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**Research Article**

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# Renal Handling of Zn-$\alpha_2$-Glycoprotein as Compared with that of Albumin and the Retinol-Binding Protein

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ABSTRACT An unusual electrophoretic pattern of the urine from a patient with malignant lymphoma was observed. One of the major proteins, identified as Zn-$\alpha_2$-glycoprotein (Zn-$\alpha_2$), was isolated from the urine and partly characterized. The Stokes radius was found to be 3.24 nm and the molecular weight, determined by sodium dodecyl sulfate polyacrylamide electrophoresis, 42,000. The plasma level in healthy individuals was 39±7 (SD) mg/liter. In 12 of 25 healthy individuals, Zn-$\alpha_2$ was measurable in the urine and was found to be 1.0±1.1 mg/liter. In 23 patients with chronic glomerulonephritis (CGN), in 9 with proximal tubular dysfunction (PTD), in 23 with various renal diseases (VRD), and in 10 with malignant lymphoma, the plasma level and the urinary excretion were compared with those of albumin (mol wt 67,000) and of the retinol-binding protein (RBP, mol wt 21,000). A close correlation was found between the urine-to-plasma (U/P) ratios of Zn-$\alpha_2$ and albumin in the patients with CGN, whereas in the PTD patients the U/P ratios of Zn-$\alpha_2$ and RBP were correlated. No significant renal arteriovenous difference in Zn-$\alpha_2$ could be demonstrated. The Zn-$\alpha_2$ excretion was increased also in two patients with malignant lymphoma and proteinuria of a tubular pattern. The plasma Zn-$\alpha_2$ varied inversely with the glomerular filtration rate in the patients with renal disease, but was normal in those with malignant lymphoma. The results are consistent with the assumption of a sieving coefficient of Zn-$\alpha_2$, substantially exceeding that of albumin, but notably lower than that of smaller low-molecular-weight proteins. An increased excretion of Zn-$\alpha_2$ may be due to increased glomerular permeability as well as to defective proximal tubular reabsorption.

## INTRODUCTION

The mechanism of renal handling of protein has been elucidated earlier by studies of proteins of such sizes as are excreted, presumably because of increased glomerular permeability (1-5), or because of decreased proximal tubular reabsorption (4, 6-16). The borderline between "glomerular" and "tubular" proteins is not properly defined. The apparently smallest protein excreted mainly because of an increased glomerular permeability is albumin, with a mol wt of 67,000, and the largest, classified exclusively as a tubular protein, is the dimer of L-chain, with a mol wt of 44,000 (15).

Glomerular and tubular proteinuria patterns are usually easily recognized by electrophoresis in various media. In a patient with malignant lymphoma we observed an electrophoretic pattern that could not be classified by conventional terms. The pattern was dominated by four proteins: albumin, a Bence Jones protein, $\beta_2$-microglobulin, and an initially unidentified band in the $\alpha$-region. Subsequent analyses showed that the latter protein was identical with Zn-$\alpha_2$-glycoprotein (Zn-$\alpha_2$),[1] and that its Stokes radius was slightly lower than that of albumin, whereas its molecular weight, determined by sodium dodecyl sulfate polyacrylamide electrophoresis, was 42,000, a value in accord with the findings of Bürghi and Schmid (17). We thought that these properties might be helpful in the investigation of renal handling of plasma proteins and therefore decided to study the excretion of Zn-$\alpha_2$ in various renal diseases. For comparison we measured the excretion of albumin (mol wt 67,000) and of the retinol-binding protein (RBP) (mol wt 21,000). A second purpose of the investigation was to find out whether the excretion of Zn-$\alpha_2$ increased in malignant lymphoma.

## METHODS

*Material.* Urine was collected from a 61-yr-old man with malignant lymphoma. The clinical features of this

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[1] *Abbreviations used in this paper:* CGN, chronic glomerulonephritis; GFR, glomerular filtration rate; PTD, proximal tubular damage; RBP, retinol-binding protein; U/P, urine-to-plasma; VRD, various renal diseases; Zn-$\alpha_2$, Zn-$\alpha_2$-glycoprotein.

TABLE I

*Pertinent Data on 46 Patients with Renal Diseases and 10 with Malignant Lymphoma*

