SUMMARY

1. The ratio of albumin clearance to creatinine clearance \((C_A/C_C)\) was determined on 129 occasions in forty patients with a wide variety of renal diseases, both 24 h collections of urine and random mid-morning samples being used. The clearance ratios derived from 24 h urine \((24\text{ h }C_A/C_C)\) and random urine \((R\text{ }C_A/C_C)\) were compared with random urine protein concentration, 24 h urine protein excretion and 24 h urine albumin excretion.

2. From these measurements 24 h \(C_A/C_C\) could only be predicted accurately by R \(C_A/C_C\), particularly when patients with postural proteinuria were excluded.

3. The diurnal variation of \(C_A/C_C\) was investigated in twelve patients with renal disease. The individual values for \(C_A/C_C\) obtained throughout the day were within the limits of prediction of 24 h \(C_A/C_C\) already established. The diurnal variation of \(C_A/C_C\) was considerably less than that of \(C_A\) or \(C_C\) alone and also less than that of the rates of protein and albumin excretion.

4. It is suggested that \(C_A/C_C\) is the best readily available measure of ‘glomerular leakiness’ and examples are given of its clinical value. In patients with renal disease 24 h \(C_A/C_C\) could be predicted, in this study, from the clearance ratio based on simultaneous random urine and blood samples.

Key words: proteinuria, albumin, creatinine, renal clearance.

Urinary protein excretion is a standard measurement in the diagnosis and management of patients with renal disease. It is particularly used as an estimate of damage to the glomerular filter although little account is usually taken of the number of functioning glomeruli contributing to the proteinuria or of the concentration of the proteins in the filtered plasma. Few attempts have been made to evaluate measures of protein excretion which take account of these two important factors (Hardwick & Squire, 1955).

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Most routine methods for the measurement of total protein in urine are inaccurate because they rely on turbidimetric comparison with an arbitrary standard, such as plasma protein (Henry, 1966). Moreover the proportions of the constituent urinary proteins may vary widely from those in plasma as a result of their different individual clearances (Joachim, Cameron, Schwartz & Becker, 1964).

The clearances of synthetic macromolecules such as polyvinylpyrrolidone (Hulme & Hardwick, 1968) or dextran (Petrie, MacLean & Robson, 1968), when related to glomerular filtration rate (GFR), give a precise measure of glomerular permeability but are too complex for clinical use. The ratio of simultaneous albumin and creatinine clearances has been suggested as a suitable index (McCrorry, Rapoport & Fleisher, 1959; Barratt, McLaine & Soothill, 1970), although tubular reabsorption of albumin and the discrepancy between creatinine clearance and GFR (Lavender, Hilton & Jones, 1969) prevent it from being an exact measure of glomerular permeability. Its clinical use has so far only been reported in children (McCrorry et al., 1959; Soothill, Barratt & McLaine, 1970).

The present study was undertaken to investigate the value of the albumin/creatinine clearance ratio in the assessment of patients of all ages with renal disease. It was compared with the standard measures of proteinuria: random urine protein content and 24 h urine protein excretion. It was also compared with measures of albuminuria including 24 h urine albumin excretion and albumin clearance. Finally the validity of the albumin/creatinine clearance ratio derived from random urine samples was assessed by comparison with the clearance ratio derived from a 24 h collection and by studies of the diurnal variation of albumin and creatinine clearances.

**PATIENTS AND METHODS**

*Patients*

Clearances based on 24 h urine collections and mid-morning random urine and serum samples were compared on 129 occasions in forty patients with a wide variety of renal parenchymal disease. Serial studies on individuals were included only where there was definite evidence of changing renal function, such as the progression of chronic glomerulonephritis (nine cases) and polyarteritis (one case), the remission of patients with acute glomerulonephritis (one case), pre- eclamptic toxaemia (four cases) and renal vein thrombosis (one case), and the response of foot-process lesion (normal light-microscopy) (four cases) and lupus nephritis (two cases) to steroids. Single studies were performed in eleven cases of chronic glomerulonephritis and in one patient with each of the following diagnoses: lupus nephritis, amyloidosis, nephrosclerosis and renal graft rejection.

