PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **182,** 531–539 (1986)

# Renal Reabsorption of Low Molecular Weight Proteins in Adult Male Rats: $\alpha_{2u}$-Globulin (42376)

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*Abstract.* Urinary proteins are reabsorbed by the renal tubule cells by two processes, the first for high molecular weight (HMW) and the second for low molecular weight proteins (LMW). The purpose of this report is to establish that $\alpha_{2u}$-globulin, the sex-dependent, major urinary protein of the adult male rat, is reabsorbed in the kidneys by the general mechanism for LMW proteins. Parameters such as clearance rates were determined to show that $\alpha_{2u}$ is reabsorbed by a process comparable to that for lysozyme. The aminoglycoside, gentamicin, was observed to inhibit the reabsorption of $\alpha_{2u}$ in a dose-dependent fashion. It increased the $\alpha_{2u}$ excretion rate from 4.2 to 13.5 μg/min; the clearance was increased from a normal of 0.33 to 0.91 ml/min. The excretion rate for $\alpha_{2u}$ was also increased by the injection of lysozyme from a normal of 7.4 to 18.1 μg/min. The effect of lysozyme was dose-dependent and reversible. Although gentamicin and lysozyme each increased the excretion of $\alpha_{2u}$, they had no effect on albumin. Both were equally effective as inhibitors of $\alpha_{2u}$ reabsorption and were 80% as effective as sodium maleate. It is suggested that $\alpha_{2u}$ is reabsorbed by a mechanism which is shared with other LMW proteins. Furthermore, this process is independent of the one which serves to translocate HMW proteins such as albumin. 

Urinary proteins are usually derived from the blood plasma by glomerular filtration followed by an escape from tubular reabsorption. Evidence indicates the existence of two general processes, one for proteins of intermediate to high molecular weight and the other for low molecular weight proteins (LMW) (1, 2). The mechanism for the LMW proteins may be subdivided into three steps: (a) selective binding to the membrane, (b) selective access to the endocytotic sites, and (c) a selective binding for specific proteins. These are part of the "selective-constraint mechanism" described by Maack and co-workers (3). The initial step may involve the relative affinities of proteins for anionic sites on the brush border membrane (4). Although cationic proteins appear to be bound preferentially, they normally represent only a small part of the urinary protein mixture (2, 5). Since the binding appears to be "cation-selective" (4), the question arises as to how the majority of the proteins which are neutral or anionic are reabsorbed. Apparently, even though differences in isoelectric point are important, they are not the exclusive factor in the reabsorption of LMW proteins (3). It is more likely that the number of free amino groups and their configuration in the protein structure are more important than a net positive charge itself (6).

Although cationic proteins such as cytochrome c or lysozyme and anionic proteins such as β-2-microglobulin, human growth hormone, and cadmium metallothionein (7–11) have been used to study protein uptake in the kidneys, they are relatively minor constituents or are not normally present in the blood plasma at all. Therefore, they may provide only a limited view of the reabsorption mechanism. A more useful model would be an endogenous anionic protein present in the blood plasma at a substantial level. Such a protein is $\alpha_{2u}$-globulin, the sex-dependent protein of the adult male rat (5). It is not only a LMW protein (19,000) (12–14) but is also present in the blood at a level of 30 μg/ml (15). Furthermore, it is an anionic protein and exists as a family of isoelectric species varying in pI from 4.6 to 5.2 (16).

The purpose of the present study is to establish whether the renal reabsorption of $\alpha_{2u}$-globulin is a mechanism specific for this protein or if it shares a general process with other LMW proteins. If the latter be the case, then $\alpha_{2u}$-globulin may serve as a valuable tool for a continuing study of the renal reabsorption of LMW proteins.

**Materials and Methods.** *Animals and materials.* Male, Sprague–Dawley rats weighing 225–300 g, fed *ad libitum* on a rat chow diet,



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were anesthetized with sodium pentobarbital (50 mg/kg, ip) prior to initiating the surgical procedure. Gentamicin sulfate, with a potency of 577 μg of gentamicin per milligram, was purchased from Sigma Chemical Co. (St. Louis, Mo.). Lysozyme (Grade I) was also from Sigma as were the UV-15 glucose assay kits and the phosphoglucose isomerase used in the determination of the GFR.