| Patient | Sex | Age | Serum creatinine | GFR | Zn-$\alpha_2$ | | $C_{Zn}/C_{Cr}$ ·10³ | Albumin | | $C_{alb}/C_{Cr}$ ·10³ | RBP | | $C_{RBP}/C_{Cr}$ ·10³ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Serum | Urine | | Serum | Urine | | Serum | Urine | |
| | | | *mg/100 ml* | *ml/min/ 1.73 m²* | *mg/liter* | | | *g/liter* | *mg/liter* | | *mg/liter* | | |
| CGN | | | | | | | | | | | | | |
| Systemic lupus erythematosus glomerulonephritis | | | | | | | | | | | | | |
| B. G. | F | 54 | 1.0 | 62 | 103 | 36 | 7.3 | 23 | 7,700 | 2.7 | 114 | 5 | 0.9 |
| M. B. | F | 49 | 0.9 | 98 | 53 | 4 | 0.4 | 43 | 170 | 0.03 | 102 | ND | — |
| D. M. | F | 41 | 0.9 | 123 | 46 | 21 | 4.7 | 24 | 1,240 | 0.54 | 86 | 1 | 0.12 |
| Membraneous glomerulonephritis | | | | | | | | | | | | | |
| D. P. | F | 18 | 1.4 | 40 | 61 | 29 | 11.2 | 30 | 3,500 | 2.8 | 123 | 11 | 2.2 |
| L. G. | F | 44 | 0.8 | 94 | 34 | 6 | 6.3 | 25 | 590 | 0.88 | 58 | ND | — |
| T. S. | M | 49 | 1.2 | 69 | 42 | 13 | 3.8 | 31 | 2,200 | 0.83 | 105 | 1.0 | 0.11 |
| Focal glomerulonephritis | | | | | | | | | | | | | |
| E. B. | F | 50 | 1.2 | 46 | 33 | 1 | 0.6 | 43 | 650 | 0.12 | 73 | ND | — |
| B. P. | M | 29 | 1.0 | 99 | 34 | 1 | 0.3 | 36 | 730 | 0.19 | 82 | ND | — |
| Proliferative glomerulonephritis | | | | | | | | | | | | | |
| S. T. | F | 25 | 1.7 | 26 | 90 | 15 | 5.7 | 35 | 8,000 | 2.5 | 115 | 2 | 0.8 |
| I. W. | M | 13 | 0.9 | 57 | 55 | 18 | 4.4 | 34 | 3,100 | 1.1 | 95 | ND | — |
| T. N. | M | 18 | 1.0 | 113 | 56 | 3 | 0.5 | 48 | 1,300 | 0.12 | 57 | ND | — |
| L. Li. | F | 19 | 0.6 | 123 | 41 | 4 | 0.7 | 42 | 2,600 | 0.36 | 68 | 1.2 | 0.12 |
| K. N. | F | 19 | 0.7 | 151 | 33 | 5 | 2.2 | 35 | 1,390 | 0.06 | 80 | 1.5 | 0.3 |
| Unclassified glomerulonephritis | | | | | | | | | | | | | |
| O. A. | M | 38 | 1.1 | 128 | 53 | 11 | 1.6 | 45 | 2,230 | 0.42 | 78 | ND | — |
| V. I. | F | 30 | 0.7 | 125 | 29 | 4 | 1.8 | 39 | 500 | 0.15 | 55 | ND | — |
| B. N. | F | 41 | 1.1 | 59 | 43 | 7 | 1.4 | 36 | 2,160 | 0.53 | 102 | 1.2 | 0.09 |
| K. P. | F | 41 | 1.2 | 82 | 65 | 61 | 6.6 | 27 | 8,250 | 2.2 | 108 | 2.3 | 0.15 |
| S. P. | M | 49 | 0.9 | 105 | 29 | 5 | 1.4 | 43 | 1,190 | 0.15 | 56 | ND | — |
| I. W. | F | 35 | 0.6 | 93 | 37 | 9 | 0.9 | 49 | 1,180 | 0.07 | 52 | ND | — |
| M. G. | F | 42 | 0.8 | 78 | 46 | 1 | 0.4 | 44 | 180 | 0.04 | 65 | 1.5 | 0.37 |
| L. R. | M | 19 | 1.1 | 71 | 29 | 23 | 12.9 | 30 | 2,700 | 3.2 | 92 | 6.7 | 0.8 |
| H. S. | M | 61 | 1.1 | 80 | 50 | 46 | 8.3 | 37 | 5,500 | 1.4 | 115 | 0.5 | 0.04 |
| K. N. | M | 63 | 16 | 3 | 99 | 3 | 10.1 | 30 | 250 | 3.3 | 196 | — | — |
| VRD | | | | | | | | | | | | | |
| Polycystic kidneys | | | | | | | | | | | | | |
| E. F. | M | 40 | 5.8 | 7.5 | 72 | 4 | 6.6 | 49 | 440 | 1.0 | 145 | 27 | 20 |
| U. P. | F | 21 | 1.0 | 70 | 50 | 5 | 1.3 | 37 | 115 | 0.04 | 75 | 25 | 0.4 |

patient have been described elsewhere (18). Three 24-h urine specimens were used as starting material for the preparation of Zn-$\alpha_2$.

The clinical material (Table I) consisted of 10 patients with malignant lymphoma and 46 with various renal diseases. 23 had histologically verified chronic glomerulonephritis (CGN) (systemic lupus erythematosus in 3, membraneous in 3, focal in 2, proliferative in 5, and unclassifiable in 10). 14 patients had various renal diseases (VRD). This group consisted of 2 patients with polycystic kidneys, 2 with gouty nephritis, and 10 with what was regarded as chronic pyelonephritis. The remaining 9 patients had proximal tubular damage (PTD) caused by chronic cadmium intoxication.

Eight patients examined with renal arteriography or phlebography because of assumed or manifest unilateral renal disease were studied by renal vein catheterization of the normal, contralateral kidney. In one of these patients both kidneys were catheterized.

Conventional criteria, including the microscopic appearance of a lymph node and/or biopsy specimen of the spleen, were used for selecting the patients with malignant lymphoma.

25 healthy individuals were selected as described earlier (19). 44 registered blood donors were also included. No records of the state of health of the latter group were available.

The glomerular filtration rate (GFR) was measured as the 24-h endogenous creatinine clearance, except in the patients with CGN, in whom determination of the creatinine clearance is known to overestimate the GFR. In these patients, the GFR was measured as inulin or $^{51}$Cr-EDTA clearance, both of which give identical results in our laboratory (20).

*Sampling.* 24-h specimens of urine were collected and representative samples were stored at −20°C until used. Blood was drawn immediately before or after the sampling period and serum was kept in the same way as the urine.

*Analyses.* The serum level of Zn-$\alpha_2$ was determined in 44 blood donors. The serum and urinary concentrations of albumin, RBP, Zn-$\alpha_2$, and creatinine were determined in the 46 patients with renal disease, in the 10 patients with malignant lymphoma, and in the 25 healthy individuals.

