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Uromucoid in the Rat: Its Isolation, Localization in the Kidney and Concentration in the Urine

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Summary. Based on the finding that rat uromucoid cross reacts with antihuman uromucoid, rat uromucoid was isolated by means of immune absorption chromatography. Using a monospecific antiserum obtained with the use of this preparation, uromucoid was shown to be present in the distal tubules of the kidney. A quantitative immunological determination was made from the urine of 30 normal rats.

Key words: Uromucoid - Rat kidney - Immune absorption chromatography - Immune histochemistry - Laurell technique.

Uromucoid is an acid protein in the urine of man and other mammals. It is also present in kidney stones and some investigators first assumed that it was one of the essential components of matrix (5). There is, however, no simple quantitative relationship between stone formation and uromucoid excretion (2). Patients suffering from kidney stones show no elevated uromucoid excretion rate.

Uromucoid is localized in the distal tubules of the kidney but its function in the kidney has not been determined (9). A suitable laboratory animal is therefore necessary for studying uromucoid excretion following experimentally induced kidney diseases. The most important prerequisite for such studies is the exact determination of the appropriate species-specific uromucoid. In this study, Wistar rats were selected since their urine shows a weak cross reaction with antihuman uromucoid. This cross reaction allows an immunohistological identification of the uromucoid in the rat kidney but not a quantitative immunological determination of its concentration. For this, the rat uromucoid must be isolated and its specific antibody produced.

A protein comparable to that of human uromucoid cannot be obtained from rat urine by previously described techniques (3). Isolation by means of anti-human uromucoid and immune absorption chromatography however, has been successful.

The following investigations describe this isolation, antibody formation, immunological localization of uromucoid in the rat kidney and its quantitative immunological determination in the urine of normal, untreated rats.

MATERIAL AND METHODS

The various steps towards isolation of the uromucoid and preparation of its monospecific antibody are presented in Figure 1. The disc electrophoretic analysis (6) of rat urine and its 5 fractions separated by gel-chromatography on Sephadex G 150, together with certain immune electrophoreses with the obtained antiserum before and after absorption are shown in Figures 2 and 3.

Using Justra and Lundgren's (1969) method (8), we obtained 34 mg of pure Immunoglobulin

