REFERENCES

Annau, E., Eperjessy, A. & Felszeghy, O. (1942). *Hoppe-Seyl. Z.* **277**, 58.
Annau, E., Eperjessy, A. & Felszeghy, O. (1943). *Hoppe-Seyl. Z.* **279**, 66.
Blakley, R. L. (1951). *Biochem. J.* **49**, 257.
Brady, R. O. & Gurin, S. (1950). *J. biol. Chem.* **187**, 589.
Burton, K. (1948). *Nature, Lond.*, **161**, 606.
Carter, H. E., Osman, E., Levine, H. & Gamm, S. (1939). *J. biol. Chem.* **128**, xiii.
Chernick, S. S., Chaikoff, I. L., Masoro, E. J. & Isaeff, E. (1950). *J. biol. Chem.* **186**, 527.
Cross, R. J., Taggart, J. V., Covo, G. A. & Green, D. E. (1949). *J. biol. Chem.* **177**, 655.
Edson, N. L. (1935). *Biochem. J.* **29**, 2082.
Edson, N. L. (1936*a*). *Biochem. J.* **30**, 1855.
Edson, N. L. (1936*b*). *Biochem. J.* **30**, 1862.
Grafflin, A. L. & Green, D. E. (1948). *J. biol. Chem.* **176**, 95.
Hogeboom, G. H. (1949). *J. biol. Chem.* **177**, 847.
Janes, R. G. & Brady, J. (1949). *Amer. J. Physiol.* **159**, 547.
Kennedy, E. P. & Lehninger, A. L. (1951). *J. biol. Chem.* **190**, 361.
Kuhn, R. & Livada, K. (1933). *Hoppe-Seyl. Z.* **220**, 235.
Lang, O. St A. K. (1939). *Hoppe-Seyl. Z.* **261**, 240.
Lang, O. St A. K. & Adickes, F. (1939). *Hoppe-Seyl. Z.* **262**, 123.
Lang, O. St A. K. & Mayer, H. (1939*a*). *Hoppe-Seyl. Z.* **261**, 249.
Lang, O. St A. K. & Mayer, H. (1939*b*). *Hoppe-Seyl. Z.* **262**, 120.
Lehninger, A. L. (1945). *J. biol. Chem.* **161**, 413.
Lehninger, A. L. (1949). *J. biol. Chem.* **178**, 625.
Lehninger, A. L. & Kennedy, E. P. (1948). *J. biol. Chem.* **173**, 753.
Lehninger, A. L. & Smith, S. W. (1948). *J. biol. Chem.* **173**, 773.
Masoro, E. J., Chaikoff, I. L., Chernick, S. S. & Felts, J. M. (1950). *J. biol. Chem.* **185**, 845.
Mazza, F. P. (1935). *Arch. Sci. biol., Italy*, **21**, 320.
Stetten, M. R. & Stetten, D. (1951). *J. biol. Chem.* **193**, 157.
Weil-Malherbe, H. & Bone, A. D. (1949). *Biochem. J.* **45**, 377.
Weinhouse, S., Millington, R. H. & Friedman, B. (1949). *J. biol. Chem.* **181**, 489.

Sedimentation and Diffusion of Human Albumins

2. NEPHROTIC HUMAN ALBUMINS AT A LOW CONCENTRATION

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(*Received* 13 *February* 1952)

Marked deviations from normal in the quantitative distribution of the serum proteins of nephrotic cases have been demonstrated by several methods, notably electrophoresis (Longsworth, Shedlovsky & MacInnes, 1939; Longsworth & MacInnes, 1940; Luetscher, 1940; Thorn, Armstrong, Davenport, Woodruff & Tyler, 1945). The presence of abnormally large amounts of lipid (Longsworth & MacInnes, 1940; Thorn *et al.* 1945), indicated the possibility of qualitative changes also. A number of different approaches have been made to decide whether there are any such differences between normal and nephrotic serum proteins, or between these and nephrotic urinary proteins.

