2. Measurements could be made 30 min. after placing a solution in the instrument.

3. Ten observations on each of a series of seven solutions of bovine plasma albumin were made, in two osmometers. The S.E. of the means of the observations ranged from 0·017 to 0·048 cm. H_2O with osmotic pressures ranging from 1·9 to 13·7 cm. H_2O.

4. The value for molecular weight of bovine plasma albumin derived by various methods from these results ranged from 66 600 to 68 800.

The authors thank Dr D. R. Stanworth for the ultracentrifuge analysis and Mr R. A. Crockson for preparing the diagrams. Most of this work was done during the tenure of a grant by one of us (D.S.R.) from the Medical Research Council.

REFERENCES

Adair, G. S. (1925). *Proc. Roy. Soc.* A, **108**, 629.
Adair, G. S. & Robinson, M. E. (1930). *Biochem. J.* **24**, 1864.
Harrington, W. F., Johnson, P. & Ottewill, R. H. (1956). *Biochem. J.* **62**, 569.
Ma, T. S. & Zuazaga, G. (1942). *Industr. Engng Chem. (Anal.)*, **4**, 280.
Rowe, D. S. (1953). *J. Physiol.* **123**, 18*P*.
Smithies, O. (1953). *Biochem. J.* **55**, 57.
Wells, H. S. (1932). *Amer. J. Physiol.* **101**, 409.

The Molecular Weights of the Proteins of Normal and Nephrotic Sera and Nephrotic Urine, and a Comparison of Selective Ultrafiltrates of Serum Proteins with Urine Proteins

By D. S. ROWE
Department of Experimental Pathology, University of Birmingham

(*Received* 27 *February* 1957)

The outstanding functional defect in the nephrotic syndrome is the severe urinary loss of protein, and the characterization of the serum and urine proteins has been recognized to be of fundamental importance in the investigation of this condition. The recent immunological demonstration of the identity of the urine proteins with some of the serum proteins (Burtin & Grabar, 1954; Gell, 1955) is cogent evidence of their derivation from that source. Electrophoretic studies have shown that these proteins have a distribution of characteristic and complementary type, the urine containing chiefly albumin with smaller quantities of α_1-, β- and γ-globulins, whereas the serum may be deficient in the albumin, α_1- and γ-globulin fractions and show an increased concentration of α_2-globulin (Hardwicke, 1954*a*). Consequent upon these protein changes, especially the fall in the albumin concentration, the colloid osmotic pressure of the plasma is greatly reduced. It has been suggested, following the Starling hypothesis, that this reduction is the cause of the oedema which is the presenting feature of the condition.

The studies of Bayliss, Kerridge & Russell (1933) on the excretion of protein by normal animal kidneys indicated a specific molecular weight, that of haemoglobin, demarcating larger protein molecules which were retained in the circulation from smaller ones which passed into the urine. More recent studies, such as those of Brewer (1951) and Wallenius (1954), on animals infused with dextran molecules of graded molecular weights, showed a progressive reduction in clearance as the molecular weight increased. Wallenius demonstrated that dextrans with average molecular weights of 10 000 and 20 000 had a clearance of about 90 and 30 % respectively relative to creatinine, whereas the preparation with an average molecular weight of 50 000 scarcely appeared in the urine. Although these results cannot be compared directly with those obtained with proteins because of the effects of charge and the differences in degree of hydration and in shape of the molecules, they do imply that normal kidneys may act as selective molecular filters over this range of molecular weights.

It is pertinent to inquire to what extent the kidney retains this selective function in conditions of heavy proteinuria. The preponderance of albumin in the urine protein, even in the face of an inverted serum albumin:globulin ratio, has suggested that some selectivity is maintained, but it seemed likely that more precise information could be obtained by comparison of the average molecular weights of the serum and urine proteins. The results presented here, which were obtained by colloid osmotic-pressure measurements, confirm the highly selective nature of the urinary loss of protein in conditions of moderate and heavy proteinuria. In addition, it has been possible to reproduce the selective protein filtration, characteristic of the nephrotic kidney, by filtering normal and nephrotic sera through suitably prepared nitrocellulose membranes.

The molecular weights of nephrotic serum and urine albumin have been investigated by several workers, with conflicting results. For example, Widdowson (1933) could detect no differences between normal serum albumin and that of nephrotic serum and urine by colloid osmotic-pressure measurements and by other methods; whereas Bourdillon (1939), from osmotic-pressure data and Charlwood (1952) from measurements of sedimentation and diffusion, claimed that nephrotic-serum and urine-albumin preparations had molecular weights respectively greater and less than that of normal serum.

