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Originals

Uromucoid Excretion in Normal Subjects, Calcium Stone Formers and in Patients with Chronic Renal Failure

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Summary. Using an electroimmunoassay technique for uromucoid in urine, the excretion of this protein has been studied in normal subjects, calcium stone formers and in patients with chronic renal failure. In the normal subjects there was no significant difference in daily excretion between males and females, but a positive correlation with urine volume was demonstrated for this group. No significant difference in daily uromucoid excretion was found between normal and stone forming subjects. In the presence of chronic renal failure uromucoid excretion was found to be reduced and correlated with overall renal function as assessed by creatinine clearance.

Key words: Uromucoid, Urine Volume, Stones, Creatinine Clearance.

The urinary mucoprotein, uromucoid (Tamm-Horsfall mucoprotein), which is derived from the ascending limb of the Loop of Henle and the macula densa region of the distal tubule (10), has been shown by immunological techniques to be a constituent of the protein matrix found in virtually all renal calculi (7, 8). It has also been shown by some workers to bind calcium ions in solution (4) and thus has been suggested as a possible factor in renal stone formation. In addition, stones which appear to consist predominantly of a mucoproteinaceous material are occasionally found (the so-called "matrix stones" (1)) and a link between uromucoid levels and the formation of these bodies may exist.

These observations have led to several studies of uromucoid excretion in the urine of stone formers. Conflicting results have been obtained in both normal and stone forming subjects, some of the discrepancies almost certainly being due to the non-specific nature of some of the assay methods used. However the development of immunological assay techniques for uromucoid (2, 6, 9) with consequent improvements in sensitivity and specificity has made a more reliable investigation of the problem possible.

In this study an electroimmunoassay procedure (2) which has been modified to give greater precision (13) has been used to study uromucoid excretion in normal subjects and in a group of patients with calcium stone disease. The effect of impaired renal function on uromucoid excretion has also been investigated.

MATERIAL AND METHODS

Uromucoid assays were performed on twenty-four hour urine collections from the following groups:

a) Thirty-six apparently healthy members of the hospital staff (24 males; 12 females). Certain members of this group were also used to study possible diurnal variations in uromucoid excretion by collecting four consecutive 6 hour specimens. In addition they were asked to collect 24 hour urines on two or more occasions while deliberately altering their fluid intake and hence urine output.

b) Forty-one adult out-patients (39 males; 2 females) with a recent history of calcium stone disease or with evidence of current calcium stone disease. All had normal values for plasma creatinine and endogenous creatinine clearance and were receiving no drug therapy for their condition. In addition three patients proven to have matrix stones at operation were studied in the post-operative period once haematuria had ceased. In an attempt to minimise the effect of bacterial activi-