*In vivo infusion system (17).* Following anesthesia, a tracheostomy was performed using PE-205 tubing. The femoral vein was exposed via an inguinal incision and was catheterized using PE-50 tubing. The urinary bladder was cannulated with PE-90 tubing. All animals were infused with a modified Krebs–Ringer solution (140 m*M* NaCl, 3.5 m*M* KCl, 5% mannitol, and 1% inulin) (17) at a constant infusion rate of 0.1 ml/min. Urine samples were collected directly from the bladder at 20-min intervals; blood samples were collected from the tail as needed. Three 20-min urine samples were routinely collected prior to the infusion of any reagent. At Time 0 the reagent, e.g., gentamicin, was infused into the femoral vein and was flushed into the system using physiological saline. The total volume of reagent and saline wash was 1 ml. The entire infusion process required 5 min. Thereafter, 20-min urine samples were collected over a total of 160 min.

*Analytical methods.* Blood and urinary inulin concentrations were determined using a modification of the method of Renschler (18) and of Frohnert *et al.* (19). Blood serum samples (100 μl) were combined with 400 μl of 0.35 *M* perchloric acid. After standing at 4°C for 10 min, the samples were centrifuged in a microfuge for 2.5 min. Aliquots (50 μl) of the supernatant solution were then used for the determination of glucose–fructose. The supernatant solutions were then heated for 5 min in a boiling water bath. Aliquots (50 μl) of the heated solutions were used for the determination of fructose. These measurements were made using a modified UV assay kit (Sigma). After dissolving the contents in 31 ml of water, 1 ml UV assay kit was transferred to a micro spectrophotometer cuvet. Then 3 μl (15 units) of phosphoglucose isomerase (the Sigma product was diluted with an equal volume of saline) was added. To this mixture were added the appropriate aliquots (50 μl) of samples. The amount of fructose released by acid hydrolysis of the inulin, that is, the difference in absorbancy before and after hydrolysis, was used to determine the inulin content. Aliquots (100 μl) of diluted urines (1–5) were also added to 400 μl of perchloric acid. This mixture was first allowed to stand at 4°C for 10 min prior to the determination of the background levels of glucose–fructose. The acid mixture was then heated at 100°C for 5 min and the determination for fructose was repeated. A standard curve was prepared using suitable dilutions of inulin. Preliminary studies showed that the presence of perchloric acid had no inhibitory effect on the enzymatic assay. Furthermore, both glucose and fructose standards yielded comparable results with this assay. Inulin concentrations were employed to calculate the glomerular filtration rate (GFR).

*Immunologic assay for $\alpha_{2u}$-globulin and serum albumin.* $\alpha_{2u}$-Globulin used in the following assays was prepared by a combination of ammonium sulfate fractionation and chromatography on CM cellulose (20). For the immunologic assays, agar radial immunodiffusion plates were prepared as described in the following. A 3% agar (Noble Agar, Difco Laboratories, Detroit, Mich.) in 0.03 *M* dibasic potassium phosphate, 0.1 *M* sodium chloride buffer (pH 8) was heated in a boiling water bath. A 5-ml aliquot was mixed with 4 ml of the buffer (56°C) and the mixture was held in a 56°C water bath for 30 sec. Antiserum (1 ml containing 5 to 15 mg of protein) was added. After rotating the tube gently, the contents were poured onto a plastic radial immunodiffusion plate (Miles Laboratories, Elkhart, Ind.). Antigen wells were cut into the agar with a 3-mm tubular cutter using a plastic template. For $\alpha_{2u}$, the wells were filled (14 μl) with diluted urine (twofold) and undiluted serum. For albumin assays undiluted urines were used. Serial dilutions of $\alpha_{2u}$ or albumin (10 to 100 μg/ml) were used as standards. After developing the plates for 24 hr at room temperature in a humid chamber, ring diameters were measured using a calibrating viewer (Model 2743, Transidyne General Corporation, Ann Arbor, Mich.). These were converted to protein concentrations using appropriate standard curves. Antisera for $\alpha_{2u}$ were

prepared against purified preparations as follows: 1 ml of $\alpha_{2u}$ (2 mg/ml) was emulsified with an equal volume of Freund's complete adjuvant (Sigma). The emulsion (0.25 ml) was injected intramuscularly into the hind limbs of a Dutch Belted rabbit; 500 $\mu$g of protein was injected. After 4 weeks, a booster shot was given using an emulsion prepared from 1 ml of $\alpha_{2u}$ (1 mg/ml) plus 1 ml of Freund's incomplete adjuvant. Further, 0.25 ml was injected intramuscularly into each hind limb; a total of 250 $\mu$g was given. The rabbits were bled 1 week later by cardiac puncture under anesthesia. Pooled blood sera were fractionated with ammonium sulfate (50% of saturation). The precipitated protein was dialyzed against 10 m*M* potassium phosphate buffer (pH = 8.00) and then lyophilized. Antiserum for albumin was obtained from Cooper Biomedical, Inc. (Malvern, Pa.).