A possible source of error in these measurements was the contamination of albumin by other serum proteins. In this work, therefore, albumin fractions have been prepared from normal and nephrotic sera and urine by preparative column electrophoresis (Flodin & Porath, 1954) and have been subjected to analysis of their purity. These specimens of albumin had molecular weights identical, within the limits of experimental error, with that of normal serum albumin.

METHODS

Collection of materials: dialysis: preservatives. Sera and urines were usually dialysed within a few hours of collection or were stored in the cold until dialysis was possible. In some experiments sodium azide, in a concentration of about 1 mg./100 ml., was added to the dialysing buffer as a preservative; much higher concentrations have been shown to have no effect on the colloid osmotic pressure of whole serum. Dialysis against several changes of buffer (pH 7·2, 0·047 M-Na_2HPO_4 + 0·020 M-KH_2PO_4 Sørensen buffer before osmometry, or pH 8·2, 0·05 M-sodium diethylbarbiturate + 0·01 M-diethylbarbituric acid for preparative electrophoresis) was continued for a minimum of 48 hr. at about 2°. A cloudy precipitate which sometimes formed in the urine was removed by centrifuging.

Estimations of concentration of protein and of osmotic pressure. The solutions were diluted, if necessary, to an appropriate protein concentration with the diffusate, and the total nitrogen concentration was estimated by a micro-Kjeldahl method (Ma & Zuazaga, 1942). Non-protein nitrogen, which was often undetectable, was estimated either after deproteinization with tungstic acid or on the final diffusate. Since significant concentration changes did not occur in the osmometers it was possible to use a series of dilutions prepared from a solution of known protein concentration and its diffusate. A factor of 6·25 was used throughout to convert protein nitrogen into protein. The osmotic pressures were measured by the method described by Rowe & Abrams (1957).

Ultrafilters. The membranes used for selective ultrafiltration were prepared in the same way as those used for the osmotic-pressure measurements (Wells, 1932), except that the water content of the final swelling mixture was reduced from 5 ml. to 2·5–3·5 ml./100 ml. of mixture. Nitrocellulose HL 120/170 (Imperial Chemical Industries Ltd.) was used. The membranes were clamped and supported between two Perspex plates (Rowe, 1956). Sufficient ultrafiltrate for protein analysis was obtained in a few hours with a membrane area of about 12 cm.^{2} and a pressure of 1 atmosphere. Ultrafiltration was performed at room temperature.

Electrophoresis. The quantitative analytical filter-paper method was that described by Hardwicke (1954*b*). Carbohydrate was detected on filter paper, after electrophoresis, by the method of Kiöw & Grönwall (1952). Immunoelectrophoretic analyses were performed by Dr P. G. H. Gell by his modification (Gell, 1955) of the technique of Grabar & Williams (1953) with antisera raised in rabbits against human serum. The preparative method was that of Flodin & Porath (1954), either starch or acetylated cellulose columns (Campbell & Stone, 1956) being used. It was necessary to concentrate the urine proteins before fractionation. The urine was placed in a dialysis sac which was alternately suspended in a current of air at room temperature and immersed in barbiturate buffer to prevent an excessive rise of electrolyte concentration. Sufficient concentration was achieved after evaporation for a few hours. After dialysis against barbiturate buffer, 4–6 ml. of an approx. 6% solution of urine or serum proteins was fractionated, bromophenol blue being added to some of the samples to indicate the position of the albumin in the column, this dye having no detectable effect on the molecular weight of normal serum albumin. A current of 30 mA at 250 V for about 30 hr., with column 45 cm. long and 3 cm. diameter, produced optimum separation of the proteins. The eluates were collected in 4 ml. fractions, the relative protein concentrations of which were assessed after a 16-fold dilution in water by their absorption at 277·5 mμ in a spectrophotometer. Before molecular-weight determinations the albumin-containing fractions were dialysed against the phosphate buffer, the nephrotic-serum albumin fractions also being concentrated by alternate evaporation and dialysis.

