Albumin Excretion as a Measure of Glomerular Dysfunction in Children

T. M. BARRATT, P. N. McLaine,* and J. F. SOOTHIll
From the Department of Immunology, Institute of Child Health, London

Barratt, T. M., McLaine, P. N., and Soothill, J. F. (1970). Archives of Disease in Childhood, 45, 496. Albumin excretion as a measure of glomerular dysfunction in children. The urine albumin/creatinine concentration ratio (UA/UC) and the albumin excretion rate per unit weight (UA/V/Wt) have been compared with the theoretically ideal parameter for measuring glomerular damage, clearance of albumin/clearance of creatinine (CA/Cc), using a simple sensitive immunochemical technique for albumin. It is shown that UA/UC on random urine specimens can be as satisfactorily used to predict CA/Cc as UA/V/Wt. Normal data over a wide range of body size of this simple parameter are presented; higher values in the newborn reflect increased permeability of the neonatal glomerulus.

Semiquantitative estimates of urine protein concentration and more precise measurements of protein excretion rates are widely used in the diagnosis and assessment of renal disease, but the choice of the most appropriate parameter has received surprisingly little critical appraisal. The glomerulus is a filter which, when damaged, may become blocked, leaky, or both. Measurements of glomerular filtration rate (GFR) have received greater attention because reduced GFR is the abnormality which usually kills. However, considerable glomerular disease may be present without abnormality of this function, but this is rarely, if ever, true of leakiness. Measurement of leakiness provides a valuable means, not only of detecting glomerular disease in an early stage, but also of evaluating its natural history and response to therapy. In addition, leakiness may itself lead to serious symptomatic disease: the nephrotic syndrome.

Symbols and Units

The following symbols and units are used throughout this paper:

- GFR = glomerular filtration rate, ml./min.
- PA = plasma albumin concentration, mg./ml.
- PC = plasma creatinine, mg./ml.
- UA = urine albumin concentration, mg./ml.
- UC = urine creatinine concentration, mg./ml.
- V = urine flow rate, ml./min.
- CA = UA/PC = albumin clearance, ml./min.
- CC = UC/PC = creatinine clearance, ml./min.
- Wt = body weight, kg.

Most quantitative studies of proteinuria have relied upon estimates of protein excretion in 24-hour urine samples, but this cumbersome technique is known to be subject to considerable collection errors, particularly in children. Though it accurately represents the loss of protein, which may be the required parameter for metabolic studies, it is an imperfect measure of the renal handling of a particular macromolecule, such as albumin, because of the heterogeneity of plasma proteins, the concentrations of which vary independently in the presence of proteinuria. The albumin clearance (CA) is theoretically a better measure of this, and is easily measured immunochemically. Blockage of glomeruli (of which creatinine clearance (CC) is an acceptable, though not perfect, estimate) may occur at the same time, and thus reduce the leak. Therefore, clearance of albumin/clearance of creatinine (CA/CC) represents nearly the ideal measure of glomerular permeability, and has the added advantage that timed urine collections are not required, though it may be optimal to collect a 24-hour urine to eliminate diurnal variation. All such parameters, based on albumin excretion, discount tubular handling of protein; this is probably avoidable only by study of infused non-protein macromolecules (Hulme and Hardwicke, 1968).
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The necessity to monitor protein excretion frequently in nephrotic children in trials of immunosuppressive agents (Soothill, Barratt, and McLaine, 1970) led us to study the parameter urine albumin/creatinine concentration ratio \((U_A/U_C)\) on random urine samples. The paper describes our experience with this parameter and its relation to the most rational measure of glomerular permeability \(C_A/C_C\) (24 hour) as well as to the more traditional urine albumin excretion per unit body weight \((U_AV/Wt\) (24 hour)).