*Calculations.* Clearance values were calculated as the ratio $U\alpha_{2u} \cdot V/P\alpha_{2u}$; $U\alpha_{2u}$ and $P\alpha_{2u}$ are the urinary and serum $\alpha_{2u}$ concentrations, respectively ($\mu$g/ml); $V$ = urinary flow rate ($\mu$l/min). GFR was calculated as the corresponding ratio using urinary and serum inulin concentrations. Statistical analyses were performed with an IBM-PC computer using the EPISTAT (public domain) program.

**Results.** Many studies of the reabsorption of LMW proteins have been performed using the antibiotic, gentamicin, and the enzyme, lysozyme. The aminoglycoside competitively inhibits the reabsorption of the cationic protein, lysozyme (21). The following experiments demonstrate that both can inhibit the reabsorption of $\alpha_{2u}$-globulin.

*Basic renal parameters: The in vivo infusion process.* In an initial 3-hr control study involving the infusion of four rats the average urinary flow rate was 93.3 ± 35.6 $\mu$l/min with a mean $\alpha_{2u}$ concentration of 51.0 ± 36.1 $\mu$g/ml and an albumin concentration of 44.0 ± 30.2 $\mu$g/ml. The excretion rates for $\alpha_{2u}$ and albumin were observed to reach constant levels by the time that the reagent was usually injected in subsequent experiments, namely Time 0 (data not included). Furthermore, there was little variation over the subsequent 160 min when 1 ml of physiological saline was injected at Time 0. The relationship of urinary $\alpha_{2u}$ concentration to urinary flow rate is shown in Fig. 1A. This, as well as an independence of urinary excretion rate from urinary volume, Fig. 1B, are comparable to the observations reported for lysozyme (8, 9).

The GFR for control rats was 1.99 ± 0.69 ml/min, Table I, and was the same as that previously published (2.00 ± 0.17 ml/min) for the continuous intravenous infusion of a mildly diuretic buffer containing 5% mannitol (17). The GFR did not change throughout an 80-min period following the intravenous injection either of 1 ml of saline or of gentamicin (20 mg/kg). The latter observation is in agreement with those of Cojocel and Hook (9) for the same dosage of the antibiotic.

*Effect of gentamicin on the excretion of $\alpha_{2u}$-globulin.* Gentamicin (20 mg/kg), injected over a 5-min period, significantly elevated the $\alpha_{2u}$ excretion rate from a normal of 4.2 ± 1.8 to 13.5 ± 4.7 $\mu$g/min ($P$ = <0.001) or an increase of over 200% (Table II). Albumin, on the other hand did not change significantly, 4.0 ± 1.7 $\mu$g/ml versus 4.3 ± 2.3 $\mu$g/ml. The variation of protein excretion rate with time post gentamicin injection is seen in Fig. 2. It is apparent that $\alpha_{2u}$ increased promptly during the first 20-min period following gentamicin whereas albumin did not change over the 2.5-hr post injection period. Figure 3 shows that the effect of gentamicin is dose-dependent, reaching maximum excretion rates at a dosage of 10 mg/kg. This figure also shows that albumin remained unaffected regardless of the dosage used. Gentamicin is known to affect glomerular sieving of proteins in addition to inhibiting tubular reabsorption of proteins (9). However, since the excretion of albumin was not affected, it seems unlikely that the aminoglycoside had an effect at the glomerular level during the time period studied. These results confirm that such LMW proteins as $\alpha_{2u}$ are reabsorbed by a system independent of the HMW proteins.

*Clearance of endogenous $\alpha_{2u}$-globulin.* Another study using gentamicin (Table I) was performed to establish its effect on $\alpha_{2u}$ clearance rates $C(\alpha_{2u})$. As in the preceding, the aminoglycoside yielded an increase in proteinuria (135.9 ± 57.7 versus the control of 75.2 ± 41.1 $\mu$g/ml) and in the excretion rate of 17.5 ± 8.4 $\mu$g/min compared with the control of 7.8 ± 4.4 $\mu$g/min ($P$ = 0.003). Clearance