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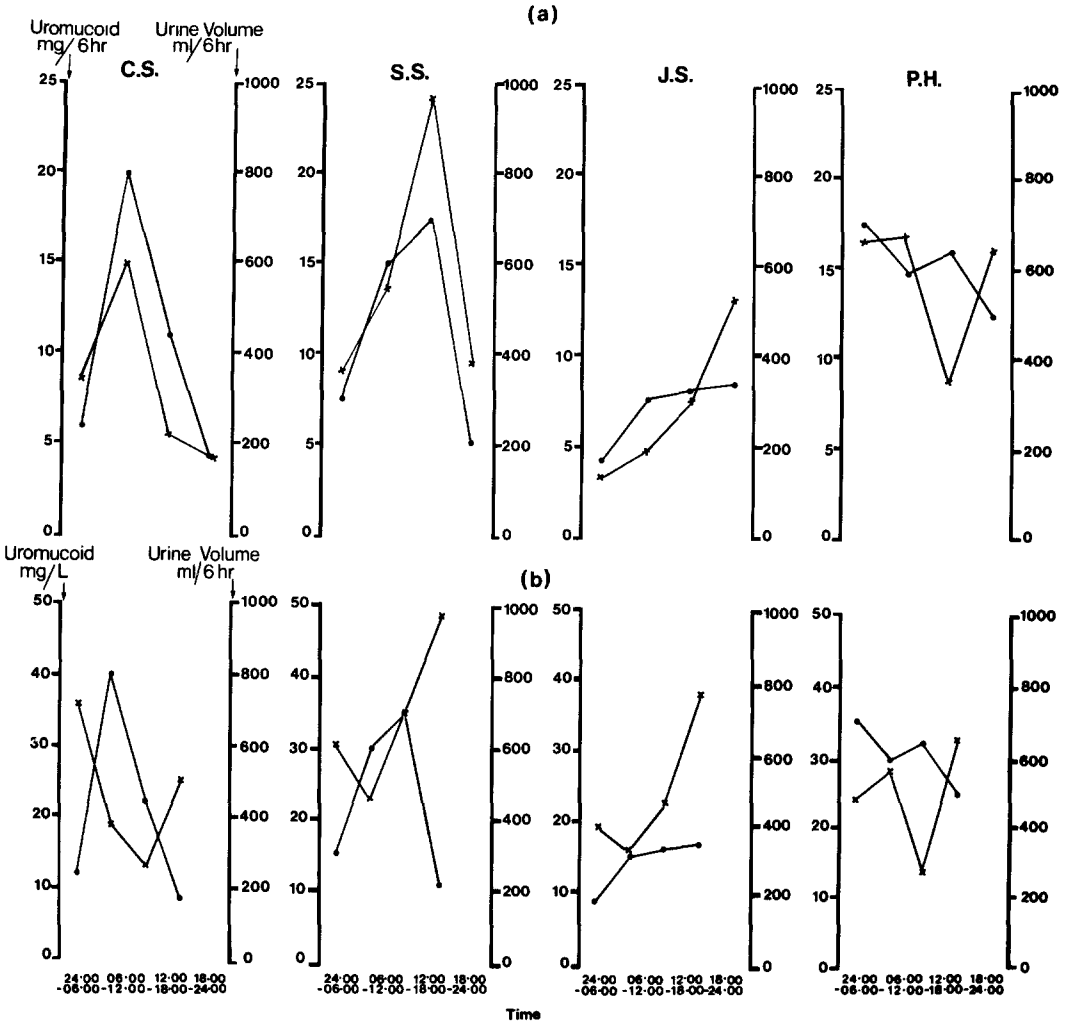

Fig. 1. Data from diurnal rhythm studies in four normal subjects expressing uromucoid as a) six-hourly excretion and b) concentration. ● —— ● = volume: X —— X = uromucoid

ty on the assay (13) these three studies were performed on short timed collections made into ice-cooled containers with preservative.

c) Twenty-nine patients in various stages of chronic renal failure (as assessed by endogenous creatinine clearance). Those with a proteinuria of greater than 0.8 g/l or with current urinary tract infection were excluded because of the assay problems they posed (see later). Patients who had undergone renal transplantation were excluded from the group as a whole but two of the patients received transplants during the study and attempts were made to study these in the post-transplant period.

Uromucoid was estimated using the technique originally described by Bichler et al. (2) but with the modifications introduced by Samuell (13). All daily urine collections were preserved with 2 ml of thymol solution (500 g/l in propan-2-ol) and analysed within 24 hours of collection. Plasma and urine creatinine was estimated using standard alkaline picrate continuous flow techniques.

Mean values are given with ± one standard deviation and the differences between means were assessed by Student's t-test.

RESULTS

a) Normal Subjects

i Diurnal Variation (Fig. 1). Studies on consecutive 6 hour collections from four normal subjects showed no obvious diurnal pattern for uromucoid excretion. Changes in urine volume did not consistently produce the expected opposite changes in