Materials and Methods

Capillary blood samples, collected by fingerprick and anticoagulated with lithium heparin, were separated and stored at \(+4^\circ\)C. Urine samples were preserved with thimerosal and stored at \(-20^\circ\)C. Random urine samples were collected in the morning, but were not necessarily the first specimens passed in the day.

Albumin was measured by a modification of the single diffusion technique (Mancini, Carbonara, and Heremans, 1965; Fahey and McKelvey, 1965). Antisera were raised in rabbits using albumin precipitated reconstituted freeze-dried electrophoretically pure human albumin (RHA 05; Behringwerke, A.G., Marburg-Lahn, Germany) as antigen. After absorption with \(\gamma\)-globulin (fraction G4; Blood Products Laboratory, Lister Institute, Elstree, Hertfordshire, England) only one precipitin line on immunoelectrophoresis with whole human serum was observed.

Freeze-dried pooled human serum was used as standard. This was calibrated in mg./ml. albumin by comparison with the Behringwerke A.G. albumin preparation. The assignation of this value is, of course, subject to many uncertainties, but this does not invalidate comparisons between separate estimates of albumin concentration.

In some urine samples of low albumin concentration, the double diffusion method was used (Soothill, 1962). Satisfactory correlation of the two methods within the range of albumin concentration 0.005-0.05 mg./ml. was observed:

\[
y = 0.74x + 0.35 \pm 0.15^* (r = 0.85; n = 16)
\]

where \(y = \log_{10} U_A\) (single diffusion) and \(x = \log_{10} U_A\) (double diffusion).

Urinary creatinine was estimated by an automated modification of the alkaline picrate method (Technicon Autoanalyser Methodology N-116). Plasma creatinine was estimated by a similar manual method after resin absorption (Stoten, 1968).

\(U_A/U_C\) was calculated as the concentration ratio of albumin and creatinine, both expressed as mg./ml. The coefficients of variation of seven replicate estimates in the same batch were: \(U_A \pm 5\%\) (single diffusion); \(U_C \pm 2\%\); \(U_A/U_C \pm 6\%\). The corresponding figures for replicate estimates in separate batches were: \(U_A \pm 16\%\); \(U_C \pm 3\%\); \(U_A/U_C \pm 14\%\).

The data were apparently consistent with a log-normal distribution. The means, ranges (\(\pm 2\) SD), and correlations were therefore calculated on logged data. Calculations of coefficients of variation were performed on unlogged data.

Results

\(U_A/U_C\) (random) was measured on random urine samples obtained from 58 individuals aged 3 days to 40 years who had no evidence of renal disease. In Fig. 1, \(U_A/U_C\) (random) is plotted as a function of body weight, and the regression equation is:

\[
\log_{10} U_A/U_C (\text{random}) = -0.52 \log_{10} Wt
\]

\(=-0.79 \pm 0.30\* (r = -0.63, p < 0.001)\).

Fig. 1.—The normal range of \(U_A/U_C\) (random) related to body weight. The correlation is significant \((r = -0.63, p < 0.001)\) and the 95% confidence limits are shown.

The negative correlation is significant; thus \(U_A/U_C\) (random) is higher in smaller children.

Means and range (\(\pm 2\) SD) of \(U_A\), \(U_C\), \(P_A\), \(P_C\), \(U_A/U_C\) (random), and \(C_A/C_C\) (random) in 8 healthy neonates, between the ages of 4 and 8 days, and for 16 healthy adults are given in Table I. Though there is no difference in \(U_A\) between the neonates and the adults, both \(U_A/U_C\) (random) and \(C_A/C_C\) (random) are approximately four times as high in the neonate, the differences being highly significant.