Hewitt (1927) was unable to differentiate between serum and urinary albumin by determinations of optical rotatory power and dispersion. Similarly, Widdowson (1933) found no differences in the optical rotation, specific refractive increments, or racemization curves. None of these properties would, however, be likely to depend very critically on molecular weight, unless the alteration in molecular weight were associated with the incorporation of material other than protein.

The finding of Alving & Mirsky (1936), that the 'albumin' fraction, separated by half-saturation of nephrotic sera with ammonium sulphate, had a low cystine content is almost certainly due to the presence, in the supernatant 'albumin' solution, of a large proportion of α-globulin rich in lipid. As Gutman (1948) pointed out, the incompleteness of such separations must be taken into account when assessing results obtained with the fractions. (See also Bradley & Tyson, 1948.)

As regards electrophoretic properties, Longsworth & MacInnes (1940) noted that the mobilities of the urinary albumins were lower than those of normal or the corresponding serum albumins. It was discovered by Luetscher (1939) that the two components which separated from both nephrotic serum and nephrotic urinary albumin during electrophoresis at pH 4 were in approximately the same relative proportions, but their ratio differed from that found with normal human albumin.

Somewhat similar phenomena have been observed by Hoch-Ligeti & Hoch (1948) during protracted electrophoresis at pH 8.

It is important to establish whether the molecular weights of nephrotic proteins are perceptibly abnormal. Although osmotic measurements by Widdowson (1933) failed to reveal any variation, Bourdillon (1939), also on the basis of osmotic measurements, claimed that the molecular weights of the serum proteins were higher, and those of the urinary proteins lower, than normal. Diffusion constants determined by Longsworth & MacInnes (1940) tended to support the contentions of Bourdillon (1939), as albumin from the urine possessed a higher diffusion constant than normal. Malmros & Blix (1946) measured the sedimentation constants of the urinary albumin in three cases, finding no detectable departure from their own normal value.

The initial concentration of protein in the urine samples varied within wide limits from one patient to another and often between samples from the same patient at different times. Consideration was given to the possibility of concentrating the urinary proteins by complete precipitation and subsequent solution in a reduced volume, by ultrafiltration (cf. Rigas & Heller, 1951), or by dialysis against gum arabic, polyvinyl pyrrolidone, or dextran solutions. As none of these methods was felt to be completely free from objection in the present instance, work was confined to samples of urine containing at least 0·5 % total protein, thus obviating the need for preliminary concentration. It did, however, mean that duplicate preparative runs were often essential, as with the sera.

Sedimentation measurements. The technique previously used (Charlwood, 1952) was adhered to, with one small exception, in spite of the appearance in the meantime of a potentially rather more accurate method of locating the peaks on the photographic plates (Kegeles & Gutter, 1951). The precision of this Fresnel interference method of Kegeles & Gutter (1951) decreases somewhat at the lower concentrations used here, the standard error reaching about 1 %. The minor alteration referred to above was the adoption of a null method in making rotor temperature measurements with the contact thermocouple and galvanometer.

Diffusion measurements. The apparatus of Creeth (1952) was used under the conditions described by Charlwood (1952). Unfortunately it was not possible to prepare and examine more concentrated solutions of nephrotic albumins, to investigate the distribution of bands in the interference pattern.