The blood samples obtained from the patients subjected to aortal and renal vein catheterization were analyzed for Zn-$\alpha_2$. The position of the catheter in the renal vein was

TABLE I—(*Continued*)

| Patient | Sex | Age | Serum creatinine | GFR | Zn-$\alpha_2$ Serum | Zn-$\alpha_2$ Urine | $C_{Zn}/C_{Cr}$ ·10³ | Albumin Serum | Albumin Urine | $C_{alb}/C_{Cr}$ ·10³ | RBP Serum | RBP Urine | $C_{RBP}/C_{Cr}$ ·10³ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *mg/100 ml* | *ml/min/ 1.73 m²* | *mg/liter* | | | *g/liter* | *mg/liter* | | *mg/liter* | | |
| VRD | | | | | | | | | | | | | |
| Gouty nephritis | | | | | | | | | | | | | |
| A. S. | M | 69 | 8.2 | 8 | 80 | 4 | 9.3 | 38 | 420 | 3.6 | 153 | 28 | 31 |
| I. H. | F | 68 | 2.5 | 27 | 61 | 19 | 7.7 | 39 | 1,500 | 1.5 | 140 | 74 | 13 |
| Chronic pyelonephritis | | | | | | | | | | | | | |
| G. E. | F | 57 | 13 | 3 | 100 | 11 | 22.6 | 41 | 1,500 | 11.3 | 220 | 74 | 170 |
| E. E. | F | 59 | 1.2 | 40 | 38 | 4 | 1.5 | 41 | 70 | 0.03 | 94 | 2.8 | 1.6 |
| E. H. | F | 19 | 2 | 37 | 83 | 4 | 1.8 | 43 | 150 | 0.12 | 138 | 2.1 | 0.5 |
| G. S. | M | 59 | 3.3 | 28 | 82 | 4 | 2.6 | 42 | 15 | 0.01 | 131 | 12 | 4.6 |
| L. V. | F | 59 | 6.4 | 8 | 114 | 2 | 2.5 | 50 | 650 | 1.2 | 196 | 67 | 45 |
| E. O. | F | 51 | 10 | 4 | 103 | 12 | 21.7 | 36 | 2,200 | 16 | 158 | 59 | 74 |
| D. P. | M | 40 | 1.8 | 50 | 61 | 13 | 3.2 | 43 | 2,300 | 0.78 | 115 | 2.2 | 0.3 |
| R. J. | F | 44 | 6.7 | 7 | 90 | 6 | 8.0 | 41 | 900 | 0.49 | 144 | 14 | 5.0 |
| L. L. | M | 35 | 1.4 | 92 | 35 | 6 | 1.0 | 45 | 180 | 0.02 | 103 | ND | — |
| K. R. | F | 51 | 1.0 | 74 | 40 | 3 | 0.6 | 51 | 60 | 0.01 | 52 | ND | — |
| PTD | | | | | | | | | | | | | |
| F. A. | M | 65 | 1.2 | 84 | 49 | 30 | 5.3 | 48 | 210 | 0.039 | 84 | 195 | 20 |
| G. L. | M | 66 | 1.3 | 62 | 40 | 11.5 | 3.4 | 47 | 25 | 0.026 | 72 | 5 | 0.8 |
| E. S. | M | 68 | 1.3 | 67 | 45 | 18 | 4.3 | 46 | 115 | 0.033 | 77 | 29 | 3.9 |
| F. B. | M | 63 | 2.1 | 26 | 54 | 11.5 | 3.7 | 44 | 85 | 0.033 | 99 | 38 | 6.6 |
| A. A. | M | 76 | 3.6 | 19 | 43 | 9 | 14 | 45 | 60 | 0.089 | 135 | 147 | 73 |
| I. N. | M | 67 | 2.1 | 30 | 54 | 26 | 12 | 47 | 70 | 0.037 | 101 | 146 | 36 |
| N. E. | M | 66 | 1.2 | 79 | 45 | 20.5 | 5.9 | 43 | 55 | 0.016 | 89 | 42 | 6 |
| G. H. | M | 76 | 1.8 | 40 | 45 | 19.5 | 9.3 | 44 | 95 | 0.046 | 117 | 82 | 15 |
| I. J. | M | 66 | 1.2 | 56 | 49 | 25 | 6.3 | 42 | 55 | 0.016 | 90 | 85 | 12 |
| Malignant lymphoma | | | | | | | | | | | | | |
| K. E. | M | 73 | 2.1 | 40 | 60 | ND | — | — | <25 | — | 38 | ND | — |
| A. A. | F | 61 | 0.6 | 95 | 49 | ND | — | — | <25 | — | ND | ND | — |
| N. N. | F | 74 | 0.8 | 45 | 62 | ND | — | — | <25 | — | 26 | 0.7 | 0.2 |
| B. N. | M | 54 | 1.0 | 110 | 53 | ND | — | — | <25 | — | 45 | ND | — |
| G. K. | M | 82 | 1.0 | 55 | 40 | ND | — | — | <25 | — | ND | ND | — |
| A. G. | F | 57 | 0.9 | 110 | 49 | ND | — | — | <25 | — | 72 | ND | — |
| T. J. | F | 56 | 1.0 | 80 | 40 | ND | — | — | <25 | — | 40 | 0.2 | 0.1 |
| H. B. | M | 64 | 0.7 | 150 | 22 | ND | — | — | — | — | ND | 1 | — |
| E. J. | M | 67 | 0.9 | 70 | 66 | 11 | 1.2 | | — | — | 22 | 5 | 1.6 |
| F. L. | M | 62 | 1.3 | 85 | 72 | 27 | 3.8 | — | — | — | 40 | 17.7 | 4.4 |

ND, not detectable.

checked by fluoroscopy and by measuring the renal extraction rate of *p*-aminohippurate, infused at a constant rate from 30 min before, and during, the sampling procedure.

*Antisera.* Antiserum against the isolated protein was reared in rabbits given 0.5 mg of the antigen emulsified with complete Freund adjuvant into the hind footpads. Booster doses were given by the same route after 3 wk. Satisfactory antibody titers were obtained 6 wk after the first injection. This antiserum was used for all the immunochemical determinations.

Anti-RBP was reared in a similar way by injecting the purified RBP isolated as described by Ravnskov (21).