RESULTS

Normal human serum

The results from three normal male human sera are given in Table 1. The molecular weights and the standard errors have been derived as described by Rowe & Abrams (1957), except that in this account the osmotic pressures have been corrected to 25°, and hence the value of $2·528 \times 10^5$ was used for RT. Linear regressions were fitted to the plots of C/π against C following Adair & Robinson (1930), since serum II showed that at high concentrations the plot of π/C against C was not linear. The molecular weight so obtained is a number-average (M_n). The small range (from 76 900 to 78 400) is a little surprising in view of the complexity of the mixture of proteins present in normal serum.

Nephrotic serum and urine proteins

Four cases, numbers 11, 12, 13 and 23, of the series reported by Squire, Blainey & Hardwicke (1957), were studied. These case numbers have been changed to numbers I, II, III and IV in this account. The patients had moderate or heavy proteinuria, with a lowered serum-albumin concentration, raised serum cholesterol, normal or only slightly

Table 1. *Osmotic pressures and number-average molecular weights of the proteins of normal serum dialysed against* pH 7·17 *Sørensen phosphate buffer*

	C Concn. of protein (g./100 ml. of solution)	π Colloid osmotic pressure (cm. H_2O at 25°)		$10^{-3}\,M_n$	10^{-3} S.E. of M_n
Normal human serum I	2·27	8·49	8·55		
	1·45	5·10	—		
	1·13	3·94	3·86	77·9	±2·5
	0·724	2·46	—		
	0·454	1·48	1·47		
	0·362	1·21	—		
Normal human serum II	6·60	34·5	—		
	4·95	22·6	22·5		
	3·29	13·3	13·3	76·9	±2·0
	1·65	6·02	5·90		
	0·825	2·85	—		
	0·413	1·39	1·40		
Normal human serum III	1·99	7·38	—		
	1·21	4·29	—		
	0·996	3·43	3·40	78·4	±1·0
	0·605	2·02	2·01		
	0·373	1·24	1·22		

raised non-protein nitrogen, a normal or moderately raised blood pressure and no excess of red blood cells in their urine. All had oedema initially.

The osmotic pressures of the proteins proved stable for many hours in the osmometers, but occasional difficulty arose in the determination of the level of the menisci of extremely cloudy sera, especially the first specimen of serum from case I. Cases I and II were studied over a period of several months, and the results are given in Table 2. When a standard error is given the calculation of average molecular weight is based on a statistical analysis similar to that used on the normal sera. Where no standard error is given the average molecular weight is calculated from a regression line of C/π against C fitted visually. This method is considered to be justified even when based on three or four observations, since the standard error of osmotic-pressure measurement has been shown to be about 0·03 cm. H_2O in this range of pressures (Rowe & Abrams, 1957), and since the standard errors of the molecular weights, where sufficient data were available for statistical analysis, were also low. These molecular-weight values are presented, with clinical and other findings, in Fig. 1, which shows that the average molecular weight of the serum proteins in these cases was variable and always greater than that of normal serum, and that the increase was apparently related to the severity of the condition. The urine proteins, in contrast, always gave average molecular weights less than that of serum albumin, implying that the globulins in this case had a smaller average size than albumin. These average molecular weights showed no systematic variation in different phases of the disease.

The serum and urine proteins for cases II and IV, which were subjected to similar analyses, also showed that the molecular weight of the serum proteins was elevated whilst that of the urine proteins was less than that of serum albumin.

The possibility of chemical or enzymic cleavage of the serum proteins in the presence of urine was investigated by incubating fresh normal serum with fresh normal urine for 5 hr. at 38°. No change was observed after a threefold dilution with urine, but after a 20-fold dilution the average molecular weight fell from 77 000 to 70 000

The more diluted proteins were subsequently concentrated by alternate evaporation at room temperature and dialysis, and although the procedure does not alter the molecular weight of serum albumin it may have denatured some of the globulin fractions. These results therefore do not exclude the possibility of the breakdown of some of the serum proteins in the presence of urine, but suggest that the effect is not a major one.