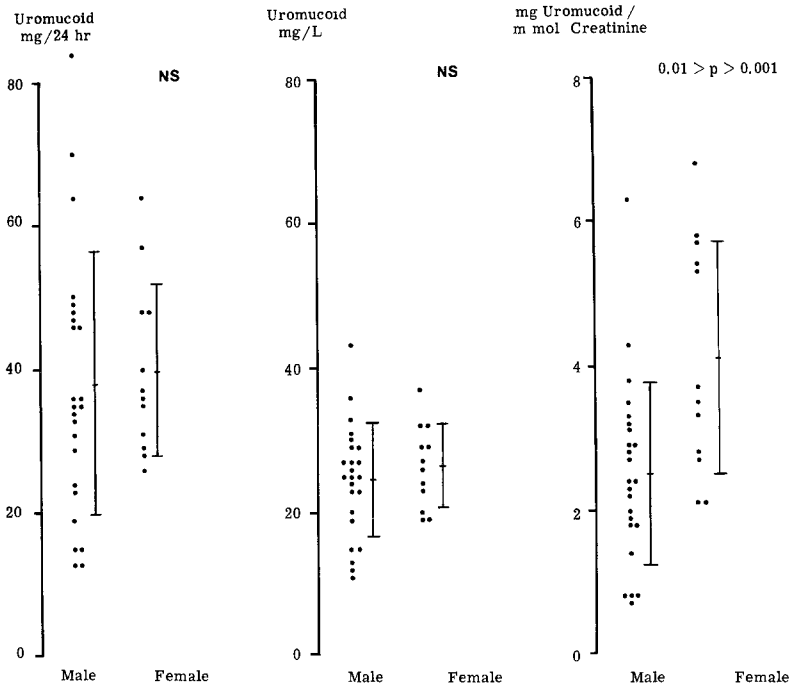

Fig. 2. Uromucoid excretion data for normal subjects. Mean values ± one standard deviation are indicated. N. S. = not significant

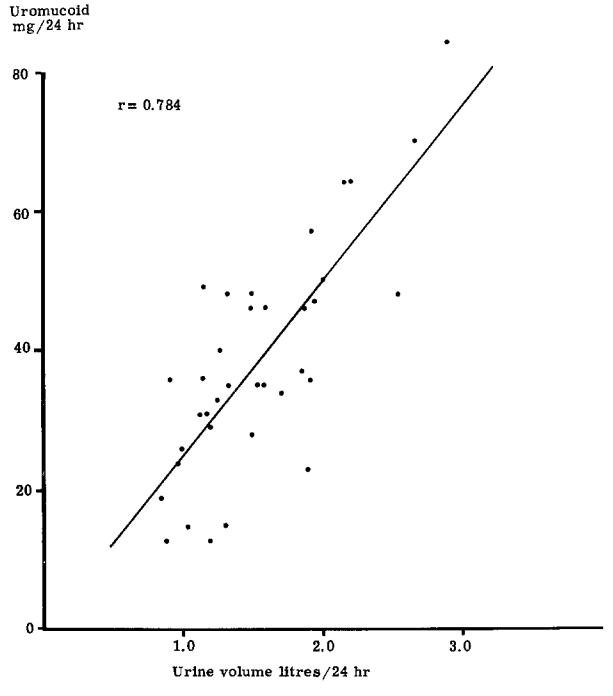

Fig. 3. Relationship between daily uromucoid excretion and urine volume in normal subjects (males and females)

uromucoid concentration and in three subjects there was a strong positive correlation between urine flow and uromucoid excretion (r = 0. 770 for these three subjects and 0. 747 for the whole group of four).

ii 24 hr Collections. The daily uromucoid excretion for the group was 38. 6 ± 16. 3 mg/24 hr with a concentration of 25. 2 ± 7. 1 mg/l. There was no significant sex difference in either case (fig. 2). Excretion expressed in relation to the creatinine level gave a value of 3. 0 ± 1. 6 mg uromucoid/ mmol creatinine but with a significantly higher value in females reflecting their lower mean creatinine excretion (10. 5 ± 3. 2 mmol/24 hr compared to 15. 6 ± 3. 3 mmol/24 hr for males).

iii Relationship to Urine Volume. The results from (ii) above again showed a significant correlation between daily uromucoid excretion and urine volume (fig. 3). Expressing these results in relation to creatinine excretion to obviate any gross collection errors still showed a positive correlation with urine volume in both males and females (fig. 4). Further to this the results of deliberately altering urine output in five normal subjects are shown in Table 1. In all cases there was a positive correlation between uromucoid excretion and the 24 hr urine volume, although the magnitude of the effect varied markedly from subject to subject.

b) Stone-Forming Subjects

The daily uromucoid excretion for the whole group was 44. 7 ± 15. 8 mg/24 hr with a concentration range of 19. 6 ± 7. 7 mg/l or 2. 94 ± 1. 07 expressed as mg uromucoid/mmol creatinine. The data is compared with that from the normal subjects in fig. 5. The only significant finding here was the

lower concentration as mg/l seen in the stone formers. The clear positive correlation with 24 hr urine volume seen in the normal subjects was not shown by this group (fig. 6. r = 0.384). There was a significantly higher mean 24 hr volume ($p < 0.001$) in the stone formers (2.47 ± 1.0 l/24 hr) than in the normal subjects (1.54 ± 0.51 l/24 hr).