In assessing \(U_A/U_C\) as a potential simple sub-
TABLE I
Parameters of Albumin and Creatinine Excretion in Healthy Adults and Neonates

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Adults (n = 16)</th>
<th>Neonates (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UA (mg./ml.)</td>
<td>0.017 (0.0056-0.051)</td>
<td>0.017 (0.0063-0.044)</td>
</tr>
<tr>
<td>UC (mg./ml.)</td>
<td>0.87 (0.31-2.40)</td>
<td>0.17 (0.075-0.40)*</td>
</tr>
<tr>
<td>PA (mg./ml.)</td>
<td>48 (34-66)</td>
<td>40 (36-45)*</td>
</tr>
<tr>
<td>PC (mg./ml.)</td>
<td>0.0087 (0.0063-0.012)</td>
<td>0.0043 (0.0028-0.0065)*</td>
</tr>
<tr>
<td>UA/UC</td>
<td>0.019 (0.007-0.049)</td>
<td>0.078 (0.015-0.39)*</td>
</tr>
<tr>
<td>CA/CC</td>
<td>3.3 (1.3-8.7)</td>
<td>11 (2.8-40)*</td>
</tr>
</tbody>
</table>

The ranges given are the means ± 2 SD calculated on logged data.
*Significantly different from the adults (p < 0.05).

Substitute for the theoretically preferable CA/CC (24 hour) as a measure of glomerular permeability, the confidence limits of the prediction of this parameter from UA/UC (random) were assessed (Fig. 2). 71 blood samples and 24-hour urine collections were obtained from 8 children known to be reliable urine collectors with the nephrotic syndrome in relapse or remission. During the 24-hour collection period, one urine sample was taken for the estimation of UA/UC (random). There is a satisfactory correlation between CA/CC (24 hour) and UA/UC (random) (Fig. 2; Table II). The slope is significantly greater than unity because high values of UA/UC (random) are associated with low values of PA. The correlation coefficient between CA/CC (24 hour) and UA/UC (random) was higher than that between CA/CC (24 hour) and UA/V/Wt (24 hour), though the difference was not significant. This supports the view that the simple test of UA/UC (random) has at least as good a predictive value of CA/CC (24 hour) as has the traditional 24-hour albumin excretion, even in selective co-operative patients, in whom error in V is minimal.

Diurnal variation of UA/UC (random) was examined in 3 nephrotic children in a stable state of relapse. UA and UC were measured on each sample passed in a 24-hour period. No consistent diurnal pattern was observed, but the coefficients of variation of UA/UC (random) were 22%, 28%, and 12%, all are significantly greater than the experimental error (±6%) and indicate some temporal variation.

The enormous range of values of the different

TABLE II
Parameters, Standard Error of Estimate, and Correlation Coefficient of Regression Equation y = ax + b.  
(Sy = standard error of the estimate of y from x)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>x</th>
<th>y</th>
<th>a</th>
<th>b</th>
<th>Sy</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>log10 CA/CC (24 hour)</td>
<td>log10 UA/UC (random)</td>
<td>1.23</td>
<td>-3.99</td>
<td>0.36</td>
<td>0.97</td>
</tr>
<tr>
<td>2</td>
<td>log10 UA/UC (24 hour)</td>
<td>log10 UA/V/Wt (24 hour)</td>
<td>1.13</td>
<td>-2.09</td>
<td>0.48</td>
<td>0.94</td>
</tr>
<tr>
<td>3</td>
<td>log10 UA/V/Wt (24 hour)</td>
<td>log10 UA/UC (random)</td>
<td>1.01</td>
<td>-1.68</td>
<td>0.35</td>
<td>0.96</td>
</tr>
</tbody>
</table>

n = 71; t1 is not significantly greater than t2 (0.10 > p > 0.05)
parameters of albumin excretion and their capacity for dramatic change are illustrated in Fig. 3, which represents the response of a child with the nephrotic syndrome to cyclophosphamide. Clearly, under these circumstances it is of minor importance which parameter is chosen for study, for changes in \( U_A \) far exceed those in \( U_C, P_A, \) or \( P_C \). In other circumstances, reliance on \( U_A \) alone would be misleading. A child with a steroid-resistant nephrotic syndrome was studied during a diuresis induced by frusemide: \( U_A \) fell from 6.8 to 0.95 mg./ml, but \( U_A/U_C \) only changed from 10.7 to 6.8 because of a parallel fall in \( U_C \) from 0.63 to 0.14 mg./ml.