Three patients with benign postural proteinuria were studied on twelve occasions while ambulant or recumbent. In one of these patients and in twelve of those with renal disease diurnal studies were performed, in which clearances were calculated from each separate voiding of urine in 24 h and from blood samples taken at 6 h intervals.

The age of the patients ranged from 6 to 65 years, serum creatinine (S_c) from 0.027 to 1.33 mmol/l (0.3-15 mg/100 ml), serum albumin (S_a) from 4.2 to 48.5 g/l (0.42-4.85 g/100 ml), C_c from 1.7 to 221 ml/min and 24 h urine total protein excretion (24 h U_PV) from 0 to 21 g.
Methods

Random urine and serum samples were obtained during the morning immediately after the completion of a 24 h urine collection.

Urine samples were preserved with sodium azide (0·015 mol/l; 1 g/l) and stored at 4°C. Serum samples were stored at -20°C.

Serum and urine albumin. This was measured by the single radial immunodiffusion technique (Mancini, Carbonara & Heremans, 1965) with slight modifications as below. Rabbit anti-human albumin serum (Behringwerke A.G.) was used throughout.

A series of standard solutions (0·04–1·2 g/l) in 0·5 mol/l NaCl was prepared; a pooled serum specimen was used as a secondary standard after calibration against standard human serum (Behringwerke A.G.). Aliquots (1 μl) of standard solutions and appropriately diluted test sera and urine samples were used in the analysis.

Very low urine albumin concentrations, which without dilution gave values lower than the lowest standard, were measured in the same manner except that three applications were made at intervals of 10 min. A range of dilute standards (0·005–0·04 g/l) was treated similarly.

Plates were incubated at room temperature for 24 h, and then dried and stained. Diameters were measured to the nearest 0·5 mm.

Serum and urine creatinine. This was measured by using an alkaline picrate method adapted or the Autoanalyser (Wootton, 1964).

Reproducibility. The between-batch coefficient of variation for serum albumin ($S_A$) and urine albumin ($U_A$) was 7·6% and was 7·5% for the derived $C_A/C_C$.

Urine total protein. This was determined by a turbidimetric technique. A portion (1 ml) of urine (diluted if necessary) was mixed with 3 ml of salicylsulphonic acid solution (30 g/l) and allowed to stand for 10 min. After gentle inversion of the container the turbidity was measured at 618 nm against a blank, in which 154 mmol/l sodium chloride was substituted for salicylsulphonic acid. A diluted pooled serum of known total protein concentration was used as a standard.

Statistical methods. Preliminary graphs and correlation coefficients were obtained with programmes 03D and 05D of the BMD Computer Series (Dixon, 1968). The rank correlation coefficients and linear regression analyses were obtained by an adaptation of the BMD and Plotband computer programs (R. E. Austin, personal communication).

RESULTS

The rank correlations between 24 h $C_A/C_C$ and five other measurements of proteinuria are shown in Table I. The other measurements have factors in common with $C_A/C_C$; this is true even of $R_U$ and 24 h $U_PV$, since albumin is the major urinary protein. The relationship between 24 h $C_A/C_C$ and $R_U$ was clearly affected by changes in urine flow rate.

Fig. 1 shows the distribution of the individual values of $C_A$ and $C_C$ and the lack of a direct relationship between them in this study.

When 24 h $U_PV$ was compared with 24 h $C_A/C_C$ (Fig. 2) it was evident that, even plotted logarithmically, their relationship was not linear, protein excretion rate increasing less at high values of $C_A/C_C$. It could also be seen that there might be 100-fold variation in an estimate of $C_A/C_C$ based on the excretion rate of protein. Little improvement of this estimate could be made by taking 24 h $U_AV$ or $C_A$ in place of 24 h $U_PV$ (Table I). These correlations were
TABLE 1. Rank correlation coefficients of 24 h and random albumin/creatinine clearance ratios with random urine protein, 24 h urine protein and albumin excretion, and albumin clearance

Data from 129 studies in all forty patients are shown in the upper half of the table; data from 117 studies in thirty-seven patients (after exclusion of three patients with postural proteinuria) are shown in the lower half of the table. \( V \) = Urine flow rate (ml/min). \( S_A \) = Serum albumin (g/l). \( S_C \) = Serum creatinine (mmol/l). \( R \) \( U_P \) = Random urine total protein (g/l). 24 h \( U_AV \) = 24 h urine albumin excretion (g). 24 h \( U_PV \) = 24 h urine total protein excretion (g). \( C_A \) = Albumin clearance = \( U_AV/S_A \) (ml/min). \( C_C \) = Creatinine clearance = \( U_CV/S_C \) (ml/min). \( R \) \( C_A/C_C \) = Albumin/creatinine clearance with random urine = \( R \) \( U_A/U_C \times S_C/S_A \). 24 h \( C_A/C_C \) = Albumin/creatinine clearance with 24 h urine = \( 24 \) \( U_A/U_C \times S_C/S_A \).