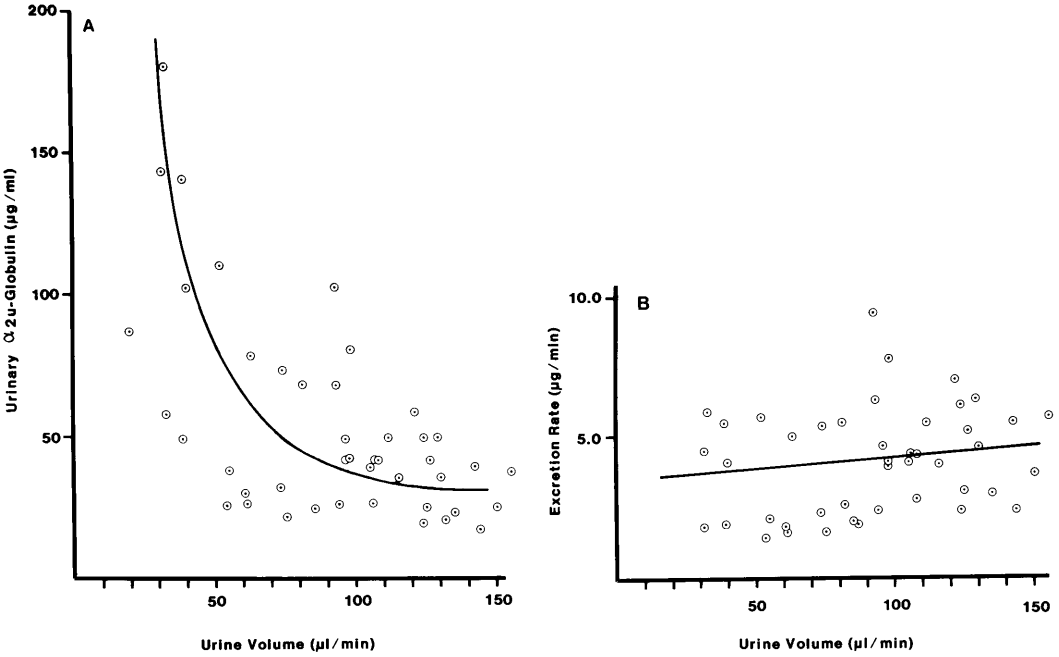


FIG. 1. (A) The relationship of urinary flow rate to urinary concentration of $\alpha_{2u}$-globulin. Points were taken from four control rats. (B) The independence of the $\alpha_{2u}$ excretion rate from the urinary flow rate.

values for $\alpha_{2u}[C(\alpha_{2u})]$ were comparable to those for exogenous lysozyme (9). Saline controls yielded a $C(\alpha_{2u})$ of $0.33 \pm 0.19$ ml/min compared with $0.91 \pm 0.29$ ml/min following gentamicin (Table I) ($P < 0.001$). The ratio $C$/GFR was increased from $0.17 \pm 0.10$ to $0.43 \pm 0.18$. These data, recorded as Experiment 1 of Table I, were often complicated by the wide ranges of $\alpha_{2u}$ excretion in different populations of rats. Thus some excreted considerably more of the protein than others. To be certain that there was no bias in the calculation of the data, a second experiment was performed in which each experimental rat served as its own control. In Experiment 2 of Table I, the excretion rates for $\alpha_{2u}$ at Time 0, that is, the 20-min period prior to the injection of the gentamicin, was observed to be comparable to the larger population of control rats of Experiment 1. Following the injection of gentamicin (20 mg/kg), samples were collected at 20-min intervals; maximum excretion data were used in the calculations for Table I. The excretion rates were somewhat, but not significantly, higher than those for Experiment 1. Both sets of observations indicate that endogenous $\alpha_{2u}$ responded to the aminoglycoside as did exogenous lysozyme (9).

*Reversible inhibition of $\alpha_{2u}$ reabsorption by excess exogenous lysozyme.* If $\alpha_{2u}$ is reabsorbed by a general translocating process for LMW proteins such as lysozyme, a more direct proof would involve inhibition following an administration of the enzyme. Egg white lysozyme was infused into adult male rats in dosages varying from 10 to 60 mg/kg of body weight. It is apparent from Table II that the excretion rate for $\alpha_{2u}$ was increased significantly ($P = 0.001$) from a normal value of $7.4 \pm 1.6$ to $18.1 \pm 4.5$ µg/min or an increase of nearly 150% when the enzyme was injected as a bolus (20 mg/kg). These data show that the effect of lysozyme was similar to that of gentamicin. Furthermore, Fig. 4 demonstrates that the inhibitory process was also dose-dependent. Measurement of urinary albumin shows that lysozyme had no effect on its excretion rate. Once again, this indicates that these two proteins are reabsorbed by different systems.

