

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 (U_A/U_C) and the albumin excretion rate per unit weight ($U_A V/Wt$) have been compared with the theoretically ideal parameter for measuring glomerular damage, clearance of albumin/clearance of creatinine (C_A/C_C), using a simple sensitive immunochemical technique for albumin. It is shown that U_A/U_C on random urine specimens can be as satisfactorily used to predict C_A/C_C as $U_A V/Wt$. 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.
- P_A = plasma albumin concentration, mg./ml.
- P_C = plasma creatinine, mg./ml.
- U_A = urine albumin concentration, mg./ml.
- U_C = urine creatinine concentration, mg./ml.

- V = urine flow rate, ml./min.
- C_A = $U_A V/P_A$ = albumin clearance, ml./min.
- C_C = $U_C V/P_C$ = 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 (C_A) is theoretically a better measure of this, and is easily measured immunochemically. Blockage of glomeruli (of which creatinine clearance (C_C) 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 (C_A/C_C) 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).

Received 5 January 1970.

*Present address: The Montreal Children's Hospital, 2300 Tupper Street, Montreal 25, Quebec, Canada.

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 ($U_A V/Wt$ (24 hour)).

Materials and Methods

Capillary blood samples, collected by fingerprick and anticoagulated with lithium sequestrene were separated and stored at +4 °C. Urine samples were preserved with thiomersalyl and stored at -20 °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 alum-precipitated reconstituted freeze-dried electrophoretically pure human albumin (RHA 05; Behringwerke, A.G., Marburg-Lahn, Germany) as antigen. After absorption with γ -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).

Urine 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 (± 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:

$$\log_{10} U_A/U_C \text{ (random)} = -0.52 \log_{10} Wt - 0.79 \pm 0.30^* (r = -0.63, p < 0.001).$$

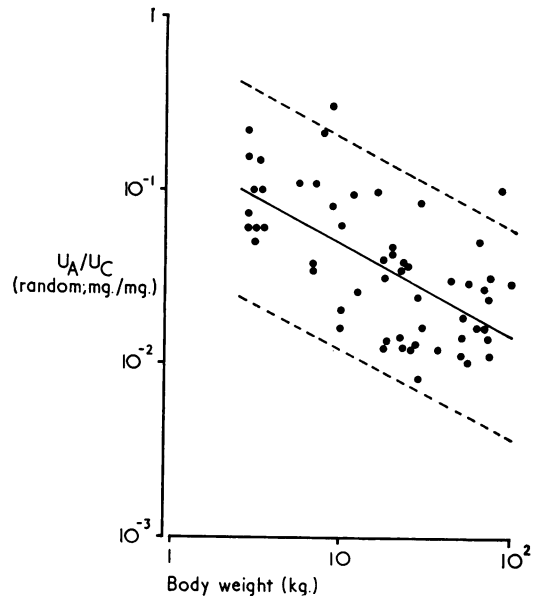


FIG. 1.—The normal range of U_A/U_C (random) related to body weight. The correlation is significant ($r = -0.63$, $p < 0.001$) and the 95% confidence limits are shown.

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

Means and range (± 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-

*Standard error of the estimate of y from x.

TABLE I

Parameters of Albumin and Creatinine Excretion in Healthy Adults and Neonates

	Adults (n = 16)	Neonates (n = 8)
U_A (mg./ml.)	0.017 (0.0056-0.051)	0.017 (0.0063-0.044)
U_C (mg./ml.)	0.87 (0.31-2.40)	0.17 (0.075-0.40)*
P_A (mg./ml.)	48 (34-66)	40 (36-45)*
P_C (mg./ml.)	0.0087 (0.0063-0.012)	0.0043 (0.0028-0.0065)*
U_A/U_C (random; mg./mg.)	0.019 (0.007-0.049)	0.078 (0.015-0.39)*
$C_A/C_C \times 10^6$ (random)	3.3 (1.3-8.7)	11 (2.8-40)*