Anti-human plasma protein, anti-human tubular protein, anti-kappa, and anti-lambda light chain were purchased from Dakopatts A/S, Copenhagen, Denmark.

Anti-inter-$\alpha$-trypsin inhibitor, anti-$\alpha_{1B}$-glycoprotein, anti-$\alpha_{1T}$-glycoprotein, and anti-$\alpha_2$-HS-glycoprotein were purchased from Behringwerke AG, Marburg-Lahn, W. Germany. Anti-Zn-$\alpha_2$ (used for identification purposes only) was obtained from Nordic Pharmaceuticals and Diagnostics, Antwerp, Belgium. Anti-antithrombin III was a gift from AS Nyegaard & Co., Oslo, Norway. Anti-antichymotrypsin and anti-C1-esterase inhibitor were kindly supplied by Drs. C.-B. Laurell, Malmö, and A. B. Laurell, Lund. Anti-albumin was also a gift from Dr. C.-B. Laurell.

*Isolation procedure.* 9 liters of urine, containing 1.2 g protein, was concentrated to 60 ml by dialysis against solid polyethylene glycol (mol wt 20,000). The concentrated urine was dialyzed against 0.02 M Tris-HCl, pH 7.4, and applied to a 2.5 × 30-cm column packed with DEAE-cellulose (Whatman DE-52, Whatman Chemicals, Div. W. & R. Balston, Maidstone, Kent, England), equilibrated with the same buffer.

The elution was performed with a linearly increasing gradient of sodium chloride. The fractions containing the $\alpha$-globulin were pooled, concentrated, and applied to a Sephadex G-100 column (Pharmacia Fine Chemicals, Uppsala, Sweden) (dimensions 2.5 × 90 cm) in 0.02 M Tris-HCl, pH 7.4, containing 0.2 M NaCl. The fractions containing the $\alpha$-globulin component were concentrated and subjected to preparative electrophoresis, followed by extensive dialysis against redistilled water, and lyophilized. The final product was pure, as judged from agarose gel electrophoresis and immunoelectrophoresis against anti-human plasma and anti-human tubular protein antiserum.

*Immunochemical analysis.* Zn-$\alpha_2$ and RBP were mea-

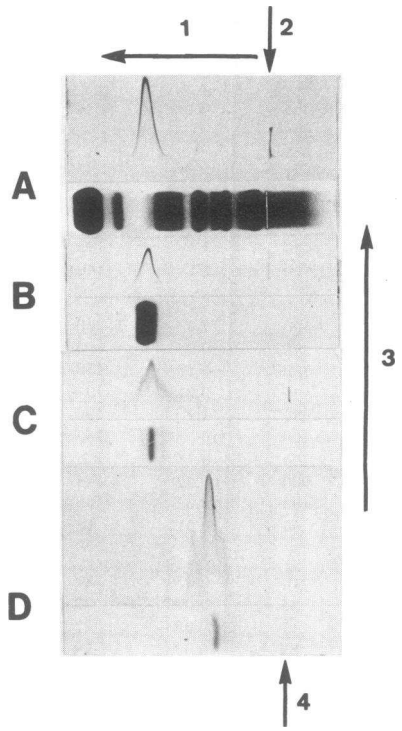


FIGURE 1 Crossed immunoelectrophoresis of: A. human plasma in agarose gel electrophoresis, with barbital buffer, pH 8.6; B. pure Zn-$\alpha_2$-glycoprotein in the same medium and buffer; C. the pure protein in agarose gel electrophoresis, but with Tris-glycine buffer, pH 8.4; and D. pure protein after isoelectric focusing in purified agarose. Arrow 1 indicates the anodal direction in the first step, and arrow 3 the anodal direction in the second step. During the latter step the samples migrated into an agarose gel containing rabbit anti-Zn-$\alpha_2$. Arrows 2 and 4 denote the application slit.

sured by electroimmuno assay (22) and albumin by single radial immunodiffusion (23). All measurements were performed on unconcentrated urine samples.

Crossed immunoelectrophoresis was carried out in accordance with Ganrot (24). Immunoelectrophoresis was performed according to Scheidegger (25).

*Other methods.* Analytical and preparative agarose gel electrophoresis was run as described by Johansson (26). Thin-layer isoelectric focusing was performed in purified 0.8% agarose containing 2% Ampholine (LKB-Produkter, Bromma, Sweden), pH 3–6, and 1% polyacrylamide (27). Before application of the sample, the gel was pre-run for 1 h at 220 V and 10 mA (27). Sodium dodecyl sulfate polyacrylamide gel electrophoresis was done as described by Weber and Osborne (28).

The Stokes radius of the isolated protein was calculated from gel filtration data, as suggested by Laurent and Killander (29, 30). After determination of the partition coefficient ($K_{av}$) on Sephadex G-100, the following equation was used, which relates the partition coefficient of a solute moving through a porous gel to the radius of an equivalent sphere:

$$(-\ln K_{av})^{0.5} = (\pi L)^{0.5} \times (r_r + r_s)$$

where $L$ is the concentration of dextran gel rods expressed as centimeters rod per cubic centimeter, $r_r$ is the radius of the rod, and $r_s$ the Stokes radius of the solute (29). Values of $L$ and $r_r$ were obtained by gel filtration of human serum albumin with a Stokes radius assumed to be 3.5 nm. The Stokes radius was also calculated (31) from the molecular weight, sedimentation coefficient, and viscosity of Zn-$\alpha_2$ as given by Bürghi and Schmid (17).

Creatinine was determined in a Technicon AutoAnalyzer (Technicon methodology N-116, Technicon Instruments Corp., Tarrytown, N. Y.).