Table 1. *Sedimentation constants of nephrotic human albumins*

Sample no.*	Buffer used in isolation	Solvent for sedimentation	Protein concn. (%)	$s_{20,w}$
S 1	Phosphate ($I=0·1$)	Phosphate ($I=0·1$)	0·25	4·21†
U 1	,, ,,	,, ,,	0·17	4·29†
S 2	Veronal ($I=0·05$)	0·2 N-sodium chloride	0·24	4·32‡†
U 2	,, ,,	,, ,,	0·19	4·30‡†
U 3	,, ,,	,, ,,	0·20	4·34
S 4	Veronal ($I=0·1$)	0·05 N-sodium chloride	0·21	4·24
U 4	,, ,,	,, ,,	0·17	4·32
S 5	,, ,,	,, ,,	0·17	4·30
U 5	,, ,,	,, ,,	0·19	4·30
S 6	,, ,,	,, ,,	0·20	4·26
U 6	,, ,,	,, ,,	0·21	4·27
S 7	,, ,,	,, ,,	0·17	4·23
U 7	,, ,,	,, ,,	0·22	4·31
S 8	,, ,,	,, ,,	0·26	4·24
U 8	,, ,,	,, ,,	0·19	4·31

* *S* and *U* refer respectively to serum and urinary albumins, whilst numbers indicate particular patients.
† Refrigeration used during these runs. ‡ Mean of two determinations.

Since neither a diffusion constant, nor a sedimentation constant, is alone sufficient for the unambiguous estimation of a molecular weight, it was considered desirable that both these quantities should be measured for the same preparations. Albumin was selected, as it constitutes the major portion of the urinary protein, it is relatively homogeneous, and it can be isolated electrophoretically without great difficulty.

EXPERIMENTAL

Electrophoretic separations were carried out exactly as described by Charlwood (1952). In several cases the albumin content of the serum was so low that duplicate preparative runs were necessary, the products being bulked prior to the subsequent analytical run. This procedure proved superior to the alternative of extending the length of a single run because, owing to the large amount of lipid-rich α-globulin, convections tended to develop in the ascending limb and were liable to prevent a complete separation.

RESULTS

Sedimentation measurements. The values in Svedberg units, obtained for the sedimentation constants, corrected to water at 20°, are shown in Table 1. In carrying out the corrections the same value of $\bar{V}$, 0·733 has been used as for normal human albumin (see Charlwood, 1952). Even if the true value of $\bar{V}$ differs considerably from this, the magnitude of the correction is little affected. This is

in marked contrast to the dependence of molecular weight on the value of $\bar{V}$ used in calculations based on the combined sedimentation and diffusion results (see Discussion). All the values for the nephrotic albumins fall within the limits defined for normal albumins under similar conditions, i.e. $4{\cdot}27_5 \pm 0{\cdot}08$ (Charlwood, 1952). The mean value for the serum albumins is $4{\cdot}26_4$ and for the urinary $4{\cdot}30_4$. Statistical analysis shows that only in the latter case is there any likelihood of these mean values representing real departures from normal, the value of the probability lying between 0·03 and 0·1, depending on whether possible effects of concentration are ignored or approximately allowed for.

values has been discussed earlier (Charlwood, 1951, 1952). Since that work was completed evidence of further discrepancies between oil-turbine and other ultracentrifuge measurements has become available (Kegeles & Gutter, 1951). However, the chief concern here is the comparison of the behaviour of normal and pathological specimens under similar conditions.

Malmros & Blix (1946) quoted 4·58, 4·54 and 4·52, for three different urinary albumins, as agreeing with their normal human albumin value, but the protein concentrations were not specified. McFarlane (1935*a*, *b*) compared corresponding serum and urinary albumins, concentrating the

Table 2. *Diffusion constants of nephrotic human albumins*

Sample no.*	Buffer used in isolation	Solvent for diffusion	Protein concn. (%)	No. of experiments	$D_{20,w}$
U 3	Veronal ($I=0{\cdot}05$)	0·2 N-sodium chloride	0·20	4	6·62
S 4	Veronal ($I=0{\cdot}1$)	0·05 N-sodium chloride	0·21	4	6·29
U 4	,, ,,	,, ,,	0·17	4	7·04
S 5	,, ,,	,, ,,	0·17	3	4·85
U 5	,, ,,	,, ,,	0·19	4	6·63
S 6	,, ,,	,, ,,	0·20	4	6·09
U 6	,, ,,	,, ,,	0·21	4	6·23
S 7	,, ,,	,, ,,	0·17	4	5·78
U 7	,, ,,	,, ,,	0·22	4	6·50
S 8	,, ,,	,, ,,	0·26	4	6·02
U 8	,, ,,	,, ,,	0·19	4	6·51

* Sample numbers denote the same materials as those in Table 1.