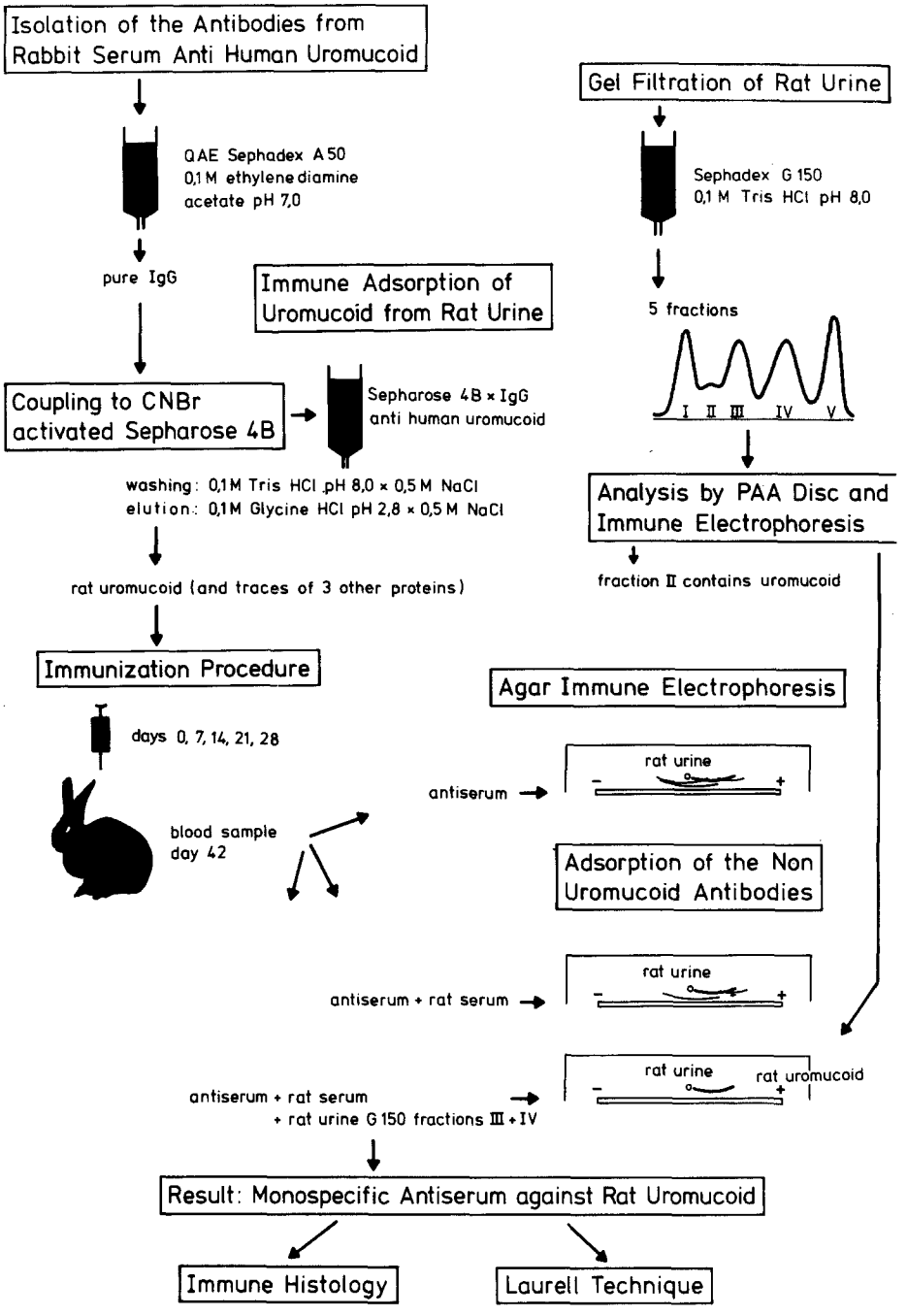

Fig. 1. Preparation of a monospecific antibody against rat uromucoid. For details, see 'Materials and Methods'

G on QAE Sephadex 50 from 2 ml commercial rabbit anti-human uromucoid serum. The coupling of this antibody with 3 g cyanbromide-activated Sepharose 4 B was conducted following the instructions given by Pharmacia-Uppsala (1972).

A column 1 cm in diameter was filled with the coupled gel-matrix equilibrated with wash buffer and charges of 5 ml of 10 x concentrated rat urine which had been incubated at room temperature for 2 hours were added. After washing the filtrate with wash buffer, the immune-adsorbed antigen was eluted. Following concentration of each eluate to about 0.05 ml, the antigen was mixed with 0.5 ml Freund's Adjuvant (complete) and injected into both hindlegs of 1 year old, 3.5 kg Alaska strain rabbits. Blood was taken from these to obtain the antiserum 42 days following injection. Scheidegger's (1955) micromethods (10) were used for the agar gel immune electrophoresis.