Highly cationic proteins such as lysozyme may complicate the results by affecting the

TABLE I. CLEARANCE RATE FOR ENDOGENOUS $\alpha_{2u}$-GLOBULIN

| Parameters | EXPT | Control | Gentamicin | P* |
|---|---|---|---|---|
| $P\alpha_{2u}$ (μg/ml) | 1[a] | 28.7 ± 19.3 | 20.5 ± 11.3 | NS |
| | 2[b] | 32.8 ± 12.3 | 22.7 ± 3.7 | NS |
| $U\alpha_{2u}$ (μg/ml) | 1 | 75.2 ± 41.1 | 135.9 ± 57.7 | 0.010 |
| | 2 | 104.2 ± 34.2 | 263.8 ± 131.9 | 0.017 |
| V (μl/min) | 1 | 113.1 ± 49.8 | 130.4 ± 26.7 | NS |
| | 2 | 100.5 ± 24.0 | 111.5 ± 24.8 | NS |
| U · V (μg/min) | 1 | 7.8 ± 4.4 | 17.5 ± 8.4 | 0.003 |
| | 2 | 10.0 ± 2.7 | 26.9 ± 5.1 | <0.001 |
| (U · V/P) $C\alpha_{2a}$ (ml/min) | 1 | 0.33 ± 0.19 | 0.91 ± 0.29 | <0.001 |
| | 2 | 0.37 ± 0.22 | 1.20 ± 0.25 | <0.001 |
| GFR (ml/min) | 1 | 1.99 ± 0.69 | 2.42 ± 0.99 | NS |
| | 2 | 1.57 ± 0.35 | 1.81 ± 0.46 | NS |
| C/GFR | 1 | 0.17 ± 0.10 | 0.43 ± 0.18 | <0.001 |
| | 2 | 0.27 ± 0.20 | 0.62 ± 0.16 | 0.010 |

[a] Experiment 1: Control includes 11 rats; 0.9% saline injected at Time 0; blood and 20-min urine collections made at 20, 40, 60, and 80 min post injection. Values given are means ± SD of the first 20-min interval only. Gentamicin (20 mg/kg) was infused at Time 0 using 11 rats; blood and urine collections made as above at 20-min intervals post injection. Values given are means ± SD for samples yielding maximum excretion rates; the majority are from the initial 20-min period.

[b] Experiment 2: A series of six rats was infused for 120 min prior to the injection of gentamicin (20 mg/kg). The 20-min samples collected prior to injection ($t_0$) were used to measure the control values (means ± SD). Three 120-min samples were collected following gentamicin injections. Values given are means ± SD for samples yielding maximum excretion rates; the majority are from the initial 20-min period.

* Statistical significance calculated between gentamicin data and corresponding control values.

TABLE II. URINARY EXCRETION OF $\alpha_{2u}$-GLOBULIN AND ALBUMIN BY ADULT MALE RATS FOLLOWING TREATMENT WITH SODIUM MALEATE, GENTAMICIN, AND LYSOZYME

| Urinary parameters | | Sodium maleate[a] | Gentamicin (20 mg/kg) | Lysozyme (20 mg/kg) |
|---|---|---|---|---|
| Volume (μl/min) | C | 77.2 ± 9.7 (9) | 65.4 ± 14.6 (7) | 95.1 ± 57.4 (5) |
| | E | 216.4 ± 31.4 (9) | 102.7 ± 25.7 (7) | 161.7 ± 37.2 (5) |
| | | $P < 0.001$ | $P < 0.010$ | NS |
| $U\alpha_{2u}$ (μg/ml) | C | 74.2 ± 30.0 (9) | 67.7 ± 33.3 (7) | 99.2 ± 45.1 (5) |
| | E | 80.2 ± 37.1 (9) | 134.6 ± 42.5 (7) | 113.2 ± 27.6 (5) |
| | | NS | $P < 0.010$ | NS |
| $UV\alpha_{2u}$ (μg/min) | C | 5.3 ± 1.8 (9) | 4.2 ± 1.8 (7) | 7.4 ± 1.6 (5) |
| | E | 17.6 ± 7.1 (9) | 13.5 ± 4.7 (7) | 18.1 ± 4.5 (5) |
| | | $P < 0.001$ | $P < 0.001$ | $P = 0.001$ |
| $U_{ALB}$ (μg/ml) | C | 54.4 ± 33.0 (9) | 67.7 ± 36.9 (7) | 42.3 ± 48.7 (5) |
| | E | 27.4 ± 9.5 (9) | 45.3 ± 25.1 (7) | 12.7 ± 4.0 (5) |
| | | $P < 0.031$ | NS | NS |
| $UV_{ALB}$ (μg/min) | C | 4.7 ± 2.7 (9) | 4.0 ± 1.7 (7) | 3.0 ± 2.6 (5) |
| | E | 5.9 ± 2.1 (9) | 4.3 ± 2.3 (7) | 2.0 ± 0.4 (5) |
| | | NS | NS | NS |

*Note.* Each value is a mean ± SD. C = control or urine samples collected at Time 0. E = experimental or urine samples collected at 20-min intervals post injection. Data were calculated using the sample providing maximum $\alpha_{2u}$ excretion rate. Number of rats used is given in parentheses. Statistical significance (*P*) calculated between experimental (E) and corresponding control (C) values.