*Calculation of protein clearance.* Urinary protein excretion was calculated as the ratio between the clearance of the specific protein ($P$) and that of creatinine (Cr), according to the formula:

$$\frac{U_P \cdot V/S_P}{U_{Cr} \cdot V/S_{Cr}} = \frac{U_P/S_P}{U_{Cr}/S_{Cr}}$$

where $V$ denotes urine output and $U$ and $S$ are the concentrations of the protein in urine and serum, respectively.

## RESULTS

*Identification of the component.* The urine from the patient with malignant lymphoma contained four major components: three of them were identified by immunoelectrophoresis and crossed immunoelectrophoresis as albumin, $\beta_2$-microglobulin, and a Bence Jones protein of type kappa. The fourth component was tested with the above-mentioned antisera. No precipitation was seen except with anti-Zn-$\alpha_2$, which gave a clear precipitate in the same position as the unknown component. This precipitate was immunologically identical with the precipitate produced by the antiserum raised against the isolated component (Fig. 1).

*The purification of the protein.* The chromatographic elution patterns are given in Figs. 2 and 3. Fig. 4 shows the agarose gel electrophoretic patterns of the material after each step. The final preparation appeared as a single, rather broad zone in agarose gel electrophoresis with barbitate buffer, pH 8.6, but as three bands in Tris-glycine buffer, pH 8.4 (Fig. 1 A and C). These three zones were also seen on isoelectric focusing in purified agarose (Fig. 1 D). Crossed immunoelectrophoresis revealed that the three components in the two cases were

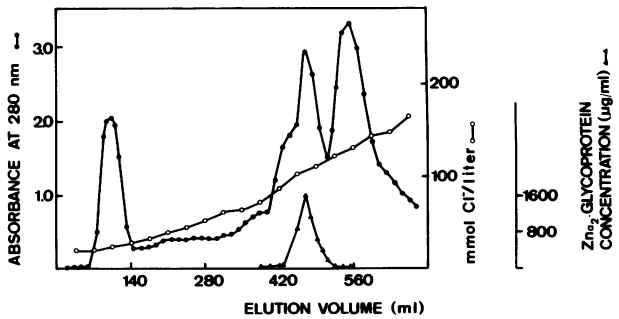


FIGURE 2 Diagram of linear gradient elution of the starting protein material (1,225 mg of total protein and 82 mg of Zn-$\alpha_2$) on a DE-52 Whatman column. Experimental details are given in the text.

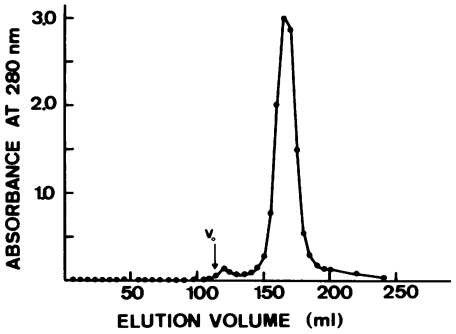


FIGURE 3 Chromatography on Sephadex G-100 of 78 mg protein containing 43 mg of Zn-$\alpha_2$ obtained by chromatography on DE-52 Whatman. $V_0$ denotes the void volume of the column. The column (2.5 × 90 cm) was equilibrated with 0.02 M Tris-HCl, pH 7.4, containing 0.2 M NaCl. Fractions of 5 ml were collected at a flow rate of 30 ml/h.

immunologically identical. In the former case only a single peak appeared (Fig. 1 D).

*Molecular weight and molecular radius.* Sodium dodecyl sulfate polyacrylamide gel electrophoresis, which revealed only a single zone, gave a mol wt of 42,000, a figure in good agreement with that (41,000) found by Bürghi and Schmid (17) by ultracentrifugation.

The Stokes radius of Zn-$\alpha_2$ was found to be 3.24 nm when determined by gel filtration. Calculation of Stokes radius from the molecular parameters (molecular weight, sedimentation coefficient, and viscosity) given by Bürghi and Schmid (17) gave 3.28 nm, close to that obtained by gel filtration.

*Serum level of Zn-$\alpha_2$.* The detection limit of the electroimmuno assay of Zn-$\alpha_2$ was 0.2 mg/liter and that of RBP, 1 mg/liter. The coefficient of variation of both methods was ±5%. The mean serum level of Zn-$\alpha_2$ in the 69 controls was 39 mg/liter ±7 (SD), a value in good agreement with that of 48±3 found by Becker et al. (32), but somewhat lower than that of 140±30 given by Poortmans and Schmid (33). In the CGN and in the VRD group the serum level of Zn-$\alpha_2$ varied inversely with the GFR (Fig. 5). When log values were used, the regression line was linear[3] (CGN, $y = 2.18 - 0.28x$, $r = -0.60$; VRD, $y = 2.19 - 0.28x$, $r = -0.83$). No correlation was found between the serum level of Zn-$\alpha_2$ in the PTD group and the GFR, probably because of the small number of observations. The serum level of RBP covered a range of 22–220 mg/liter and varied with the GFR in all three groups (CGN, $y = 2.47 - 0.29x$, $r = -0.71$; VRD, $y = 2.44 - 0.26x$, $r = -0.81$; PTD, $y = 2.47 - 0.30x$, $r = -0.80$).

[3] Logarithmic values of all parameters (the serum levels of the proteins, the protein/creatinine clearance ratios, and the GFR) were used in all statistical calculations. The original values of the parameters are given in Table I.

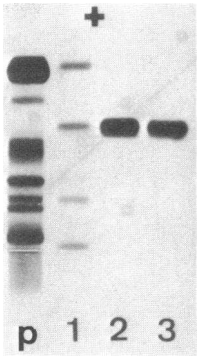


FIGURE 4 Agarose gel electrophoretic patterns during the preparation of Zn-$\alpha_2$. 1. Concentrated urine from the patient (see text). 2. The pooled, concentrated fractions after DE-52 Whatman cellulose chromatography. 3. The pooled, concentrated fractions after Sephadex G-100 chromatography. Human plasma is given as reference (p).