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

stitute for the theoretically preferable C_A/C_C (24 hour) as a measure of glomerular permeability, the confidence limits of the prediction of this parameter from U_A/U_C (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 U_A/U_C (random). There is a satisfactory correlation between C_A/C_C (24 hour) and U_A/U_C (random) (Fig. 2; Table II). The slope is significantly greater than unity because high values of U_A/U_C (random) are associated with low values of P_A . The correlation coefficient between C_A/C_C (24 hour) and U_A/U_C (random) was higher than that between C_A/C_C (24 hour) and $U_A V/Wt$ (24 hour), though the difference was not significant. This supports the view that the simple test of U_A/U_C (random) has at least as good a predictive value of C_A/C_C (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 U_A/U_C (random) was examined in 3 nephrotic children in a stable state of relapse. U_A and U_C were measured on each sample passed in a 24-hour period. No consistent

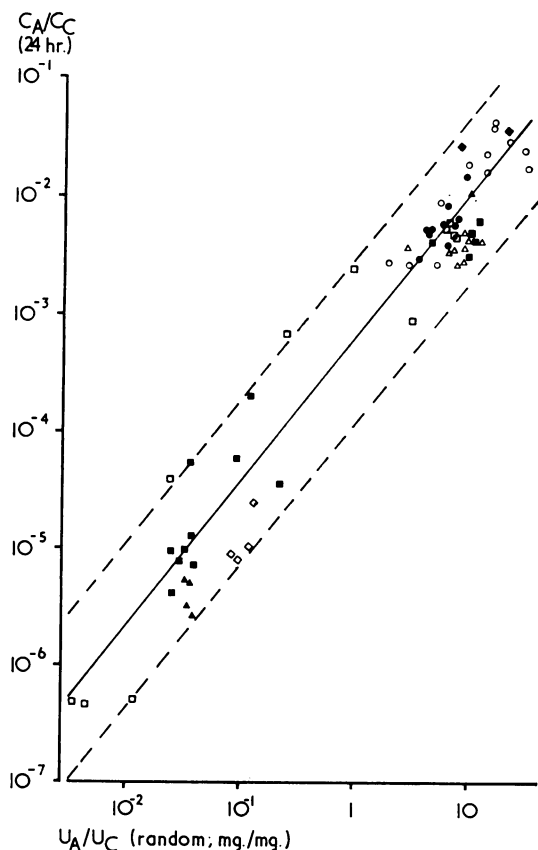


FIG. 2.—The relation between C_A/C_C (24 hour) and U_A/U_C (random) in 71 samples from 7 children with the nephrotic syndrome. Separate symbols indicate individual cases. The 95% confidence limits are shown.

diurnal pattern was observed, but the coefficients of variation of U_A/U_C (random) were 22%, 28%, and 12%; all are significantly greater than the experimental error ($\pm 6\%$) 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 $y = ax + b$.
(S_y = standard error of the estimate of y from x)

	y	x	a	b	S_y	r
1	$\log_{10} C_A/C_C$ (24 hour)	$\log_{10} U_A/U_C$ (random)	1.23	-3.99	0.36	0.97
2	$\log_{10} C_A/C_C$ (24 hour)	$\log_{10} U_A V/Wt$ (24 hour)	1.13	-2.09	0.48	0.94
3	$\log_{10} U_A/VWt$ (24 hour)	$\log_{10} U_A/U_C$ (random)	1.01	-1.68	0.35	0.96

$n = 71$; r_1 is not significantly greater than r_2 ($0.10 > p > 0.05$)

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.

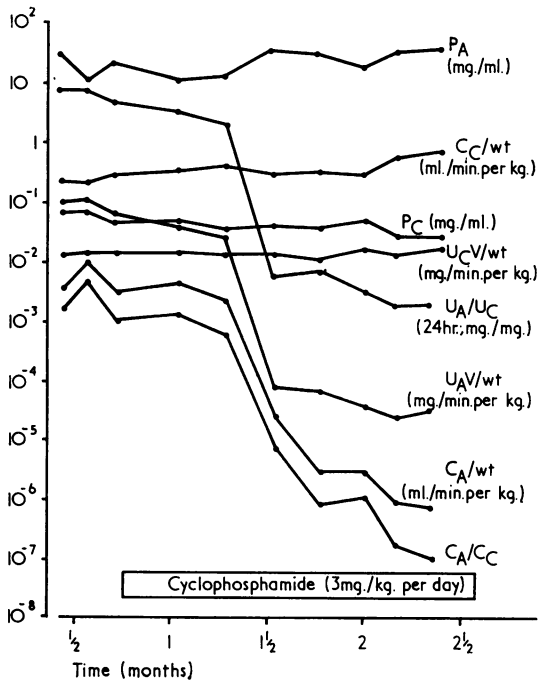


FIG. 3.—Changes in the parameters studied in a boy with the nephrotic syndrome associated with minimal renal histological abnormality during response to cyclophosphamide.