Ultrafiltration experiments

An attempt was made to reproduce the selective protein transmission of the nephrotic kidney by preparing nitrocellulose membranes of such permeability that the serum proteins appeared in low concentrations in their ultrafiltrates. The molecular weights and electrophoretic distributions before and after ultrafiltration are given in Table 3. Bovine plasma albumin (Armour Batch no. 18643) was not detectably altered by the procedure, since the four values obtained on the ultrafiltrate lay within the 95% confidence limits of the C/π against C plot of the original material. [This albumin was a different

Table 2. *Osmotic pressures and number-average molecular weights of the proteins of nephrotic serum and urine*

Material	Date	C Concn. of protein (g./100 ml. of solution)	π Colloid osmotic pressure (cm. H_2O at 25°)		$10^{-3} M_n$	10^{-3} S.E. of M_n
Nephrotic serum case I						
A	11. i. 55	1·33	2·11	2·23	177	±4·1
		0·996	1·58	1·58		
		0·666	0·96	1·00		
		0·333	0·50	0·49		
B	17. iii. 55	0·937	1·56	—	162	—
		0·669	1·11	—		
		0·535	0·86	—		
C	5. v. 55	1·99	6·13	6·09	99	±1·0
		0·996	2·78	2·79		
		0·499	1·32	1·35		
D	26. v. 55	2·29	7·38	7·32	94	—
		1·14	3·37	—		
		0·915	2·71	—		
		0·572	1·61	—		
E	20. vi. 55	2·70	9·03	—	95	—
		1·08	3·15	—		
		0·54	1·51	—		
Urine case I						
A	23. ii. 55	1·07	4·67	—	64	—
		0·714	3·09	—		
		0·357	1·43	—		
B	17. iii. 55	0·858	4·45	—	57	—
		0·572	2·73	—		
		0·286	1·31	1·31		
C	26. vi. 55	0·943	4·00	—	67	
		0·471	1·89	—		
		0·378	1·49	—		
Nephrotic serum case II						
A	18. ii. 55	1·93	5·62	—	102	—
		1·45	4·09	—		
		0·966	2·61	—		
		0·483	1·22	—		
B	3. vi. 55	3·16	8·32	8·31	126	±1·0
		1·58	3·64	3·65		
		0·631	1·32	1·35		
Urine case II						
A	16. ii. 55	1·38	6·95	—	56	—
		0·917	4·51	—		
		0·458	2·17	—		
		0·229	1·01	—		
B	8. iii. 55	0·912	4·17	—	59	—
		0·683	3·10	—		
		0·456	1·99	—		
		0·228	0·98	—		
C	26. iv. 55	1·37	6·60	—	63	—
		0·685	3·07	—		
		0·342	1·38	—		
D	3. vi. 55	1·59	7·59	7·62	58	±0·9
		0·635	2·85	2·84		
		0·317	1·43	1·39		

sample from that described by Rowe & Abrams (1957), having a significantly greater average molecular weight. Ultracentrifugal analysis showed a small component sedimenting faster than the main peak.]

The proteins appearing in the ultrafiltrates from normal and nephrotic sera resembled nephrotic urine proteins in electrophoretic distribution, and their average molecular weights were also similar to those of the urine proteins. The average molecular weights of the proteins appearing in the ultrafiltrates of nephrotic urine showed a fall. The reduction of 5000 in the urine of case IID was significant since the four points obtained all lay below the 95% confidence limits of the C/π against C plot of the original urine protein. The smaller reduction in the molecular weights of the urine proteins compared with the nephrotic serum proteins was associated with smaller alterations in their electrophoretic distributions.

Fig. 2 shows the results of an immunoelectrophoretic analysis of the serum and urine proteins from case III and their ultrafiltrates, with an antiserum containing no anti-albumin (The serum had been stored frozen for a few weeks, which resulted in

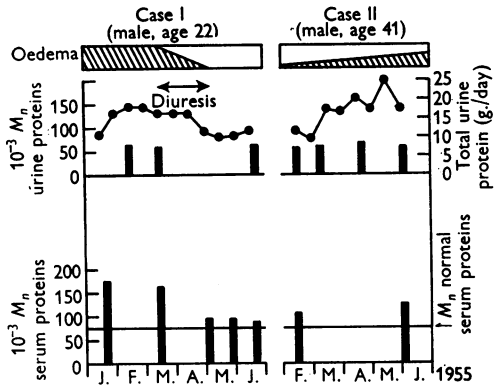


Fig. 1. Average molecular weights of the serum and urine proteins from two cases of the nephrotic syndrome, shown in relation to the daily urine protein loss and the severity of the oedema. Heights of the blocks represent the average molecular weights of the proteins, and the filled-in points indicate the daily urine protein loss (means of 7-day periods).