Attempts to measure uromucoid in urines collected as described from the three patients with matrix stones proved unsuccessful, unsuitable precipitin peaks being obtained in the assay. Urine infection may have been responsible (13) but the poor renal function in these three subjects (creatinine clearance values of 10, 18, and 16 ml/min respectively) was probably a contributory factor (see later).

Table 1. Changes in uromucoid excretion observed in five normal subjects as the result of deliberate variation in fluid intake and hence urine flow

Subject	Volume l/24 hr	Uromucoid mg/24 hr	Uromucoid mg/l	Creatinine m mol/24 hr	Uromucoid mg/m mol creatinine
S. S.	0.85	5.8	6.8	12.9	0.45
	1.40	29.3	21.0	14.0	2.1
	3.46	56.0	16.2	13.8	4.0
P.H.	1.73	27.3	15.8	13.1	2.1
	6.38	60.3	9.5	12.8	4.7
J.S.	1.64	18.5	11.3	12.0	1.5
	3.42	23.4	6.8	12.0	1.9
R.K.	0.82	20.0	24.3	12.8	1.6
	3.75	34.5	9.2	12.0	2.8
C.S.	1.70	23.2	13.6	16.0	1.5
	3.0	50.4	16.8	13.0	3.8

c) Renal Failure Patients

Attempts to study this group revealed the problems of assaying uromucoid in the presence of significant proteinuria. Urine protein levels of greater than 0.8 g/l produced progressive lowering and distortion of the precipitin peaks (fig. 7). This was thought to be due to possible competitive binding between uromucoid and other proteins for the sodium dodecyl sulphate (S. D. S) used in the assay to cleave the uromucoid macromolecule into immunologically active sub-units (12). This was confirmed by being able to restore normal precipitin peak characteristics by increasing the concentration of S. D. S. used in the assay (fig. 8). However, to vary the S. D. S. concentration depending on the urine protein level was considered too inconvenient since it would mean assaying each urine with its own set of standards prepared in the relevant S. D. S. concentration. This is necessary because precipitin peak height is dependent on S. D. S. concentration even in the absence of protein (2, 13). It was because of this problem that patients with a proteinuria of greater than 0.8 g/l were excluded from the study.

In 12 of the 29 patients with chronic renal failure uromucoid could be detected and assayed normally, while in the remaining 17 the precipi-

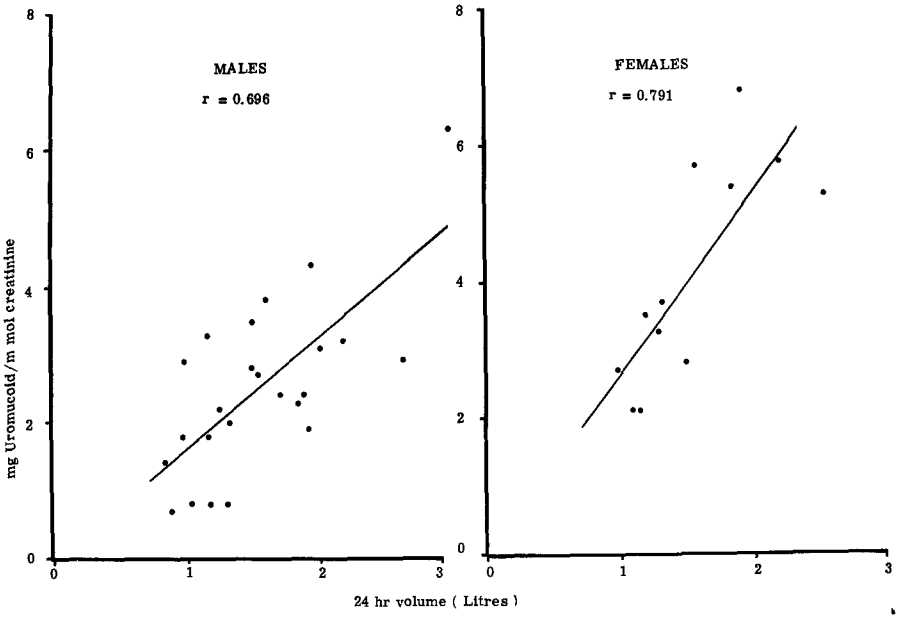

Fig. 4. Relationship between uromucoid excretion as mg/mmol creatinine and urine volume in normal subjects. Results are separated into males and females because of the demonstrated sex difference (Fig. 2)