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<tr>
<th></th>
<th>( R ) ( U_P )</th>
<th>24 h ( U_PV )</th>
<th>24 h ( U_AV )</th>
<th>( C_A )</th>
<th>( R ) ( C_A/C_C )</th>
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<td>24 h ( C_A/C_C )</td>
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<td>0.84</td>
<td>0.83</td>
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<td>( R ) ( C_A/C_C )</td>
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<td>0.83</td>
<td>0.81</td>
<td>0.85</td>
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<td>Patients with postural proteinuria excluded</td>
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<td>24 h ( C_A/C_C )</td>
<td>0.72</td>
<td>0.79</td>
<td>0.77</td>
<td>0.82</td>
<td>0.99</td>
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<tr>
<td>( R ) ( C_A/C_C )</td>
<td>0.73</td>
<td>0.79</td>
<td>0.77</td>
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**FIG. 1.** Comparison of albumin clearance with creatinine clearance in 129 studies in forty patients. Logarithmic scales.
not improved by excluding the twelve studies in the three patients with postural proteinuria.

The relationship between 24 h C_A/C_C and R C_A/C_C is shown in Fig. 3. It can be seen that the clearance ratio derived from a random urine was closely related to that derived from a complete 24 h collection. When the patients with postural proteinuria were excluded the correlation of these parameters increased. However, it should be noted that a given value for R C_A/C_C is compatible with at least a twofold variation in 24 h C_A/C_C. Both 24 h C_A/C_C and R C_A/C_C have the factor S_C/S_A in common. Therefore 24 h U_A/U_C and R U_A/U_C were also compared and were also found to be very closely correlated (rank correlation coefficient 0·98 whether patients with postural proteinuria were included or not).

Fourteen studies of the diurnal variation of C_A/C_C were performed. In one patient with postural proteinuria all the values obtained during recumbency were within the 95% confidence limits of the prediction of 24 h C_A/C_C from the mid-morning R C_A/C_C derived from the 117 studies in patients with definite renal pathology. During a normal day’s mobilization this patient showed a striking discrepancy between random and 24 h clearance ratios. In the other twelve patients all the individual values for C_A/C_C obtained throughout a normally active day were within the limits of prediction of 24 h C_A/C_C already established (Fig. 3). In these twelve patients it was found that the diurnal variation of C_A/C_C was considerably less than that of the albumin and creatinine clearances alone (mean coefficients of variation: C_A, 52%; C_C, 46%; C_A/C_C, 17%) and also less than the diurnal variation of the other indices of proteinuria.
DISCUSSION

In this study excellent agreement was found between $R \frac{C_A}{C_C}$ and 24 h $\frac{C_A}{C_C}$, particularly when patients with benign postural proteinuria were excluded. In patients with definite renal disease the diurnal variation of $\frac{C_A}{C_C}$ was small despite appreciable variation in $C_A$ itself and in the other measurements of protein excretion. The initial investigation of all patients with unexplained proteinuria is usually to determine whether it can be related to posture. It would therefore seem reasonable to use $R \frac{C_A}{C_C}$ in place of 24 h $\frac{C_A}{C_C}$ as an index of 'glomerular leakiness' in longitudinal studies where the diagnosis of definite renal disease has been made. The clearance ratio derived from random urine and serum samples has the advantage of being available from a single out-patient visit, thus avoiding both the delay and inconvenience of 24 h urine collection.