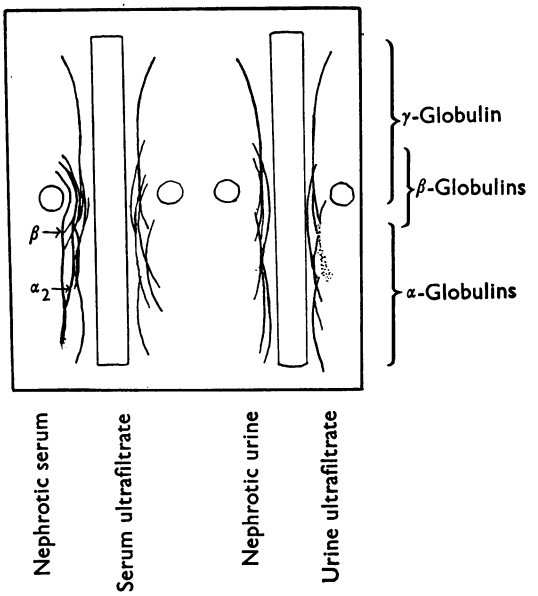


Fig. 2. A line diagram of an immunoelectrophoretic analysis of nephrotic serum and urine from case IV and the proteins appearing in their ultrafiltrates through a selective nitrocellulose membrane. Arrows indicate an α_2-globulin, present in high concentration in the serum and in low concentration in the ultrafiltrate and the urine, and the β-lipoprotein, apparently absent from the serum ultrafiltrate and the urine. The urine ultrafiltrate showed a relatively increased α_1-globulin concentration.

Table 3. *Effect of selective ultrafiltration of bovine plasma albumin and of serum and urine proteins on their number-average molecular weights and electrophoretic analyses*

Material	$10^{-3}M_n$	Electrophoretic analysis as percentage composition				
		Albumin	α_1	α_2	β	γ
Bovine plasma albumin	70±0·4	—	—	—	—	—
Ultrafiltrate	70	—	—	—	—	—
Normal serum II	77±2·0	66	3	7	12	12
Ultrafiltrate 1	64±0·5	73	6	6	8	7
Ultrafiltrate 2	68	75	13	3	7	2
Case I serum B	162	—	—	—	—	—
Ultrafiltrate	74	—	—	—	—	—
Case III serum	123	27	6	38	19	10
Ultrafiltrate	68	70	10	6	14	0
Case II urine C	63	66	14	4	9	7
Ultrafiltrate	47	73	11	4	5	7
Case II urine D	58±0·9	65	8	7	10	10
Ultrafiltrate	53	67	13	5	9	6

a partial denaturation of the β-lipoprotein, as shown by the continuation of its line of precipitation into the α_1 region.) Several proteins which are present in the serum are reduced or absent in its ultrafiltrate, which has a spectrum comparable in number of components with that of nephrotic urine. The urine proteins ultrafiltered through the same membrane show an almost unchanged pattern, but have the α_1-globulin concentration relatively increased. Comparable results were obtained from ultrafiltrates of other serum and urine proteins.

Osmotic pressures of nephrotic serum and urine albumin compared with that of normal serum albumin

Typical protein concentrations appearing in the eluate fractions from normal and nephrotic serum and nephrotic urine are illustrated in Figs. 3–5, the individual peaks being identified by subsequent paper electrophoresis in parallel with the original serum or urine. The α_1-globulin did not appear as a separate peak in these experiments. Table 4 gives the results of measurements of colloid osmotic

Table 4. *Osmotic pressures of the albumin fractions from serum and urine and the assessment of their contamination with globulins*

