2u} *excretion*. It is generally conceded that the kidneys are an important site for the catabolism of proteins of small molecular weight [14]. During protein deprivation, the kidneys may assume a major function of amino acid salvage and prevent an undesirable loss in the urine of small-molecular-weight proteins. This is suggested by the almost total reabsorption of the protein in rats on a 0% casein diet.

Table 2. Reabsorption of α_{2u} in the kidneys of rats fed different protein diets

Diet[a]	Serum concentration of α_{2u}[b]			Urinary excretion[d]	Renal removal rate	Reabsorbed
	Initial *μg/ml*	1 hour after nephrectomy *μg/ml*	Change *μg/ml*	*μg/hr/100 g body wt*	*μg/hr/100 g body wt*	%
20% casein						
1	46 (2)	136 (2)	90	150	351	57
2[c]	45 ± 12 (8)	130 ± 26 (8)	85	—	—	—
50% casein						
1	36 (2)	133 (2)	97	278	378	26
2[c]	31 ± 2 (5)	122 ± 34 (5)	91	—	—	—
0% casein						
1[c]	28 ± 0 (4)	46 ± 8 (4)	18	2	70	97

[a] Rats were kept on each diet for 10 days prior to nephrectomy; number of animals are denoted in parentheses.
[b] Based on blood samples obtained at 0 and 1 hour after nephrectomy
[c] Data from Neuhaus and Flory [4].
[d] Data from Table 1

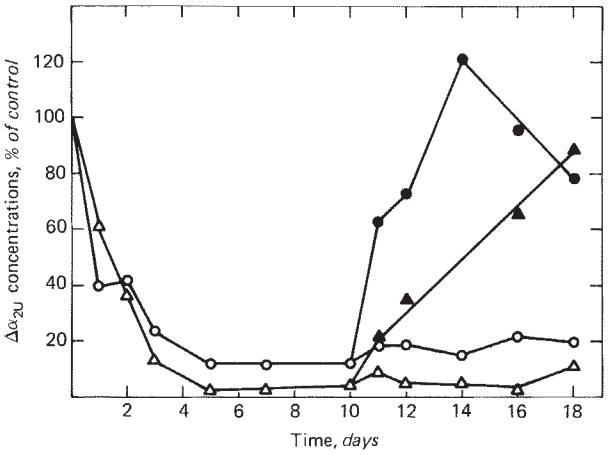

Fig. 1. *Changes in urinary excretion of* α_{2u} *and renal* α_{2u} *levels plotted as percent of control values (20% casein diet) following protein depletion (0% casein) and repletion (20% casein).* Urinary α_{2u} of protein-deprived rats is represented by △—△; renal concentrations by ○—○. Rats refed a 20% casein diet are represented by ▲—▲ for the urinary excretion and ●—● for the renal levels. Each point is the average of three rats.

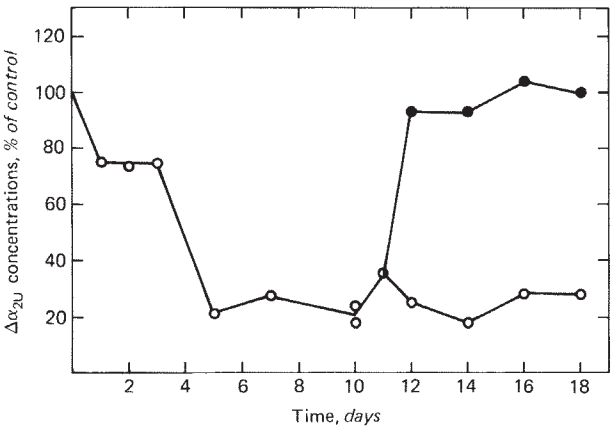

Fig. 2. *Changes in serum* α_{2u} *plotted as percent of control values (20% casein diet) following protein depletion (0% casein) and repletion (20% casein).* Protein-deprived rats are denoted by *open circles*; rats refed 20% casein, *closed circles*. Each point is the average of three rats.

A role of renal reabsorption in controlling the loss of α_{2u} during protein deprivation might best be visualized by refeeding animals previously kept on a protein-free diet. In this study, rats were kept on the 0% casein diet (depletion) for 10 days followed by a repletion period of 8 days on a 20% casein diet. Two sets of control rats were retained throughout, one on the 20% and the other on the 0% casein diet.

Figure 1 shows the decline in α_{2u} excretion and renal α_{2u} concentration, which dropped to minimal levels during the first 5 days on the 0% casein diet. Repletion with 20% casein on day 10 resulted in a rapid restoration of the renal concentration, reaching a maximum greater than the normal by the fourth day of repletion (day 14). The α_{2u} of the urine, on the other hand, gradually attained normal values over a period of 8 days (day 18). Figure 2 shows a very rapid restoration of serum α_{2u} concentration within 48 hours. Thereafter, the serum protein was normal until the end of the experiment (day 18).