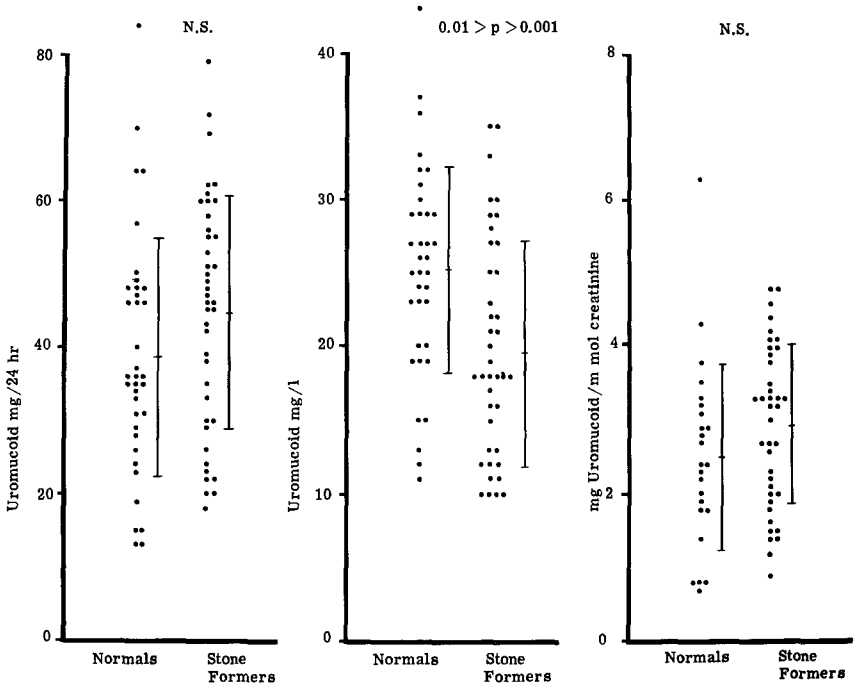

Fig. 5. Comparison between uromucoid excretion in normal subjects and in stoneformers. Only males are included where uromucoid is expressed as mg/mmol creatinine. Mean ± one standard deviation are indicated. N. S. = not significant

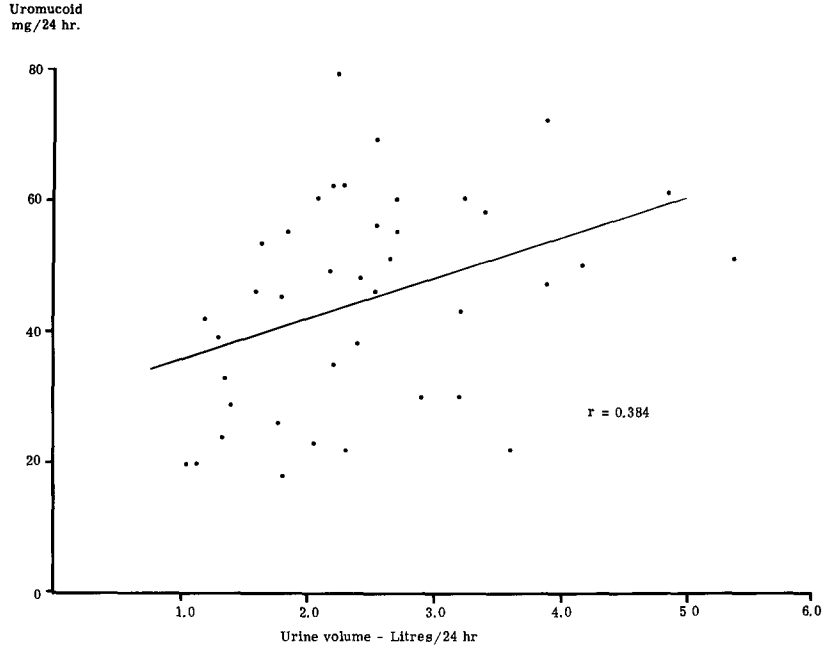

Fig. 6. Relationship between urine volume and daily uromucoid excretion in stone forming subjects

tin peaks formed were either very low, making accurate quantitation impossible, or were absent. The creatinine clearance values in the former group were 51 ± 20.4 ml/min while in the latter group they were significantly lower at 8.0 ± 4.2 ml/min. In the 12 patients with measurable uromucoid the 24 hr excretion correlated positively with both volume and creatinine clearance although more strongly with the former (fig. 9). Urine volumes in the 17 patients in which uromucoid could not be measured were 2.06 ± 0.89 l/24 hr and were not significantly lower ($p = > 0.5$) than in the other 12 patients (1.88 ± 0.88 l/24 hr).