![Image](http://adc.bmj.com/...)

**Fig. 3.—Changes in the parameters studied in a boy with the nephrotic syndrome associated with minimal renal histological abnormality during response to cyclophosphamide.**

There are situations in which the choice of the appropriate parameter of albuminuria is critical to the correct interpretation of the changes in glomerular permeability. Fig. 4 illustrates a sixfold fall in \( C_A/C_C \) during cyclophosphamide treatment of a child with glomerulonephritis and nephrotic syndrome which is associated with a rise in \( P_A \) in \( C_A/Wt \), but no change in \( U_A/V/Wt \). A child with the congenital nephrotic syndrome unresponsive to treatment (Fig. 5) shows a fall in \( C_A/Wt, C_U/Wt, \) and \( U_A/V/Wt \) and a rise in \( P_A \), while \( C_A/C_C \) is relatively unchanged. In these two circumstances, changes in \( U_A/U_C \) only partially reflect changes in \( C_A/C_C \) and any parameter other than \( C_A/C_C \) would be misleading.

**Discussion**

The properties of the glomeruli as filters of macromolecules and the role of the tubules in protein reabsorption have been recently reviewed (Hardwicke et al., 1970). Substances as large as insulin pass the glomerular membrane as readily as water and their concentration in Bowman's capsule fluid is the same as in plasma water (Richards, 1938), but albumin is almost completely retained; micropuncture techniques have shown an albumin concentration of less than 0.025 mg./ml. in early proximal tubular fluid in most dogs (Dirks, Clapp, and Berliner, 1964). Permeability studies (Hulme and Hardwicke, 1968) with polydispersed synthetic macromolecules fit the mathematical description of Landis and Pappenheimer (1963) of an isoporous membrane, and suggest an effective pore size for macromolecules which would just retain albumin.
Glomerular disease is associated with abnormal function consistent with the superimposition of one or more populations of pores of larger size (Hulme and Hardwicke, 1968). Such a description may well be a simile rather than anatomical reality, but it is clear that leakiness of glomeruli in disease is best described by the comparison of the passage of molecules which should just be retained with that of water. The relative clearances of infused inert macromolecules of suitable sizes would provide the ideal description of glomerular permeability, but are not practical for routine clinical use. The validity of measurement of excretion of a protein as a measure of glomerular function is limited by tubular reabsorption, the amount of which is still unknown. Apart from this, albumin, which is measurable by a simple sensitive immunochemical technique in normal urine, is ideal as the substance which should be just retained.

The clearance of creatinine is known to overestimate GFR in man (Shannon, 1935), particularly in the presence of heavy proteinuria (Berlyne et al., 1964), but the errors arising from this are relatively small compared with the enormous range of excretion of albumin in renal disease, and its great practical advantage of relatively constant endogenous production outweighs the disadvantages for repeated clinical measurement. \( C_A/C_C \) therefore represents the rational parameter for measuring glomerular damage against which other measures may be compared; this ratio has been suggested before (McCory, Rapport, and Fleisher, 1959) but has not been appraised in detail.

Ignoring the contribution of tubular function to albumin and creatinine excretion, \( C_A/C_C \) measures the albumin concentration gradient across the glomerular membrane:

- **Filtered albumin** = \( U_A/V \)
- **Filtered water** (GFR) = \( U_C/V/P_c \)

\[ \begin{align*}
  & \text{Albumin concentration in glomerular filtrate} \\
  & = \frac{U_A}{V} \\
  & \times \frac{U_C}{P_c} \\
  & = C_A/C_C
\end{align*} \]