Four precipitates were obtained from rat

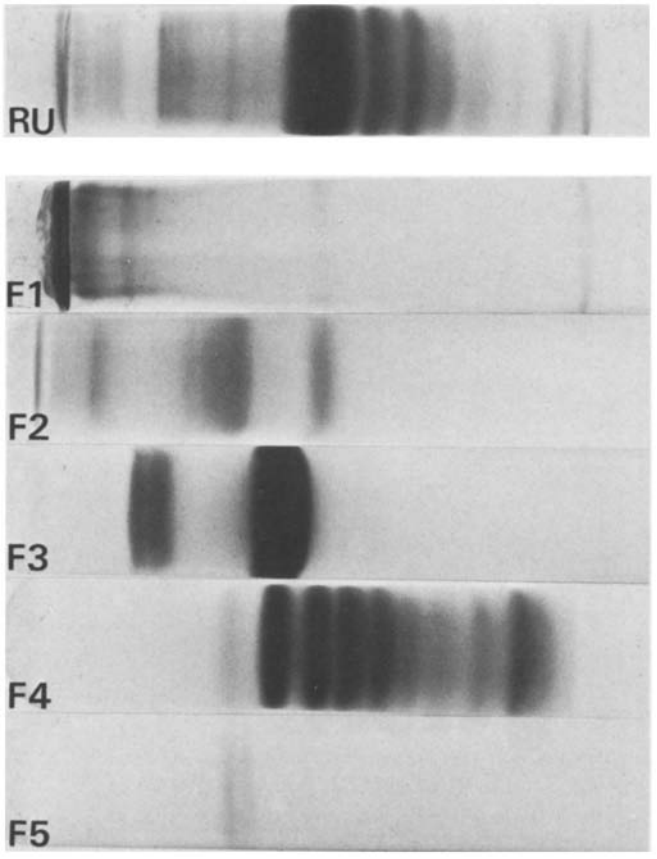

Fig. 2. Polycrylamide disc electrophoresis of rat urine (RU) and of its five fractions (F_1 - F_5) obtained from Sephadex G 150 chromatography. F_1 is the fraction with the highest molecular weight. F_2 contains uromucoid. F_3 and F_4 were used for the purfication of the antiserum. F_5 contains substances of low molecular weight i.e. amino acids. Staining with Amido Black 10 B.

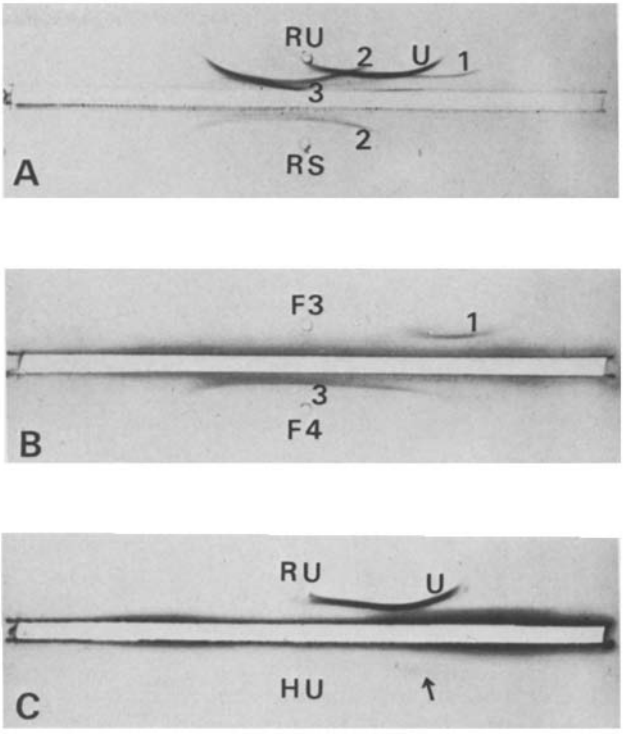

Fig. 3. Agar immune electrophoreses: Fig. 3 A, rat urine (RU) and rat serum (RS) against unabsorbed antiserum. Fig. 3 B, fractions 3 and 4 (F_3, F_4) against the unabsorbed antiserum, Fig. 3 C, rat urine (RU) and human urine (HU) against the antiserum absorbed with rat serum and fractions 3 and 4 from the gel chromatography of rat urine. U = rat uromucoid, 1-3 = precipitates of antibodies which are absorbed by rat serum and fractions 3 and 4. Note the weak precipitate of human uromucoid with the antibody against rat uromucoid (arrow). Staining with Comassie Brilliant Blue

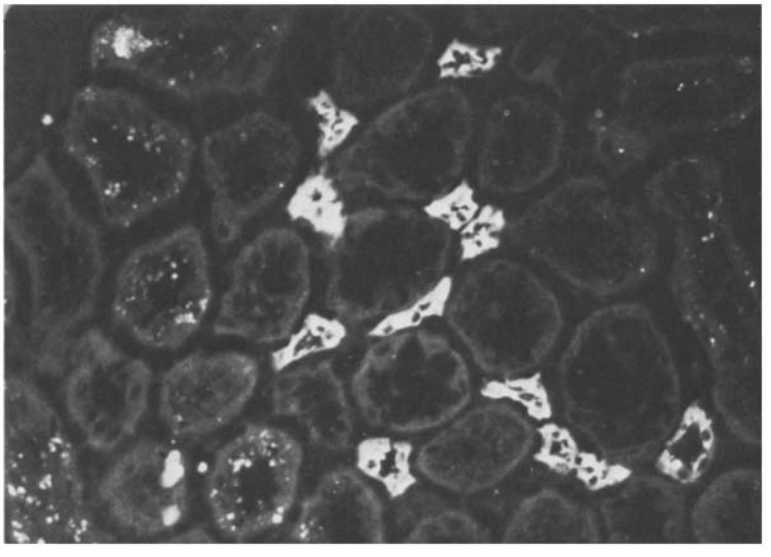

Fig. 4. Immune histological localization of uromucoid in the distal tubules of the rat kidney by fluoresceine isothiocyanate conjugated antibody against rat uromucoid