[a] Pooled data for ip (400 mg MAL/kg) and iv (150–300 mg MAL/kg) studies. Urine samples were collected at 20-min intervals. Data were calculated using the sample providing maximum $\alpha_{2u}$ excretion rate.

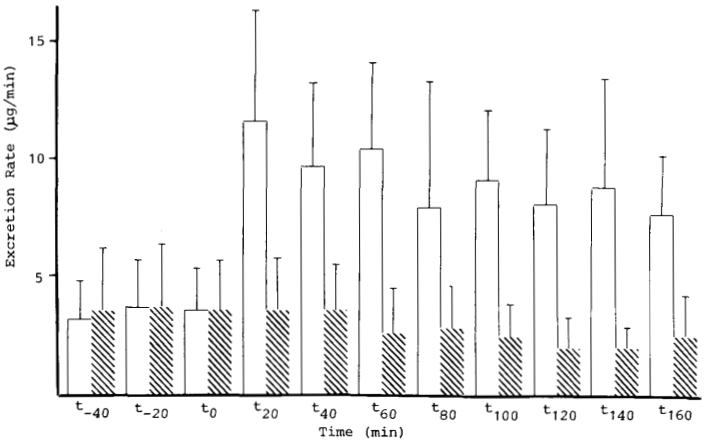


FIG. 2. The response of $\alpha_{2u}$-globulin excretion rate to the iv injection of gentamicin (20 mg/kg) at Time 0. Open bars represent the urinary excretion rate of $\alpha_{2u}$ while shaded bars are albumin. Four adult male rats were used for this study. Each point represents the mean ± SD.

glomerular filtration as well as the tubular reabsorption (21, 22) or by damaging the structure and function of the cells (3). Glomerular damage, as by puromycin aminonucleoside, is known to cause a massive excretion of albumin without affecting $\alpha_{2u}$ (23). Therefore, since the excretion of albumin was not affected by the lysozyme, an action at the glomerular level seems unlikely. However, to support this conclusion, a study was initiated to demonstrate the reversibility of the lysozyme effect on the excretion of $\alpha_{2u}$-globulin. Figure 5 provides representative results involving the administration of three doses (20 mg/kg each) of lysozyme. It is clear from this figure that the effect of lysozyme on $\alpha_{2u}$ excretion is reversible.

*Rate of $\alpha_{2u}$-globulin reabsorption.* The increase in $C$/GFR ($\alpha_{2u}$) from a normal of 0.17–0.27 to a gentamicin-induced value of 0.43–

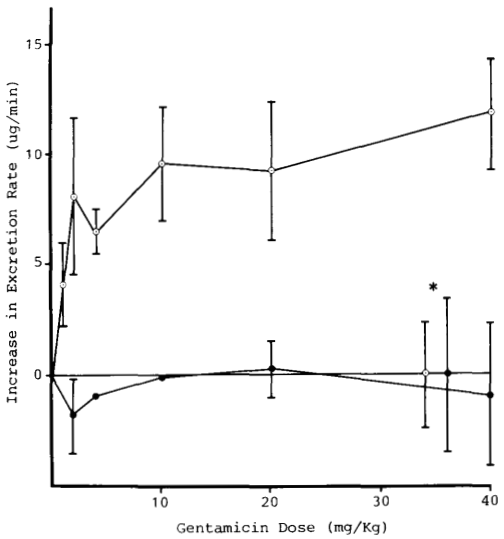


FIG. 3. Effect of varying dosages of gentamicin on the excretion of $\alpha_{2u}$ and albumin. Increases in excretion rate represent maximum changes during 20-min collection periods post gentamicin (control = zero time). Open circles are for $\alpha_{2u}$; closed are for albumin. Data are mean changes ± SD (three to seven rats). *SD for control values.