Results for the two patients who received renal transplants are shown in fig. 10. The number of specimens that could be assayed was restricted because of post-operative haematuria. A significant improvement in renal function appeared necessary before measurable uromucoid levels were produced.

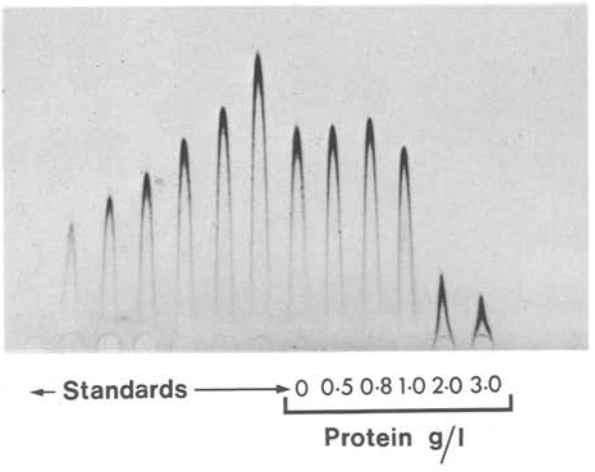

Fig. 7. Electroimmunoassay plate showing effect of urine protein concentration on precipitin peaks produced. Increasing amounts of human albumin were added to a protein free urine prior to assay

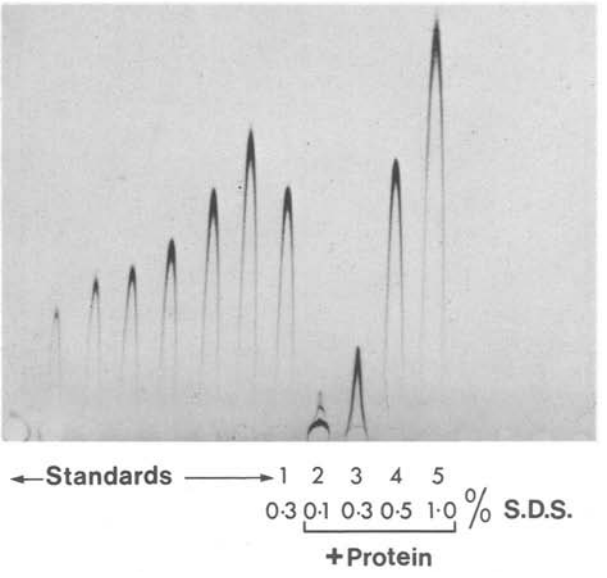

Fig. 8. Electroimmunoassay plate showing the restoration of normal precipitin peaks achieved by increasing the S. D. S. concentration used in the routine assay (peaks 2-5). The protein content was 2. 0 g/l. Peak 1 is the same urine without added protein