Discussion

Of the various tissues tested for α_{2u}, the sex-dependent protein of the adult male rat, only the liver, serum, kidneys, urine, and salivary glands possess demonstrable amounts [15, 16]. The unusually high concentration of α_{2u} in the kidneys, constituting as much as 3 to 6% of the total soluble proteins, is impressive and raises questions either as to its function in this organ or the possible role of the kidneys in controlling its excretion. Roy and Raber [17], using immunofluorescent techniques, localized large quantities of α_{2u}, presumably resulting from reabsorption, in the cells of the renal tubules, proximal as well as distal. The sex-dependent protein has a molecular weight of 16,000 to 20,000 daltons [10, 18], and, therefore, according to the limits of glomerular permeability established for dextran (mol wt, 8,000 to 95,000 daltons) should pass readily through the glomeruli [19]. The anticipated filtration and tubular reabsorption of α_{2u} is also in accord with observations of the fate in the nephron of proteins of varying molecular size [14, 20–26]. The ultimate fate of reabsorbed proteins, however, appears to be uncertain, whether catabolized to amino acids or returned to the bloodstream intact. It appears that the primary fate of such proteins as lysozyme, Bence Jones protein, and the L chains of immunoglobulins, as well as serum albumin is catabolic; they are not recycled to the bloodstream [14, 20, 24–26]. Yet, studies of the half-life of ^{125}I-albumin following injection into bilaterally nephrectomized rats showed that its metabolism was unchanged compared with that occurring in intact rats [27]. This indicated that the kidneys are not a major factor in protein catabolism. Furthermore, according to Maack and Kinter [28], catabolism and reabsorptive transport need not be mutually exclusive processes. Intact lysozyme (mol wt, 14,000 daltons) was transported intact across the renal tubule-cell membranes of the flounder nephron despite the fact that these cells possess the biochemical machinery to catabolize proteins. In the mouse kidney, ^{125}I-

lysozyme was found distributed between renal phagosomes and the cytosol. Maack, Mackensie, and Kinter [29] suggested that the catabolism in phagosomes of reabsorbed proteins is an emergency measure functional during protein overload. Thus, there is evidence to show that protein, absorbed by the tubule cells, need not be catabolized to amino acids but may be restored to the circulation [9, 30].

Our current data show that the kidneys regulate the urinary excretion of α_{2u}, by affecting its reabsorption. Thus, in the normal rat on a 20% casein diet, 60% of the α_{2u} filtered was reabsorbed. Under these conditions, the male rat had a serum concentration of 3.0 ± 0.7 mg/100 ml and excreted 11.3 ± 8.4 mg/day. During protein deprivation, the excretion of the urinary protein was nearly eliminated. A comparison of the renal removal rate calculated from the accumulation of α_{2u} following nephrectomy with the excretion of this protein in the urine indicated that the kidneys reabsorbed practically all (97%) of the α_{2u} filtered; only 0.2 mg was excreted daily. Based on these figures, the renal uptake was approximately 6 mg of α_{2u} per day, which is 65% below the normal of 17 mg (based on a 60% reabsorption and an excretion of 11 mg daily). As seen in Table 1, the actual renal α_{2u} concentration was reduced by 86%. Simultaneously, the total urinary protein was also decreased; protein-depleted rats excreted only 7 mg/day compared with a normal of 38 to 46 mg [4]. The α_{2u} represented only 7% of the total protein excreted compared with the normal proportions of 23 to 35%. This suggests that the reabsorption of α_{2u} may be a selective process. The serum concentration of α_{2u} also was reduced, however, by 60%, indicating a reduced hepatic synthesis. Furthermore, the accumulation of α_{2u} in the serum of nephrectomized rats on a protein-free diet was reduced by 80% (Table 2). Therefore, the excretion of the sex-dependent protein was affected both by a reduced hepatic synthesis and an increased renal reabsorption. Together, these two factors may have resulted in what seems to be a selective reduction in α_{2u} excretion. At the present time, there is no evidence to show that the reabsorption is specific for α_{2u} as opposed to a general process for all plasma proteins found in the glomerular filtrate. The increased reabsorption of α_{2u} under conditions of protein deprivation may serve the purpose either of salvaging the protein itself or its constituent amino acids, which the animal can ill afford to lose.