The albumin/creatinine clearance ratio could not be predicted in this study from classical measures of proteinuria, including random urine protein and 24 h urine protein excretion. Nor could it be accurately predicted from the 24 h urine albumin excretion or albumin clearance. There was no clear relationship between the albumin clearance and creatinine clearance alone.
Protein excretion rate may be an inadequate measure of 'glomerular leakiness' because it may be independently affected by improvement or deterioration in the GFR, and may also be affected by the plasma protein concentration (as shown by the non-linearity of the relationship between 24 h U_pV and 24 h C_A/C_C in Fig. 2). Also, drugs which are used in the treatment of patients with renal disease have effects on GFR and plasma protein concentration independent of their effect on 'glomerular leakiness' (McMahon, Gordon, Kenoyer & Keil, 1960).

The use of albumin/creatinine clearance ratios in longitudinal studies is exemplified in Figs. 4 and 5. In the patient with acute proliferative glomerulonephritis (Fig. 4), the initial increase in serum albumin and decrease in protein excretion could have been due to a fall in GFR, shown by the falling creatinine clearance. The progressive decrease in albumin/creatinine clearance ratio demonstrated that there was an improvement in 'glomerular leakiness' substantiated by his eventual recovery. In a case of lupus nephritis (Fig. 5) the urine protein excretion increased markedly at 1 month and at 5 months. On the first occasion this gave a spurious impression of increasing 'glomerular leakiness' and on the second it was associated with increased 'glomerular leakiness'. On both occasions there was an increase in GFR,

**Figure 4.** Longitudinal study in patient with acute proliferative glomerulonephritis. Serum albumin, creatinine clearance, 24 h urine protein excretion and 24 h albumin/creatinine clearance ratio during 8 months after the onset of the disease are shown.
shown by a rising creatinine clearance. This could have accounted for the increasing proteinuria without any change in 'glomerular leakiness'. The change in creatinine clearance might have been due to a genuine improvement in renal disease or, initially, to the independent effect of prednisone in increasing GFR. In these two patients, as in the others, the random value of the ratio corresponded closely with the clearance ratio based on a 24 h urine collection. Thus 24 h urine collection could have been omitted.

The main failings of the albumin/creatinine clearance ratio as a measure of 'glomerular leakiness' are that it is based on relatively imprecise measures of glomerular permeability and GFR. Because of tubular reabsorption of albumin (Dirks, Clapp & Berliner, 1964) it could be particularly misleading when minor glomerular damage produces a small increase in true glomerular permeability without a change in GFR. When there is a larger glomerular leak per surviving nephron tubular reabsorption of protein is presumably saturated and the albumin/
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creatinine clearance ratio should give a better approximation of the true glomerular handling of albumin.

Endogenous creatinine clearance does not accurately parallel GFR in man; indeed it may overestimate it by as much as a factor of 2 (Lavender et al., 1969). This error is, however, small in comparison with the logarithmic changes in albumin clearance. Contrary to previous reports, the discrepancy between creatinine clearance and GFR is not related to proteinuria (Hilton, Lavender, Roth & Jones, 1969).

Lauson, Forman, McNamara, Mattar & Barnett (1954) suggested the albumin/inulin clearance ratio as an index of the minimum glomerular permeability to albumin. Because of difficulties in measurement of albumin, the clearance of a dye (T-1824) was substituted. Presumably this method did not gain wide acceptance because it required a continuous infusion technique. McCrory et al. (1959) suggested the albumin/creatinine clearance ratio as an index of the response of nephrotic children to steroids, with an electrophoretic method used to measure albumin. Barratt et al. (1970) studied the relationship between albumin/creatinine clearance ratio and urine albumin/creatinine ratio in children, using a more precise immunological determination of albumin, as in the present study. They showed that these indices were at least as accurate as standard measures of proteinuria in predicting glomerular damage. They did not make a systematic study of the relationship between 24 h and random albumin/creatinine clearance ratios. However, the present study corroborates their suggestion that random albumin/creatinine clearance ratio is an ideal clinical measure of glomerular leakiness. The same group (Soothill et al., 1970) reported the use of this parameter in a controlled trial of immunosuppressive agents in children with the nephrotic syndrome.

The present study suggests that the albumin/creatinine clearance ratio is suitable as an index of 'glomerular leakiness' in renal disease in all age groups. The samples are easy to collect and the estimations are relatively simple and precise. Automated nephelometry (Larson, Orenstein & Ritchie, 1971) could increase the speed and convenience of the test.

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REFERENCES


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