Material and eluate fraction no.	*C* Concn. of protein (g./100 ml. of solution)	*π* Colloid osmotic pressure (cm. H_2O at 25°)		Carbohydrate	Globulin components (immuno-electrophoretic analysis)
Normal serum I, fractions 4 and 5	3·20	15·0	—	Nil	—
	0·641	2·49	2·55		
	0·320	1·20	1·19		
Normal serum II, albumin peak fractions	1·49	6·26	6·25	Nil	Nil
	0·745	2·94	2·94		
	0·373	1·42	1·40		
Normal serum IV, albumin peak fractions	0·731	2·91	2·80	Nil	—
	0·365	1·42	—		
Nephrotic serum case III A, fractions 4 and 5	0·460	1·71	1·70	Nil	Nil
Nephrotic serum case III B					
Fraction 8	0·288	1·09	—	Nil	Nil
Fraction 9	0·688	2·65	—	Nil	Trace α_1
Fraction 10	0·439	1·73	—	Nil	α_1
Urine case III A					
Fraction 8	0·293	1·14	1·13	Nil	Nil
Fraction 9	0·586	2·30	2·29	+	Trace α_1
Urine case III B					
Fraction 17	0·392	1·49	1·50	Nil	Nil
Fraction 18	0·613	2·44	2·44	Nil	Nil
	0·307	1·17	—		
Fraction 19	0·728	3·12	3·06	Trace	α_1
	0·359	1·51	—		
Fraction 20	0·659	2·92	2·83	+	α_1
	0·329	1·45	—		
Nephrotic serum case IV					
Fraction 7	0·525	1·99	—	Nil	Nil
	0·262	0·95	—		
Fraction 8	0·917	3·62	—	Nil	Nil
	0·459	1·68	—		
Fraction 9	1·25	5·20	—	Nil	α_2
	0·624	2·47	—		
Fraction 10	0·933	4·05	—	Nil	α_2
	0·466	1·88	—		
Urine case IV					
Fraction 11	0·437	1·69	—	Nil	Nil
Fraction 12	0·390	1·49	—	Trace	Trace α_1
Fraction 13	1·03	4·28	—	Nil	α_1
	0·513	2·04			

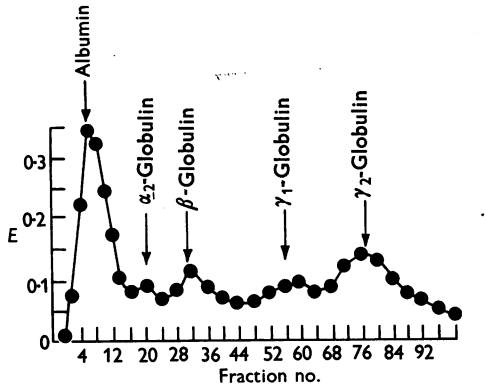


Fig. 3. Elution of normal serum protein fractions from an acetylated cellulose column after zone electrophoresis (normal serum I).

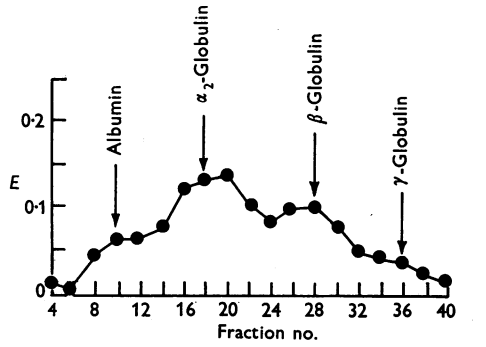


Fig. 4. Elution of nephrotic serum proteins as in Fig. 3 (serum case IIIB).

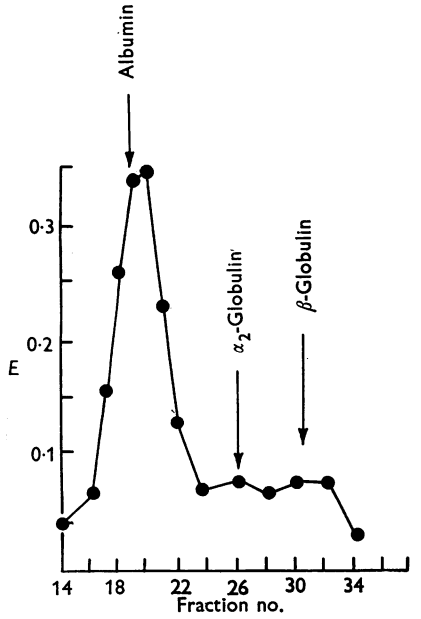


Fig. 5. Elution of nephrotic urine proteins as in Fig. 3 (urine of case IIIB).