High protein diets are known to increase the general loss of plasma proteins in the urine, a phenomenon which at first was thought to be the consequence of an exacerbation of the spontaneous nephrotic syndrome and, therefore, contingent on an increased renal pathology [1, 31–33]. Subsequently, it was shown that high protein diets not only increased the excretion of total urinary protein but also of α_{2u} [4]. Because the ratio of the two remained in the normal range (approximately 30%), it appeared likely that the increased excretion of sex-dependent protein could be related to the general, elevated loss of plasma proteins [4].

Because the increased loss of proteins was restored to normal upon refeeding the regular diet [4], it seems highly unlikely that this is a consequence of increased renal pathology rather than a readily reversible physiologic process. Thus, it might reflect an increase in the GFR, which is known to occur in dogs on high-protein diets [34]. Measurements of the GFR in rats based on the urinary excretion of creatinine and the plasma creatinine concentrations showed, however, no changes resulting from the 50% casein diet. Therefore, the excretion of α_{2u} could not be ascribed to an elevated GFR. A second explanation for the increased excretion would be a compensatory hepatic biosynthesis of α_{2u}. But, when rats on a 50% casein diet were totally nephrectomized and the accumulation of α_{2u} in the plasma was measured over a period of 4 hours, the increase in α_{2u} was normal (Table 2) [4], indicating the absence of an elevated synthesis or hepatic secretion of the protein. A third explanation for the elevated excretion of α_{2u} would be a compensatory reduction in the renal reabsorptive process. Assuming that the plasma volume remained in the normal range [10], the renal removal rate and hence the tubular protein load should have been the same as for the control rats on the 20% casein diet. Because the urinary excretion was increased by 80%, the renal reabsorption of filtered protein was decreased from a normal of 60% to 25% or less. In other words, this would represent a daily renal uptake of 7 mg compared with the normal of 17 mg, or a reduction of 60%. This is comparable to the 70% decrease in renal concentration obtained from the analytic data (Table 1). Thus, it appears that the increased urinary excretion of α_{2u} observed in rats on a high-protein diet is consistent with a reduced reabsorption of protein from the glomerular filtrate rather than a substantive change in tubular load. It is presumed that the reduced uptake of α_{2u} is not a

selective process but affects other plasma proteins as well. If only protein reabsorption is affected by high-protein diets and not glomerular filtration, then it would be expected that the percentage of α_{2u} in total urinary protein would be normal, as was reported previously [4].

Protein depletion-repletion studies, Figs. 1 and 2, showed that the serum α_{2u} concentrations of rats on a protein-free diet rapidly returned to normal after restoration of the 20% casein diets. Perhaps the serum level of α_{2u} is the critical factor which must be restored in the adult male rate following repletion. If, however, the urinary excretion had paralleled the serum levels, the loss of protein might have placed an undue metabolic burden on the animal at a time when the amino acid supplies were marginal. Thus, the kidneys prevented a waste of this small protein (or its constituent amino acids) by retaining for the first 4 days after repletion a highly active reabsorptive process. This is evident from the fact that the renal α_{2u} concentrations rapidly reached normal and even higher levels whereas the normal urinary excretion was delayed until after 8 days. Although the nature of the mechanism controlling protein reabsorption by the tubule cells is unknown, it is attractive to speculate that the α_{2u} is reabsorbed and recycled intact to the bloodstream.

On the basis of the data presented, it is proposed that the kidneys are an important factor in controlling the loss of the sex-dependent protein, α_{2u}, in the urine. During a dietary protein deficit, the reabsorption of α_{2u} was almost total. On repletion, the renal uptake of protein continued for 4 days after restoration of the normal diet, thereby reducing excessive losses of protein. When the available amino acid supply was in excess of that required (high-protein diet), the excretion of α_{2u} and total urinary protein was increased by a compensatory reduction in tubular reabsorption.

Summary

The kidneys are an important factor in controlling the excretion of the sex-dependent protein, α_{2u} globulin, in the urine. On a protein-free diet (0% casein), when the plasma level was reduced by 60%, the daily excretion of α_{2u} in the urine was reduced from a normal of 11 mg to 0.2 mg; 97% of the α_{2u} filtered by the kidneys was reabsorbed. When a normal-protein diet (20% casein) was restored, the serum α_{2u} levels were returned to normal within 48 hours. The urinary excretion, on the other hand, remained below normal until the eighth day after repletion, whereas the renal concentrations of α_{2u} reached higher than normal values by the fourth day. Thus, during protein deficiency the kidney reduced excessive losses of protein in the urine by an almost total reabsorption of α_{2u}. On a high-protein diet (50% casein), the serum levels of α_{2u} were normal, yet its excretion was elevated 80% above the normal (20 mg/day compared with 11 mg/day). To effect this increased loss, the reabsorption of the protein was reduced to 26% of the normal.

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