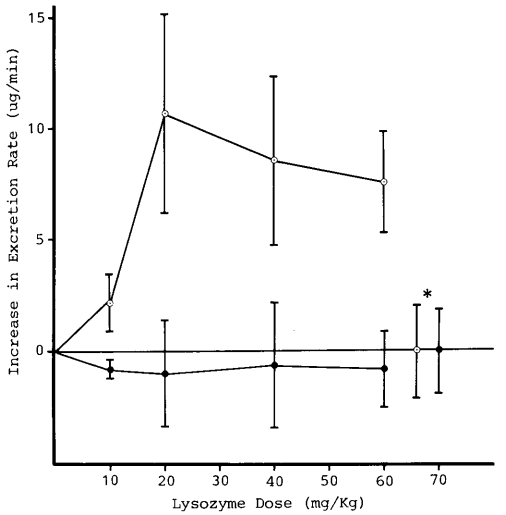


FIG. 4. Effect of varying dosages of lysozyme on the excretion rate for $\alpha_{2u}$-globulin and albumin. See legend, Fig. 3, for description of points. Each point represents data from three to five rats.

0.62, the dose dependency of the effects of lysozyme and gentamicin, the reversibility of the action of lysozyme, and also the lack of any effect on albumin excretion indicate that these substances inhibit the renal reabsorption of $\alpha_{2u}$-globulin and do not affect the glomerular process. To compare quantitatively the inhibitory effects of gentamicin and lysozyme on $\alpha_{2u}$, an estimated filtered load was obtained by measuring the excretion rate following inhibition of the reabsorption with sodium maleate (MAL). Earlier studies with intact rats indicated that treatment with MAL blocks tubular reabsorption of protein resulting in a transient state of albuminuria, gamma-globulinuria, increased urinary transferrin, as well as $\alpha_{2u}$-globulinuria (23, unpublished results). To achieve a maximal inhibition of reabsorption, MAL was administered to rats during the continuous infusion process as used in the preceding experiments. However, the dosage needed could not be given intravenously without toxic effects. Therefore, two protocols were used: (a) iv injection using reduced dosages of 150–300 mg/kg, and (b) the usual 400-mg/kg dosage was injected intraperitoneally. In the latter case no toxic effects were noted although a maximum response was not observed during the first 20-min collection period. The data showed that the mean maxi-

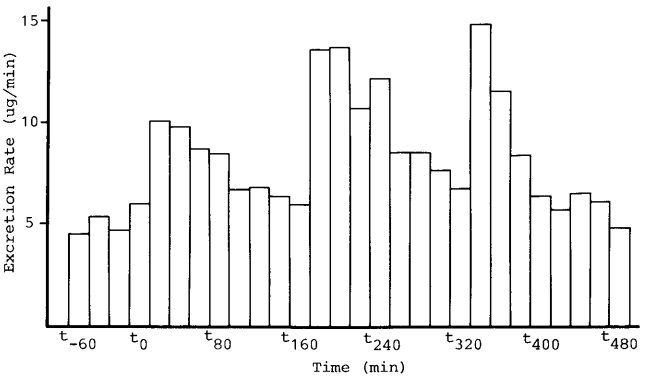


FIG. 5. Reversibility of the stimulated $\alpha_{2u}$ excretion rate following three infusions of lysozyme. Lysozyme (20 mg/kg) was infused at $t_0$, $t_{160}$, and $t_{320}$.

mum excretion rate was 12.3 ± 1.8 for ip compared with 20.2 ± 7.3 μg/min for iv administration. The two values were not significantly different ($P > 0.100$). Therefore, an overall mean value of 17.6 ± 7.1 μg/min (Table II) was taken as the estimated total filtered load for $\alpha_{2u}$. The mean excretion rate prior to MAL administration (Time = 0) was 5.3 ± 1.8 μg/min. Therefore, the difference of 12.3 μg/min represents an apparent maximum reabsorption rate and was 70% of the filtered load itself. This value confirms those reported for the intact rat (15, 23) and is the same as that obtained for lysozyme (9). The mean maximum excretion rates (Table II) obtained with gentamicin (20 mg/kg) and lysozyme (20 mg/kg) were 13.5 (control = 4.2 μg/min) and 18.1 μg/min (control = 7.4 μg/min), respectively, representing reabsorption rates of approximately 10.0 μg/min. Gentamicin and lysozyme were equally effective and 80% as effective as MAL. Table II shows that MAL had no action on the excretion of albumin which contradicts the results previously reported for intact rats; the reason for this discrepancy is not yet understood. MAL, by both protocols, yielded a striking glucosuria which reached maximum levels at 20–60 min post administration (data not included).