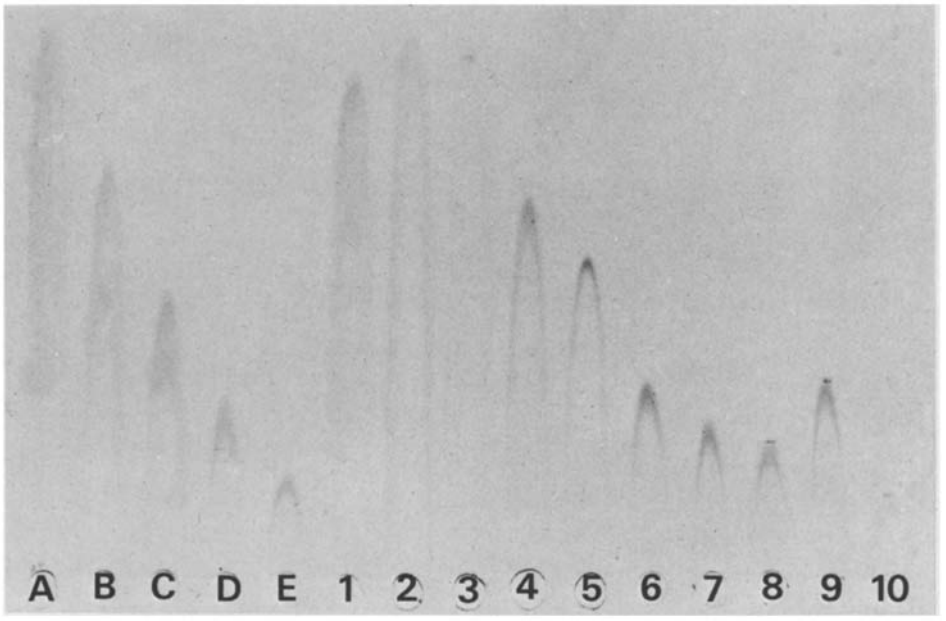

Fig. 5. Quantitative determination of rat uromucoid with the unidimensional Laurell technique to demonstrate the suitability of the prepared antiserum for this method. A-D: Urine standards in geometrical dilution series. A corresponds to 80 µl, B = 40 µl, C = 20 µl, D = 10 µl, E = 5 µl of the urine produced in 24 hours of a single normal rat. 1-10: Uromucoid from 40 µl samples (8 µl 5 x concentrated) of the 24 hour urine of a single rat before (1-3) and after (4-10) an experimental lowering of the uromucoid excretion rate

urine and the developed antiserum (Fig. 3 A), the heaviest of these being uromucoid (U), the other three (1-3) being much lighter. Precipitate 2 could also be obtained from rat serum (Fig. 3 A), as could precipitate 1 in fraction 3 of the rat urine filtration (Fig. 3 B) and precipitate 3 in fraction 4 of its gel filtration (Fig. 3 B). As a result, after absorption of the obtained antiserum with rat serum and fractions 3 and 4, a monospecific antibody against rat uromucoid (Fig. 3 C) could be isolated. The absorbed antiserum showed a weak cross reaction with human urine (Fig. 3 C). The preparation of fluorescein-isothiocyanate (FITC)-conjugated antibodies has already been described in detail elsewhere (4), as has the quantitative determination of uromucoid in human urine using the unidimensional Laurell technique (3). In contrast to human urine, rat urine can be concentrated without the addition of sodium dodecylsulphate (SDS). 0.5 ml antiserum were added to each agarose plate (20 ml gel). Glycin-Tris-barbital mixed buffer, pH 8.8 (1) was used and 8 µl 15 x concentrated rat urine was added to the start point. The current was held at 40 mA for 3 hours. For staining, Comassie Brilliant Blue was used.