The serum levels in the patients with malignant lymphoma were within normal limits.

*The urinary excretion of Zn-$\alpha_2$.* In 13 of the 25 healthy individuals, the concentration of Zn-$\alpha_2$ in the urine was below the detection limit of the method used, whereas the mean urinary concentration in the remaining cases was 1.0 mg/liter (range 0.2-8.5 mg/liter). The mean calculated Zn-$\alpha_2$/creatinine clearance ratio × $10^3$ in these 13 individuals was 0.2, and the mean amount of Zn-$\alpha_2$ excreted per gram creatinine was 0.6 mg. Measurable amounts of Zn-$\alpha_2$ were found in urine from all the patients with renal disease. The highest levels (50-60

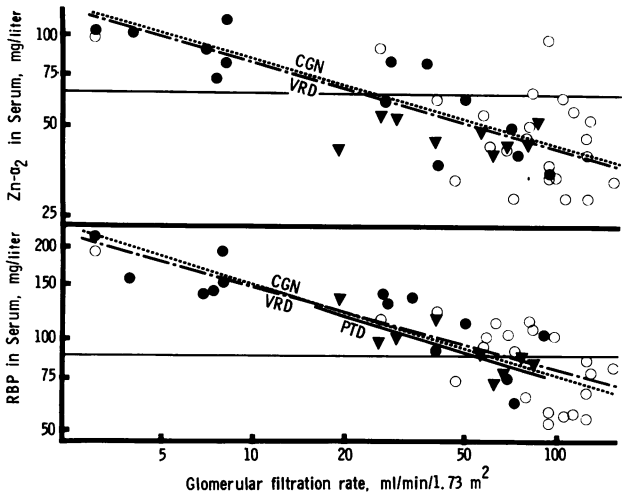


FIGURE 5 Correlation between the GFR and the plasma level of Zn-$\alpha_2$ (upper) and that of RBP (lower). ○ Patients with CGN; ▼ patients with PTD; ● patients with VRD. - - - -, Regression line of CGN; ——, regression line of PTD; –·–·–, regression line of VRD. The horizontal lines denote the upper limits of the normal means±2 SD.

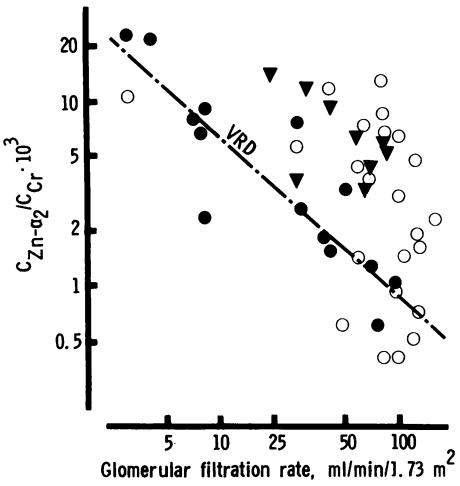


FIGURE 6 Correlation between the GFR and the Zn-$\alpha_2$/creatinine clearance ratio. A significant correlation was found only in the VRD group. Symbols as in Fig. 5.

mg/liter) were found in patients with severe uremia and in those with nephrotic syndrome.

*Correlation between Zn-$\alpha_2$ excretion and GFR.* The Zn-$\alpha_2$/creatinine clearance ratio varied inversely with the GFR in the VRD group ($y = 3.67 - 0.86x$; $r = -0.88$) but not in the CGN or the PTD group (Fig. 6).

*Correlation between Zn-$\alpha_2$ and albumin excretion.* A highly significant correlation was found between the urine-to-plasma (U/P) ratio of Zn-$\alpha_2$ and albumin in the CGN ($y = 1.21 + 0.70x$, $r = 0.87$) and the VRD group ($y = 1.96 + 0.40x$, $r = 0.90$), whereas the correlation was not significant in the PTD group ($y = 2.54 + 0.52x$, $r = 0.54$). The clearance of Zn-$\alpha_2$ was two- to fourfold that of albumin in CGN and VRD, but about 200-fold in PTD (Fig. 7).

*Correlation between Zn-$\alpha_2$ and RBP excretion.* The concentration of RBP in the urine was high (range 5-195 mg/liter) in the patients with PTD, but low (0-11 mg/liter) in those with CGN. The excretion of RBP in patients with VRD covered a wide range (0-74 mg/liter).

U/P ratios of Zn-$\alpha_2$ and RBP varied closely with each other in VRD ($y = 1.65 + 0.36x$, $r = 0.79$) and in PTD ($y = 1.81 + 0.33x$, $r = 0.86$). In the latter group the clearance of Zn-$\alpha_2$ was slightly higher than that of RBP at low proteinuric rates, but with increasing severity of proteinuria the clearance of RBP rose to more than 10-fold that of Zn-$\alpha_2$. No significant correlation was found in the CGN group ($y = 2.15 + 0.29x$, $r = 0.31$) (Fig. 8).

*Correlation between the excretion of RBP and albumin.* In the VRD group, but not in the others, there was a significant correlation between the U/P ratios of RBP and albumin ($y = 1.96 + 0.40x$, $r = 0.90$). The RBP clearance was 200–1,000 times that of albumin in the PTD and 10–100 times that in the VRD group (Fig. 7).

*Protein excretion in patients with malignant lymphoma.* In 2 of the 10 patients with this disease, the excretion of Zn-$\alpha_2$ was increased. The excretion of RBP was increased in these two patients as well as in a third. Unfortunately, urinary albumin was not determined in these two patients, but an earlier examination with Albustix (Ames Co., Inc., Div. of Miles Labs., Slough, England) had shown mild albuminuria in both. In seven patients the excretion of albumin in the urine was within normal limits and no Zn-$\alpha_2$ or RBP was detectable in the urine.