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_C/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_C/Wt ,

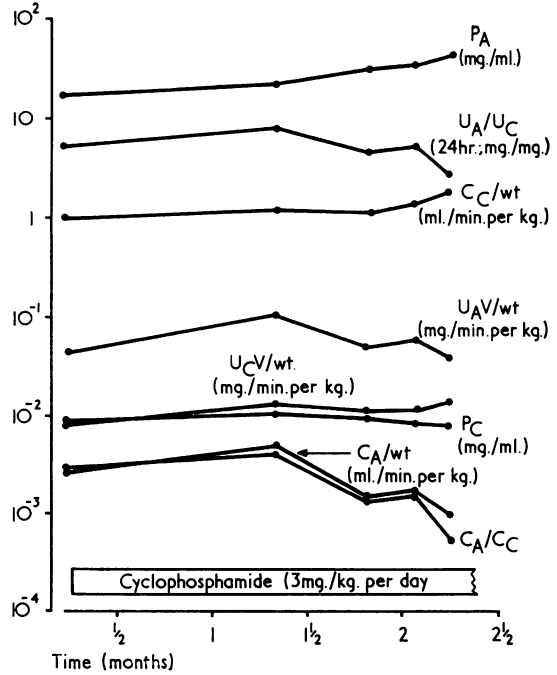


FIG. 4.—Data from a 35 kg. girl with the nephrotic syndrome and glomerulonephritis, plotted similarly to Fig. 3.

$U_A/V/Wt$ 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.

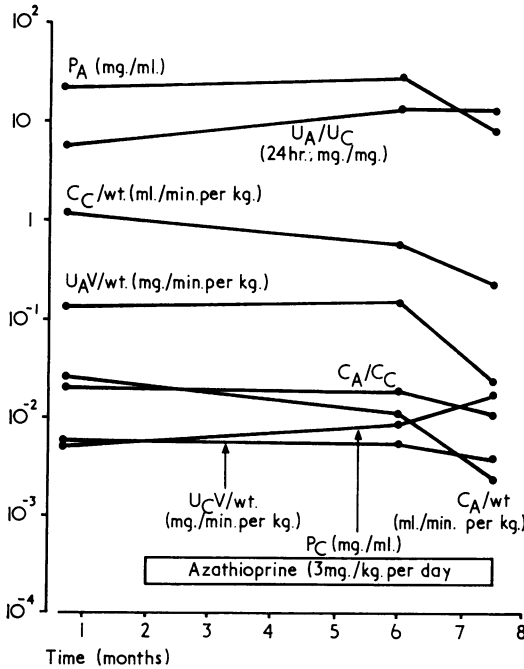


FIG. 5.—Data from a 6.5 kg. boy with congenital nephrotic syndrome, plotted similarly to Fig. 3.

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 (McCroory, Rapoport, 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:

$$\text{Filtered albumin} = U_A V$$

$$\text{Filtered water (GFR)} = U_C V / P_C$$

\therefore Albumin concentration in glomerular filtrate

$$= U_A V / \frac{U_C V}{P_C}$$

\therefore Albumin concentration gradient, filtrate/plasma

$$= \frac{U_A V}{P_A} / \frac{U_C V}{P_C} \\ = C_A / C_C$$

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:

$$C_A / C_C = U_A / U_C \times P_C / P_A$$

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:

$$C_A / C_C = U_A V / Wt \times \frac{P_C / U_C V}{P_A / Wt}$$

The data presented in Table II show that U_A/C_C (random) provides as reliable a prediction of C_A/C_C (24 hour) as does $U_A V/Wt$ (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

well, for creatinine production is relatively constant (Behrendt, 1962):

$$U_c V/Wt \simeq 0.01 \text{ mg./kg. per min.}$$

$$\therefore U_A V/Wt \text{ (mg./kg. per min.)} \simeq$$

$$U_A/U_c \text{ (mg./mg.)} \times 0.01.$$

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).