**Discussion.** Plasma proteins that pass through the glomerular filter are reabsorbed by the proximal tubule cells by two systems: one translocates high molecular weight and the other transports LMW proteins across the brush border membranes (1, 2). All evidence presented so far, including those in this report, support the independence of these two processes. Thus, the infusion of lysozyme and gentamicin increased the excretion rate of $\alpha_{2u}$-globulin without affecting that of albumin (Table II).

The process for the renal reabsorption of LMW proteins is considered to be of low affinity, high capacity, and of low specificity (7, 10). The mechanism is complex and may involve such factors as (a) binding to membrane sites, (b) access to the endocytotic sites, and (c) sites selective for specific proteins (3). It has been the purpose of the present study to establish whether or not the sex-dependent, anionic urinary protein, $\alpha_{2u}$-globulin, shares a common translocating process with LMW proteins or whether it utilizes an independent mechanism specific for this protein.

A protein often used to study the LMW translocating process is lysozyme. It is normally present in rat plasma at a concentration of only 4.46 ± 0.31 μg/ml. The filtered load was calculated to be 4.44 ± 0.97 μg/min while the amount reabsorbed was 4.42 ± 0.96 μg/min. Therefore, 99.5% of the filtered load was reabsorbed by the kidneys. The clearance rate [$C$(lzm)] for endogenous lysozyme was calculated to be 0.0039 ± 0.0025 ml/min; the $C$/GFR ratio was 0.0053 (8). When the enzyme level was increased to 255 ± 31 μg/ml by injecting egg white lysozyme, then the relative reabsorption rate declined and excretion was increased. The clearance rate for lysozyme at the elevated plasma level was much higher than with the endogenous concentration, namely 0.21 ± 0.02 ml/min (9). The injection of gentamicin (30–60 mg/kg) raised the $C$(lzm) to 0.65 ± 0.03; the $C$/GFR ratio was increased from a control of 0.23 to 0.8–1.01. Thus the reabsorption and excretion of lysozyme depends on the plasma concentration and is a constant fraction of the filtered load (6, 22).

Endogenous $\alpha_{2u}$-globulin, normally present in the plasma at 30 μg/ml, has a renal clearance of 0.33 ± 0.19; gentamicin raised this value to 0.91 ± 0.29 ml/min. Furthermore, the $C$/GFR ratio was increased from 0.17 ± 0.10 to 0.43 ± 0.18. These data are comparable to those for the elevated level of the heterologous lysozyme. Cojocel and Hook calculated a lysozyme reabsorption of 70% of the filtered load (9). For $\alpha_{2u}$ the filtered load was estimated as 17.6 ± 7.1 μg/min which is the excretion rate following treatment with MAL. The mean excretion rate prior to MAL administration (time = 0) was 5.3 ± 1.8 μg/min. Therefore, the difference of 12.3 μg/min represents an apparent maximum reabsorption rate which was 70% of the filtered load. These results are comparable to those for the elevated levels of lysozyme (9). Based on the comparisons made in this study, it is suggested that $\alpha_{2u}$ is reabsorbed in the kidneys by the same mechanism as lysozyme.

As already indicated, the initial step in the reabsorption of LMW proteins appears to be an interaction of positively charged proteins or groups with anionic sites on the brush border membrane. Since lysozyme, a highly cationic protein, almost completely prevented the reabsorption of anionic $\alpha_{2u}$ in a dose-dependent and reversible manner, it is suggested that

the enzyme competed with the endogenous protein in the initial binding step. It is now apparent that other LMW proteins of equal or higher isoelectric point also compete with $\alpha_{2u}$ (unpublished results). The fact that a variety of LMW proteins displace $\alpha_{2u}$ supports the concept that they are internalized by a common pathway. The binding of a protein to the brush border membrane sites may depend more upon the number of free amino groups and their configuration in the protein molecule than upon the net charge of the protein itself (6, 7). Therefore, the reabsorption of the anionic $\alpha_{2u}$-globulin may be dependent upon its lysyl residues and their specific configuration in the molecule. It is suggested that $\alpha_{2u}$-globulin can serve as an important marker protein for a continuing study of the renal reabsorption mechanism for LMW proteins.

This study was supported by a grant from the Parsons Endowment Research Fund of the School of Medicine, The University of South Dakota, and from a grant for kidney research provided by the Fraternal Order of the Eagles. The author is especially grateful to Sara and Susan Tiahrt for their excellent technical assistance and to Dr. Michael L. Kauker, Department of Physiology and Pharmacology, The University of South Dakota School of Medicine, for his review of this manuscript and for helpful discussions during the progress of the project.

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Received February 4, 1986. P.S.E.B.M. 1986, Vol. 182.
Accepted May 8, 1986.