Diffusion measurements. Table 2 gives the diffusion constants of the nephrotic albumins, reduced to water at 20° and expressed in terms of the unit of 10^{-7} cm.^{2} sec.$^{-1}$. In a previous paper it was shown that corresponding normal albumins have a mean diffusion constant of 6·32 (Charlwood, 1952). Taking into account the small, real differences which exist between normal preparations and the errors of measurements, on the basis of 95% fiducial limits, values obtained by the present method and lying within the range 6·11–6·52 may be classed as normal. Of the five serum albumin values shown in Table 2 only one falls within this range, a second slightly below the lower limit, whilst the other three are definitely low. Of the six urinary albumins three gave high diffusion constants, two fall practically on the upper limit of the range and only one appears quite normal. In every case the diffusion constant of the serum albumin was lower than that of the corresponding urinary component.

DISCUSSION

Sedimentation results. Previous sedimentation experiments with nephrotic albumins are confined to oil-turbine ultracentrifuge measurements by McFarlane (1935*a*, *b*) and Malmros & Blix (1946). The question of the absolute accuracy of oil-turbine

latter by means of ammonium sulphate precipitation. There was some suggestion that sedimentation was respectively slower and faster than usual, but in practically every case the differences did not exceed the probable experimental error. Although this is interesting in view of the present results, the main conclusion must be that there is no really valid evidence for deviations from normal as regards sedimentation.

Diffusion results. The diffusion of albumin from one case of nephrosis was studied by Longsworth & MacInnes (1940). Otherwise the only work described on this subject is that of McFarlane (1935*a*, *b*) who observed the spreading of sedimenting boundaries. In two nephrotic sera the rate of spreading of the albumin boundary was abnormally high; but, since the '*X*' protein peak was not visible, this could be ascribed to the polydispersity of the material in the boundary under consideration. In one case the corresponding urinary protein showed about the same degree of spreading, whilst in the other it was lower, although still greater than normal. It does not seem possible to draw definite conclusions from McFarlane's observations, owing to the reservation expressed concerning the identity of the material responsible for the boundaries. Measurements of diffusion constants from ultracentrifuge patterns are prone to err on the high side should the material

be at all polydisperse and are liable to be influenced by vibrational disturbance. Consequently, reliance can be placed only on measurements made under conditions free from such objections.

The diffusion constants obtained by Longsworth & MacInnes (1940) for a normal human serum and a nephrotic urinary albumin, when corrected to 20°, become 6·39 and 6·72 respectively. Their normal value is consistent with determinations made by the present technique (Charlwood, 1952). In addition, their urinary albumin diffused more rapidly than normal, in accord with the conclusions based on Table 2.

Molecular weights. On the assumption that the present results may be used without appreciable error in place of $s^0_{20,w}$ and $D^0_{20,w}$ (Charlwood, 1952), molecular weights may be calculated, the partial specific volume, $\bar{V}$, being the only remaining factor needed. It would have been impossible to determine this quantity with the requisite accuracy from the small amounts of material isolated in this work. Consequently, for the purpose of comparison, it has been necessary to assume that $\bar{V}$ is the same as for normal albumin (cf. Longsworth & MacInnes, 1940). Unless a significant part of the pathological albumin molecule consists of material which is not protein, this assumption is probably justified. Then, taking $\bar{V} = 0{\cdot}733$, the molecular weights calculated for the nephrotic serum albumins range from the normal value of about 62 000 (Charlwood, 1952) to 80 000, whilst the urinary ones lie between normal and 55 000. The limitations implicit in these statements must be emphasized. The molecular weights apply only to the albumin fraction which is isolated by electrophoresis under the present conditions and they are conditional on the correctness of $\bar{V}$. It should be noted, however, that the type of error most likely to be incurred is that which would be occasioned if the molecules of pathological albumin, particularly the serum albumin, included lipid portions. Then $\bar{V}$ would actually be higher and the above molecular weight estimates would be too low.