It is apparent that \( C_A/C_C \) has the great practical advantage that, as the \( V \) term cancels, timed urines are not required. \( U_A/U_C \) will correlate with \( C_A/C_C \) except in so far as \( P_A \) or \( P_c \) vary:

\[ C_A/C_C = \frac{U_A}{U_C} \times \frac{P_c}{P_A} \]

Changes in plasma concentrations are usually of a much smaller magnitude than changes in urine concentrations, but are not negligible, as shown in Fig. 4. \( U_A/U_C \) would, in fact, be expected to correlate better with \( C_A/C_C \) than would \( U_C/V/Wt \), for the further variable \( U_C/V/Wt \) (which includes urine collection errors) is introduced into the equation:

\[ C_A/C_C = \frac{U_A}{U_C} \times \frac{P_c}{P_A} \times \frac{Wt}{Wt} \]

The data presented in Table II show that \( U_A/U_C \) (random) provides as reliable a prediction of \( C_A/C_C \) (24 hour) as does \( U_C/V/Wt \) (24 hour), in spite of some temporal \( U_A/U_C \) variation in the random samples. The use of a creatinine correction to avoid the necessity for timed urine collections is, of course, not new, but has not, to our knowledge, been systematically applied to protein excretion. It has, for example, been used in studies of calcium (Nordin, 1959) and purine (Kaufman, Greene, and Seegmiller, 1968) excretion.

A conceptually separate reason for measuring albumin excretion is the study of the metabolic effects of its loss and the relation of this loss to synthesis, on which the serum albumin concentration, and therefore the symptoms of the nephrotic syndrome, depend (Squire, Blainey, and Hardwicke, 1957). Here \( U_A/V/Wt \) is probably the most appropriate parameter, and \( U_A/U_C \) will correlate...
that in approximately 1962): (Behrendt, 1962):

\[
U_C V/Wt \approx 0.01 \text{ mg.}./\text{kg. per min.}
\]

\[
\cdot U_A V/Wt \text{ (mg.}./\text{kg. per min)} \\approx

U_A/U_C \text{ (mg.}./\text{mg.}) \times 0.01.
\]

On this basis a value for \( U_A/U_C \) of 1.0 is approximately equivalent to an albumin excretion of 15 mg./kg. per day, i.e. 1 g./day in the 70 kg. adult.

Calculation from Table II (equation 3) suggests that in our children a value of \( U_A/U_C \) (random) of 1.0 was approximately equivalent to \( U_A V/Wt \) (24 hour) of 30 mg./kg. per day. This discrepancy is partly due to the fact that the majority of the children in this study had creatinine excretion rates greater than 0.01 mg./kg. per min.

Table I illustrates that \( C_A/C_C \) is greater in the healthy newborn than in the healthy adult. The data imply increased glomerular filtration of albumin, decreased tubular reabsorption, or both. The latter is unlikely since the clearance of lysozyme (a low molecular weight protein normally reabsorbed by the tubules) relative to that of creatinine is similar in neonates and adults (Barratt and Crawford, 1970), suggesting that the function of tubular protein reabsorption is relatively mature in the neonate. The increased values of \( C_A/C_C \) presumably therefore reflect increased permeability of the neonatal glomerulus, a conclusion which could not have been reached from a study of urine albumin concentrations alone.

The advantage, particularly to the paediatrician, of a parameter which eliminates the need for both timed urine and blood collections, and at the same time compensates for body size is readily apparent. The immunochemical gel diffusion technique for measuring albumin concentration requires the minimum of equipment, and antisera are available commercially. Urine creatinine estimations are no problem to the automated biochemistry laboratory. The albumin/creatinine concentration ratio offers both simplicity and precision in the monitoring of renal disease. Now that renal disease is amenable to a considerable number of possible lines of treatment, their economic appraisal becomes important, and this parameter is proving valuable in this role (Soothill et al., 1970).

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References


Correspondence to Dr. T. M. Barratt, Department of Immunology, Institute of Child Health, 50 Guilford Street, London W.C.1.