RESULTS AND DISCUSSION

Although the cross reactions between rat urine and antihuman uromucoid (4) and between human urine and anti-rat uromucoid (Fig. 3 C) indicate a partial immunological identity which suggests similar configurations, we found evidence that the molecules differ from one another in at least one property. Rat uromucoid was found in fraction 2 of urine gel filtration on Sephadex G 150. This means that the native uromucoid must have a molecule size below the filtration limit of G 150, i.e. does not aggregate from oligomeres as does human uromucoid. We found no further uromucoid in the SDS splitting of fraction 1 after gel filtration. As a result, the addition of SDS to rat urine before preparation was not necessary. On the other hand, human uromucoid with SDS addition and rat uromucoid without SDS addition show the same electrophoretic mobility in agar gel electrophoresis (Fig. 3 C). To date, it has not been possible to relate rat uromucoid to one of the disc electrophoresis bands or to fraction 2 of the gel filtration (Fig. 2).

Immunohistology (Fig. 4) confirms the previous finding that in the rat, uromucoid is localized in the distal tubules and is probably synthesized there (4).

The quantitative immunological determination of rat uromucoid using the unidimensional Laurell technique (Fig. 5) gave an excretion of 197 ± 56 units in 30 rats, i.e. excretion varies by ± 28 %.

Using quantitative uromucoid determination as well as immunohistology, these experiments on the rat have enabled an animal 'model' to be developed on which changes in this acid mucoprotein in the urine can be studied.

This development is of great clinical importance since it enables statements to be made regarding the function of uromucoid with respect to the tubular cells and also its behaviour in the urine. For example, in chronic kidney infections, especially of the tubule region (pyelonephritis), a significantly reduced uromucoid excretion is typical. Fletcher (1972)

is of the opinion that uromucoid forms the glycocalyx of the tubular cells (7). It is possible that a connection exists with electrolyte transport. However, as our immunohistological studies have shown, the whole cell is filled with uromucoid, and this substance may well act as a protection for the tubular cell.

Using these methods for quantitative uromucoid determination in rats, a second series of experiments will be conducted to determine the possible function of uromucoid in both the tubular cell and the urine.

REFERENCES

1. Axelsen, N.H., Kroll, J., Weeke, B.: A Manual of Quantitative Immunoelectrophoresis. Methods and Applications. Oslo: Universitetsforlaget 1975
2. Bichler, K. -H.: Uromucoidausscheidung bei Normalpersonen und Steinbildnern. In: Fortschritte der Urologie und Nephrologie vol. 5. Eds: Vahlensieck, W., Gasser, G., p. 32. Darmstadt: Steinkopff 1975
3. Bichler, K. -H., Haupt, H., Uhlemann, G., Schwick, H.G.: Human uromucoid. I. Quantitative immunoassay. Urological Research 1, 50 (1973)
4. Bichler, K. -H., Kirchner, C.: Immunhistologische Darstellung von Uromukoid in der Niere des Menschen und der Ratte. Urologe 14, 246 (1975)
5. Boyce, W.H.: Proteinuria in kidney calculous disease. In: Proteins in Normal and Pathological Urine. Eds: Manuel, Y., Revillard, J.P., Betuel, H., p. 235. Basel: Karger 1970
6. Davies, B.J.: Disc electrophoresis. II. Method and application to human serum proteins. Annals of the New York Academy of Sciences 121, 404 (1964)
7. Fletcher, A.P.: The tamm and horsfall glycoprotein. In: Glycoproteins. (Ed: Gottschalk, A.) p. 892. Amsterdam: Elsevier 1972
8. Joustra, M., Lundgren, H.: Preparation of freeze-dried monomeric and immunochemically pure IgG by a rapid and reproducible chromatographic technique. In: Protides of the Biological Fluids. Proc. 17. Colloqu. Bruges 1969, 511-515
9. McKenzie, J.K., McQueen, E.G.: Immunefluorescent localization of Tamm-Horsfall mucoprotein in human kidney. J. clin. Path. 22, 334 (1969)
10. Scheidegger, J.J.: Une micro-methode de l'immuoélectrophorèse. International Archives of Allergy and Applied Immunology 7, 103 (1955)

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