The osmotic pressure measurements of Bourdillon (1939) gave a molecular weight of 72 000 for normal human albumin, 57 000–67 000 for different nephrotic urinary albumins and 99 000–122 000 for nephrotic serum albumins. Bourdillon was careful to point out that his 'albumin' was defined as that part of the protein which remained in solution after half-saturation with ammonium sulphate. As is now well known (Gutman, 1948), this fractionation is imperfect and the albumin from nephrotic serum is accompanied by much α-globulin. The inferences from Bourdillon's measurements are, therefore, somewhat inconclusive. The objection of possible contamination with α-globulin does not apply to the electrophoretically isolated materials, as the analytical experiments failed to reveal any globulin impurity. The degree of resolution was sufficiently high to have detected, in most cases, the proportion of globulin which would be required to account for high values of molecular weight.

The general conclusions are that the mean molecular weights of nephrotic serum albumins are often higher than normal, the urinary usually lower. Deductions which can be made from these observations depend on whether the serum (or urinary) albumin is polydisperse or of uniform molecular weight. The evidence of Luetscher (1939), Hoch (1948, 1950) and Hoch-Ligeti & Hoch (1948) from electrophoresis and the diffusion characteristics of normal human albumin (Charlwood, 1952) make the former alternative by far the more probable. The combination of techniques used here does not give any simple average of molecular weight of polydisperse material (Ogston, 1949).

It would be expected that any smaller molecules would pass more readily through the kidney unless specific effects predominated. This agrees with the observed lower mean molecular weight of the urinary albumins and also with the fact that the albumin to globulin ratio is higher in nephrotic urine than in serum (Malmros & Blix, 1946). However, Rigas & Heller (1951) found that it is apparently the globulin components which predominate in the small quantities of urinary protein encountered in normal subjects.

The further possibility, mentioned by McFarlane (1935*b*), that differences in the urinary protein might be due to chemical or biochemical action of constituents of the urine, whilst by no means excluded, has not been discussed, because the abnormally high molecular weights found for the serum albumins help to make such a hypothesis superfluous.

Thus, although the nephrotic albumins are definitely abnormal, the evidence is not sufficient to decide whether the cause lies in an unusual distribution of normal albumin components or in the presence of new components. Present techniques do not permit fractionation of the albumin. If this should become possible and the fractions be distinguishable immunologically, it might be feasible to demonstrate the existence, or otherwise, of abnormal molecules.

SUMMARY

1. The albumin fractions of a number of nephrotic sera and urine samples have been isolated electrophoretically.

2. Sedimentation constants for these preparations in the concentration range 0·2–0·3 % agreed with determinations previously made on normal human albumins.

3. In the Gouy diffusiometer the nephrotic serum albumins generally diffused more slowly, and the urinary albumins more rapidly, than normal.

4. It has been demonstrated that nephrotic albumins are abnormal. Probably the mean molecular weights of nephrotic serum and urinary albumins are respectively higher and lower than normal.

The author wishes to express his thanks to Prof. E. C. Dodds, F.R.S., for his help and interest. He acknowledges very gratefully the co-operation of Prof. J. R. Marrack, M.A., M.D., Prof. M. L. Rosenheim, M.D., F.R.C.P., members of their departments and Dr D. N. Baron in making available the clinical material. Helpful suggestions regarding the manuscript have been made by Dr J. M. Creeth. This work has been carried out during the tenure of an Imperial Chemical Industries Research Fellowship in the University of London.