*Renal extraction of Zn-$\alpha_2$.* In three patients the concentration of Zn-$\alpha_2$ in the renal vein was lower than that in arterial blood; in four it was higher; and in two it was equal in both types of blood. The differences were smaller than the coefficient of variation of the method

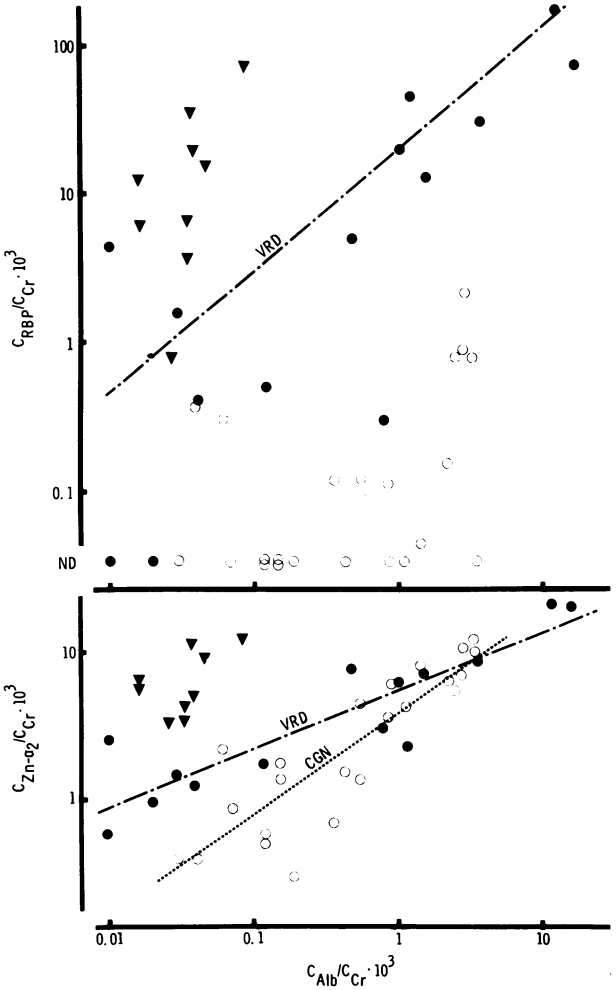


FIGURE 7 Correlation between the albumin/creatinine clearance ratio and (upper) the RBP/creatinine clearance ratio, and (lower) the Zn-$\alpha_2$/creatinine clearance ratio. ND, not detectable. Symbols as in Fig. 5.

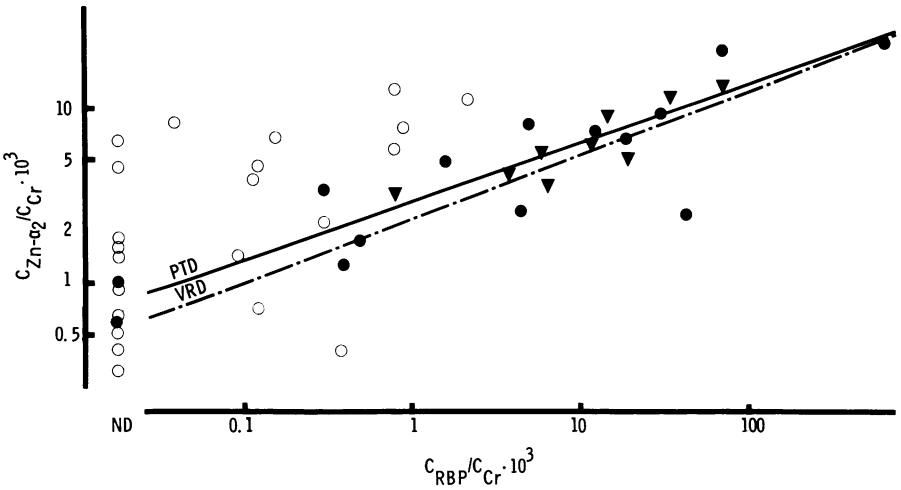


FIGURE 8 Correlation between the RBP/creatinine clearance ratio and the Zn-$\alpha_2$/creatinine clearance ratio. ND, not detectable. Symbols as in Fig. 5.

and the mean of all renal venous blood levels of Zn-$\alpha_2$ was equal to that of the arterial levels.

## DISCUSSION

The glycoprotein designated Zn-$\alpha_2$ (because of its precipitability with $Zn^{2+}$-ions) was first isolated and characterized by Bürghi and Schmid (17), who used pooled human plasma as starting material. It occurs normally in small amounts in human plasma and urine (34) as well as in saliva and sweat (33), but its biological function is unknown. In urine the level is notably higher after strenuous physical exertion (33). The present isolation procedure yielded an immunologically pure preparation, which, however, showed a pattern of heterogeneity in agarose gel electrophoresis and isoelectrofocusing experiments, a pattern not earlier reported for Zn-$\alpha_2$. Although the cause of the heterogeneity has not been studied, it was not an unexpected finding in view of the common microheterogeneity found for carbohydrate-rich proteins. It should be pointed out that no molecular size heterogeneity of the isolated protein could be discovered.

Because of its rather small molecular size, increased amounts of Zn-$\alpha_2$ were expected in the urine from patients with renal disease. Since the Stokes radius of Zn-$\alpha_2$ (3.24 nm) was only slightly lower than that of albumin (3.50 nm), while its molecular weight of 41-42,000 was close to that of the L-chain dimer (44-45,000), which has a Stokes radius of 2.8 nm (35), it was of interest to determine the role of increased glomerular permeability and a decreased tubular reabsorption for the urinary excretion of Zn-$\alpha_2$.