We acknowledge the help of the medical and nursing staff at The Hospital for Sick Children in undertaking

this study, of Dr. B. E. Clayton for facilities for creatinine determination, of the Wellcome Trust for a grant to P. N. McLaine during the period of study, and of the Medical Research Council for support of the Research Group in Immunology.

REFERENCES

- Barratt, T. M., and Crawford, R. (1970). Lysozyme excretion as a measure of renal tubular dysfunction in children. *Clinical Science*, in the press.
- Behrendt, H. (1962). *Diagnostic Tests in Infants and Children*, 2nd ed. p. 165. H. Kimpton, London.
- Berlyne, G. M., Varley, H., Nilwarangkur, S., and Hoerni, M. (1964). Endogenous-creatinine clearance and glomerular-filtration rate. *Lancet*, **2**, 874.
- Dirks, J. H., Clapp, J. R., and Berliner, R. W. (1964). The protein concentration in the proximal tubule of the dog. *Journal of Clinical Investigation*, **43**, 916.
- Fahey, J. L., and McKelvey, E. M. (1965). Quantitative determination of serum immunoglobulins in antibody-agar plates. *Journal of Immunology*, **94**, 84.
- Hardwicke, J., Cameron, J. S., Harrison, J., Hulme, B., and Soothill, J. F. (1970). Proteinuria studied by clearances of individual macromolecules. In *Proteins in Normal and Pathological Urine*, pp. 111-149. Ed. by Y. Manuel, J. P. Revillard, and H. Betuel. Karger, Basle.
- Hulme, B., and Hardwicke, J. (1968). Human glomerular permeability to macromolecules in health and disease. *Clinical Science*, **34**, 515.
- Kaufman, J. M., Greene, M. L., and Seegmiller, J. E. (1968). Urine uric acid to creatinine ratio: a screening test for inherited disorders of purine metabolism. *Journal of Pediatrics*, **73**, 583.
- Landis, E. M., and Pappenheimer, J. R. (1963). Exchange of substances through the capillary walls. *Handbook of Physiology*, section 2, *Circulation*, ed. by W. F. Hamilton, vol. 2, pp. 961-1034. American Physiological Society, Washington, D.C.
- McCrorry, W. W., Rapoport, M., and Fleisher, D. S. (1959). Estimation of severity of the nephrotic syndrome in childhood as a guide to therapy and prognosis. *Pediatrics*, **23**, 861.
- Mancini, G., Carbonara, A. O., and Heremans, J. F. (1965). Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2**, 235.
- Nordin, B. E. C. (1959). Assessment of calcium excretion from the urinary calcium/creatinine ratio. *Lancet*, **2**, 368.
- Richards, A. N. (1938). Processes of urine formation. *Proceedings of the Royal Society. Series B. Biological Sciences*, **126**, 398.
- Shannon, J. A. (1935). The renal excretion of creatinine in man. *Journal of Clinical Investigation*, **14**, 403.
- Soothill, J. F. (1962). Estimation of eight serum proteins by a gel diffusion precipitin technique. *Journal of Laboratory and Clinical Medicine*, **59**, 859.
- , Barratt, T. M., and McLaine, P. N. (1970). Controlled studies of the treatment of steroid resistant and steroid responsive relapsing nephrotic syndrome. *Proceedings of the IVth International Congress of Nephrology*. Karger, Basle.
- Squire, J. R., Blainey, J. D., and Hardwicke, J. (1957). The nephrotic syndrome. *British Medical Bulletin*, **13**, 43.
- Stoten, A. (1968). A micromethod for creatinine using resin to remove interfering substances. *Journal of Medical Laboratory Technology*, **25**, 240.

Correspondence to Dr. T. M. Barratt, Department of Immunology, Institute of Child Health, 30 Guilford Street, London W.C.1.