REFERENCES

Alving, A. S. & Mirsky, A. E. (1936). *J. clin. Invest.* **15**, 215.
Bourdillon, J. (1939). *J. exp. Med.* **69**, 819.
Bradley, S. E. & Tyson, C. J. (1948). *New Engl. J. Med.* **238**, 223.
Charlwood, P. A. (1951). Thesis. University of London.
Charlwood, P. A. (1952). *Biochem. J.* **51**, 113.
Creeth, J. M. (1952). *Biochem. J.* **51**, 10.
Gutman, A. B. (1948). *Advanc. prot. Chem.* **4**, 155. New York: Academic Press Inc.
Hewitt, L. F. (1927). *Biochem. J.* **21**, 1109.
Hoch, H. (1948). *Biochem. J.* **42**, 181.
Hoch, H. (1950). *Biochem. J.* **46**, 199.
Hoch-Ligeti, C. & Hoch, H. (1948). *Biochem. J.* **43**, 556.
Kegeles, G. & Gutter, F. J. (1951). *J. Amer. chem. Soc.* **73**, 3770.
Longsworth, L. G. & MacInnes, D. A. (1940). *J. exp. Med.* **71**, 77.
Longsworth, L. G., Shedlovsky, T. & MacInnes, D. A. (1939). *J. exp. Med.* **70**, 399.
Luetscher, J. A., jun. (1939). *J. Amer. chem. Soc.* **61**, 2888.
Luetscher, J. A., jun. (1940). *J. clin. Invest.* **19**, 313.
McFarlane, A. S. (1935*a*). *Biochem. J.* **29**, 1175.
McFarlane, A. S. (1935*b*). *Biochem. J.* **29**, 1202.
Malmros, H. & Blix, G. (1946). *Acta med. scand.* Suppl. no. 170.
Ogston, A. G. (1949). *Biochem. J.* **45**, 189.
Rigas, D. A. & Heller, C. G. (1951). *J. clin. Invest.* **30**, 853.
Thorn, G. W., Armstrong, S. H., jun., Davenport, V. D., Woodruff, L. M. & Tyler, F. H. (1945). *J. clin. Invest.* **24**, 802.
Widdowson, E. M. (1933). *Biochem. J.* **27**, 1321.

Studies in the Biochemistry of Micro-organisms

89. METABOLIC PRODUCTS OF *PENICILLIUM MULTICOLOR* G.-M. AND P. WITH SPECIAL REFERENCE TO SCLEROTIORIN

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(*Received* 12 *March* 1952)

During the routine testing of a number of moulds for new metabolic products five strains of *Penicillium multicolor* Grigorieva-Manilova and Poradielova came under observation. Four of these five strains produced in the mycelium a yellow colouring matter which was extractable with light petroleum and which bore a strong resemblance to a chlorine-containing compound obtained from *P. sclerotiorum* van Beyma by Curtin & Reilly (1940) and named by them sclerotiorin(e). Sclerotiorin had already been obtained independently in these laboratories in 1940 by Dr R. F. Hunter from the mycelium of *P. sclerotiorum*. This finding was not recorded in the literature since publication was forestalled by Curtin & Reilly (1940), but there was no doubt from the source of the substance, its composition (particularly its chlorine content) and a comparison of properties that Dr Hunter's isolate was identical with sclerotiorin. Since Hunter's preparation was still available it was compared with the substance isolated from the various strains of *P. multicolor* and identity was established.

One of the strains of *P. multicolor* (no. N.R.R.L. 2324, Northern Regional Research Laboratory, Peoria, Illinois) afforded, in addition to sclerotiorin in the mycelium, a culture solution which on acidification to about 0·5N with hydrochloric acid gave a deposit of crystalline needles. This apparently new product, denoted as product I, was somewhat soluble in water but was reprecipitated on acidification. It had m.p. 110·5° and gave in aqueous solution a cherry-red colour with ferric chloride. From the analysis and determination of the equivalent the new product had the probable empirical formula