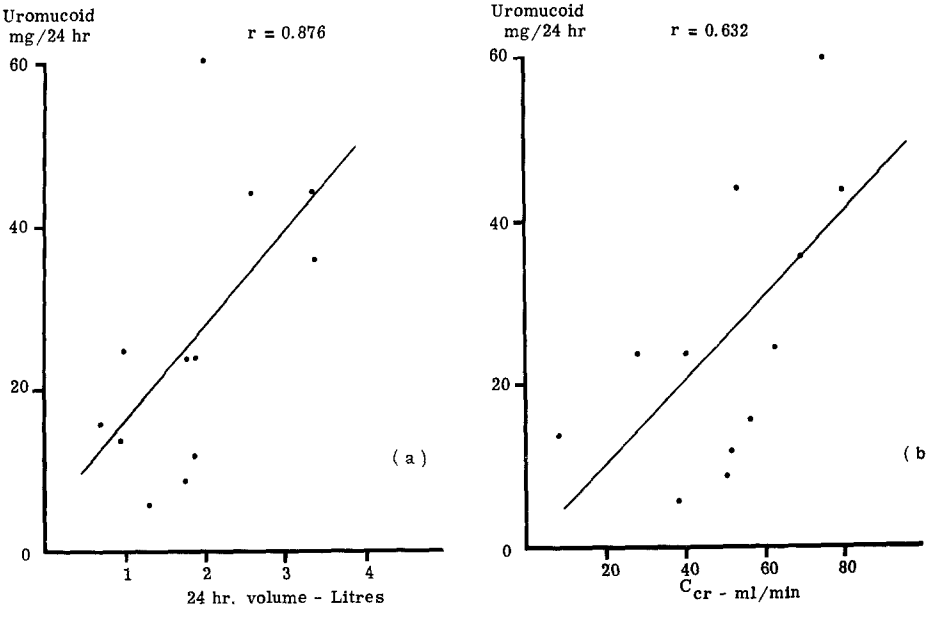

Fig. 9. Daily uromucoid excretion related (a) to volume and (b) to creatinine clearance, in the renal failure patients in which uromucoid could be quantitated

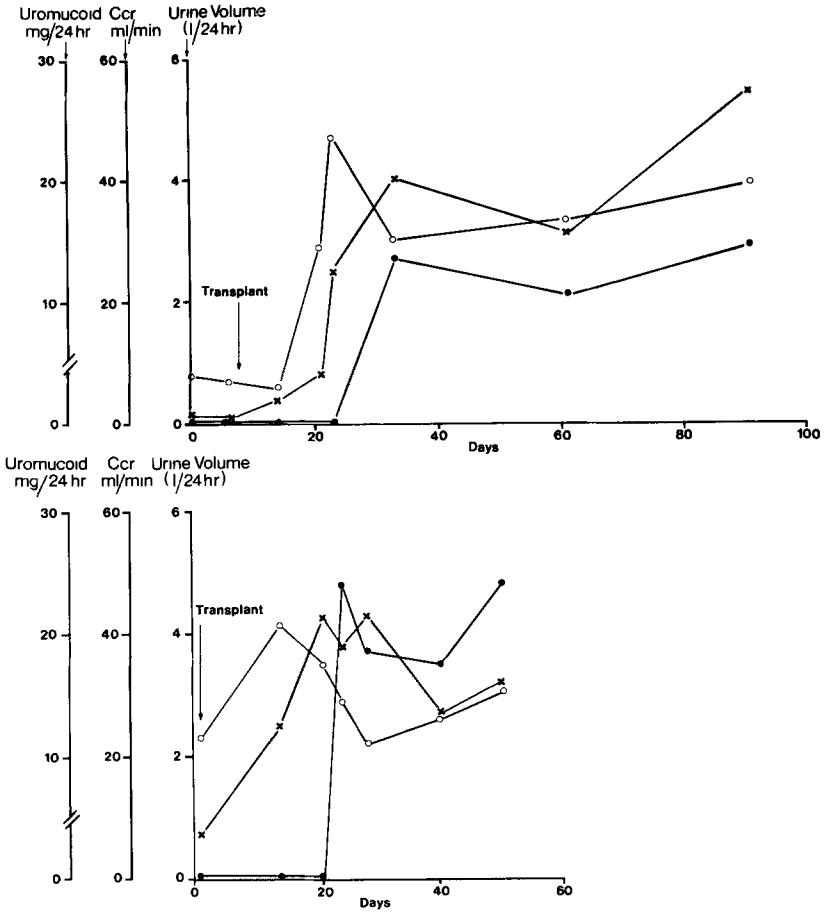

Fig. 10. Changes in uromucoid excretion, urine output and creatinine clearance in two patients who underwent renal transplantation. ● —— ● = uromucoid: X —— X = creatinine clearance: O —— O = urine volume

DISCUSSION

The non-specific nature of the assay techniques used in some of the earlier studies on uromucoid excretion makes comparison with the present study difficult and it is possibly only worthwhile comparing results with those workers who have used immunological quantitation.

The figures for daily uromucoid excretion in normal subjects found here are slightly lower than those reported by Bichler et al. (3) using electroimmunoassay, Mazzuchi et al. (9) using immunodiffusion and Grant and Neuberger (6) using radioimmunoassay. The latter group also reported no significant sex difference in daily excretion which agrees with the present study, while the other groups found a higher excretion in males. None of these workers expressed uromucoid excretion in relation to creatinine levels although in one study (9) the higher excretion found in males was still evident after adjustment for body surface area. This contrasts with the present findings when these are related to creatinine excretion, a higher figure for females being found. The failure to demonstrate any clear circadian rhythm for uromucoid excretion in this study is in agreement with other workers.