There is first of all reason to believe that changes in glomerular permeability interfere with the degree of Zn-$\alpha_2$ excretion, since the patients with glomerular damage showed an increased excretion of the protein, which was closely correlated with the increased excretion of albumin, but showed no correlation between the excretion of albumin and the low molecular weight RBP. The last-mentioned finding also rules out the possibility that the correlation between the excretion of albumin and Zn-$\alpha_2$ is due to competition for tubular reabsorption.

Incomplete tubular reabsorption may be another cause of an increased Zn-$\alpha_2$ excretion. Since it is generally accepted that impairment of reabsorption of normally filtered protein is the cause of low molecular weight proteinuria in patients with proximal tubular damage, the constant finding of an increased Zn-$\alpha_2$ excretion and the close correlation between Zn-$\alpha_2$ and RBP excretion in such patients strongly suggests that also Zn-$\alpha_2$ excretion may be caused by this mechanism.

The increased excretion of Zn-$\alpha_2$ in patients with PTD also indicates that at least part of the Zn-$\alpha_2$ normally passes the glomerular basement membrane. If the reabsorption of albumin and Zn-$\alpha_2$ is decreased to the same extent in patients with PTD, the glomerular permeability of Zn-$\alpha_2$ should be about 200 times that of albumin, since the U/P ratio of Zn-$\alpha_2$ in the PTD patients was about 200 times the U/P ratio of albumin. If we assume that the tubular reabsorption is completely abolished in the PTD patient with the highest U/P ratio of Zn-$\alpha_2$ observed ($14 \cdot 10^{-3}$), it would mean that the calculated sieving coefficient would be 0.014 and that the glomerular filtration of Zn-$\alpha_2$ would be at least 1.4% of the filtration fraction.

The assumed low glomerular filtration of Zn-$\alpha_2$ is compatible with the absence of any measurable differences in renal arteriovenous concentration. With a calculated sieving coefficient of Zn-$\alpha_2$ of 0.014, a renal arteriovenous

concentration difference of about 0.3% should be obtained with a filtration fraction of about 0.2. This small difference is not measurable by the technique used by us. Thus, even if the permeability of the glomerulus to Zn-$\alpha_2$ far exceeds that to albumin, it is much lower than that to the low-molecular-weight proteins usually studied, some of which have renal extraction rates approaching or exceeding the filtration fraction (36–41).

The high U/P ratios of Zn-$\alpha_2$ compared with those of albumin observed in all patients studied are notable in view of the small difference in Stokes radius between these two proteins. Evidently, molecular characteristics other than molecular size determine how they are handled by the kidneys. Recent studies (42) demonstrating great differences between the fractional clearance of charged and uncharged dextran of the same effective molecular radius suggest that the molecular charge may be of importance.

Still more interesting was the finding that the patients with tubular damage exhibited U/P ratios of Zn-$\alpha_2$ exceeding those of albumin by about 200 times, whereas the patients with glomerular damage only had U/P ratios of Zn-$\alpha_2$ clearance values 5- to 10-fold those of albumin. Two possible, not mutually exclusive, explanations may be considered. The first explanation is based on the findings in the above-mentioned studies on the effect of molecular charge (42). Since the isoelectric point of albumin is lower than that of Zn-$\alpha_2$, a loss of fixed negative charges from the glomerular capillary wall in glomerulonephritis may explain why the excretion of albumin increases more than Zn-$\alpha_2$ excretion in glomerulonephritis compared with the situation in PTD. The second explanation may be that the tubular protein reabsorption is a selective process with a preference for Zn-$\alpha_2$ over albumin. If so, Hardwicke and Squire's (43) widely accepted view of nonselective protein reabsorption should be reconsidered.

Owing to the considerable glomerular filtration of low molecular weight proteins, their catabolism depends largely on the kidneys (9, 14-16, 38). This explains the invariable inverse ratio between the plasma level of these proteins and the GFR (12, 19, 44). The inverse relation between plasma Zn-$\alpha_2$ and the GFR indicates that the kidneys also play a significant role in the catabolism of Zn-$\alpha_2$. Judged from comparison with the plasma level of Zn-$\alpha_2$ and RBP, the degree of renal catabolism of these two proteins is apparently largely equal, but it should be recalled that RBP normally occurs in plasma associated with prealbumin. This complex has a molecular size preventing a more substantial glomerular filtration of RBP. Since it is mainly free plasma RBP that increases in renal failure (45-47), the slope of the curve for the ratio between plasma RBP and GFR would probably become much steeper if only free RBP was considered. For the same reason, the U-P ratios of RBP may be underestimated.

The high level of Zn-$\alpha_2$ found in saliva and sweat (33) suggests the existence also of a renal tubular secretion. However, it seems highly improbable that tubular secretion should increase and vary so closely with the increased glomerular permeability or proximal tubular damage.

The finding of Zn-$\alpha_2$ in large amounts in the urine from a patient with malignant lymphoma initially prompted us to undertake this study, in the hope that this protein might in some way provide a clue to the understanding of malignant lymphoma. However, as no substantially raised plasma levels could be found in any of the 10 patients with this disease, and as the urinary concentration was elevated only when other proteins could be found in the urine, we feel that the urinary appearance of Zn-$\alpha_2$ was not related to the disease per se, but probably due to impairment of renal function.

In conclusion, it would appear that the underlying mechanisms of increased urinary Zn-$\alpha_2$ excretion are best explained in the following way: Normally a small fraction, probably less than 1% of the plasma Zn-$\alpha_2$, is filtered at the glomerulus, after which it is almost completely reabsorbed. When the proximal tubules are damaged, reabsorption is insufficient, and an increased amount is excreted in the urine, but since only a small amount is filtered, its clearance does not by far reach the values of other, smaller low-molecular-weight proteins. The filtered fraction increases when the glomerular permeability is increased, as in glomerulonephritis. Owing to the simultaneously increased filtration of other proteins, especially albumin, the tubular reabsorptive capacity is saturated, and the result is an increased urinary excretion of Zn-$\alpha_2$.

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