The correlation between daily uromucoid excretion and urine volume demonstrated here in normal subjects is not in agreement with the conclusions of Bichler et al. (3) or Grant et al. (5) although the published data from the former group seem to suggest such a relationship. Other groups have certainly reported marked variations in daily uromucoid output but provide no data concerning any change in urine volume.

The lack of any significant difference in daily uromucoid excretion between normal subjects and calcium stone formers in in agreement with Bichler et al. (3) and contrasts sharply with the elevated levels in stone formers reported using non-specific assay techniques. Why daily uromucoid output should correlate well with urine volume in the normal subjects and not in the stone formers is not clear although it may be related to the significantly higher volume range in the latter group. However the data suggests that differences in excretion might exist between the two groups at extremes of the urine output range and it would be interesting to know the effect of lowered urine vol-

ume on uromucoid excretion in the stone formers.

Uromucoid excretion in renal failure seems to have been little studied using immunological techniques, but the reduced levels in chronic renal failure and the positive correlation with creatinine clearance found in this study agree substantially with the findings of Grant et al. using radioimmunoassay (5). An earlier study using a salt precipitation technique (11) produced similar conclusions. Low levels of uromucoid have been reported in the urine of patients with renal tubular acidosis and staghorn calculi (3). While such patients have not been included in the above study, it is felt that the proven unreliability of the electroimmunoassay technique in the presence of infection may have been a contributory factor.

From this study no obvious relationship seems to exist between uromucoid excretion and calcium stone formation. However the findings suggest that a knowledge of both urine output and renal function is necessary when interpreting data concerning uromucoid excretion.

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REFERENCES

1. Allen, T.D., Spence, H.M.: Matrix stones. Transactions of the American Association of Genito-Urinary Surgeons 57, 77 (1965)
2. Bichler, K.H., Haupt, H., Uhlemann, G., Schwick, H.G.: Human uromucoid - quantitative immunoassay. Urological Research 1, 50 (1973)
3. Bichler, K.H., Kirchner, Ch., Ideler, V.: Uromucoid excretion of normal individuals and stone formers. British Journal of Urology 47, 733 (1976)
4. Boyce, W.H., Garvey, F.K., Norfleet, C.M.: Ion-binding properties of electrophoretically homogenous mucoproteins of urine in normal subjects and in patients with renal calculus disease. Journal of Urology 72, 1019 (1954)
5. Grant, A.M.S., Baker, L.R.I., Neuberger, A.: Urinary Tamm-Horsfall glycoprotein in certain kidney diseases and its content in renal and bladder calculi. Clinical Science 44, 377 (1973)
6. Grant, A.M.S., Neuberger, A.: The development of a radioimmunoassay for the measurement of urinary Tamm-Horsfall glycoprotein in the presence of sodium dodecyl sulphate. Clinical Science 44, 163 (1973)
7. Keutel, H.J.: Fractionation of kidney stone matrix. Urologia Internationalis 19, 233 (1965)
8. Keutel, H.J., King, J.S., Boyce, W.H.: Further studies of uromucoid in normal and stone urine. Urologia Internationalis 17, 324 (1964)
9. Mazzuchi, N., Pecarovich, R., Ross, N., Rodriguez, I., Sanguinetti, C.M.: Tamm-Horsfall urinary glycoprotein - quantitation by radial immunodiffusion: Normal patterns. The Journal of Laboratory and Clinical Medicine 84, 771 (1974)
10. McKenzie, J.K., McQueen, E.G.: Immunofluorescent localisation of Tamm-Horsfall mucoprotein in human kidney. Journal of Clinical Pathology 22, 334 (1969)
11. McKenzie, J.K., Patel, R., McQueen, E.G.: The excretion rate of Tamm-Horsfall urinary mucoprotein in normals and in patients with renal disease. Australasian Annals of Medicine 13, 32 (1964)
12. Pitt-Rivers, R., Impiombato, F.S.A.: The binding of sodium dodecyl sulphate to various proteins. Biochemical Journal 109, 825 (1968)
13. Samuell, C.T.: A modified electroimmunoassay technique for uromucoid in urine. Clinica Chimica Acta, in press (1978)

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