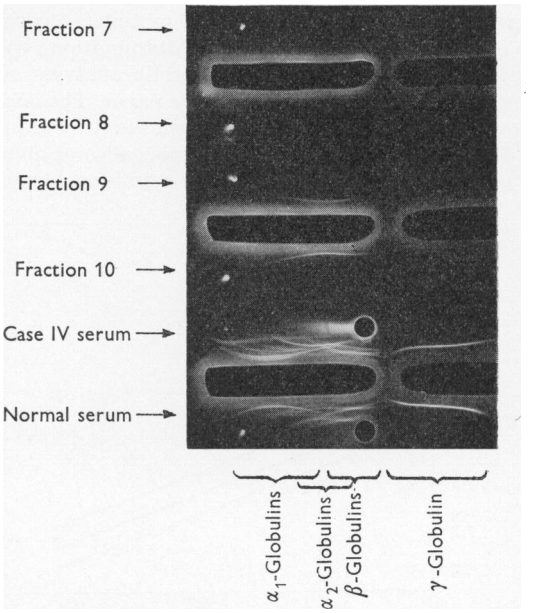


Fig. 6. An immunoelectrophoretic analysis of the albumin-containing fractions from nephrotic serum (case IV), an antiserum containing no anti-albumin being used. Analysis of the whole serum and a normal serum demonstrates the presence of precipitins to α_1- and α_2-globulins in the antiserum; α_2-globulin is detected as an impurity in the fractions 9 and 10.

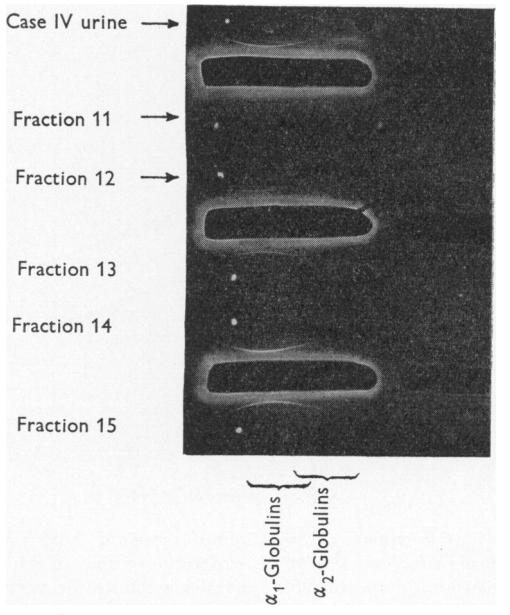


Fig. 7. An immunoelectrophoretic analysis of the albumin-containing fractions from nephrotic urine (case IV), similar to Fig. 9. α_1-Globulin is detected in fraction 12 onwards.

pressure and protein concentration on the albumin-containing fractions from these proteins, together with assessments of their contamination with globulins by immunoelectrophoretic analyses and by carbohydrate staining on filter paper. The latter method was used since the most likely contaminating protein, α_1-globulin, contains several glycoprotein components. Illustrative immunoelectrophoretic analyses, with an antiserum containing no anti-albumin, are shown in Figs. 6 and 7. These serum fractions differed from the remainder in that the first contaminating protein to appear was an α_2-globulin.

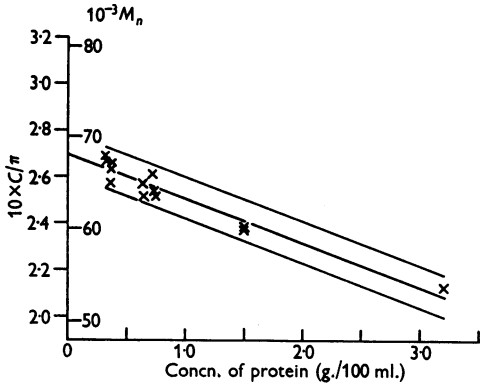


Fig. 8. C/π versus C for albumin prepared from three normal sera. The regression line and the 95% confidence limits of the observations are shown.

Fig. 8 is a plot of C/π against C for the pure albumin from the three normal individuals. A regression line has been fitted and the 95% confidence limits are also shown. The equation of the regression is

$$C/\pi \times 10 = 2{\cdot}697\ (\pm 0{\cdot}040) - 0{\cdot}188\ C,$$

$$\text{Mol.wt.} = 68\,200 \pm 1000.$$

The results from nephrotic serum albumin are plotted in Fig. 9, which also shows the 95% confidence limits of the measurements on normal serum albumin. Albumin fractions in which no globulin could be detected have been plotted as crosses, and the fractions with globulin contamination have been plotted as filled-in circles. Fig. 10 is a similar plot for the albumin of nephrotic urine. Both plots indicate that there are no significant differences between the values of C/π for normal and nephrotic albumin, and their molecular weights must therefore be regarded as identical within the experimental limits of these observations. The globulin-contaminated specimens, especially those from the urines, tend to have lower values of C/π at any given concentration, and so probably have a lower average molecular weight.

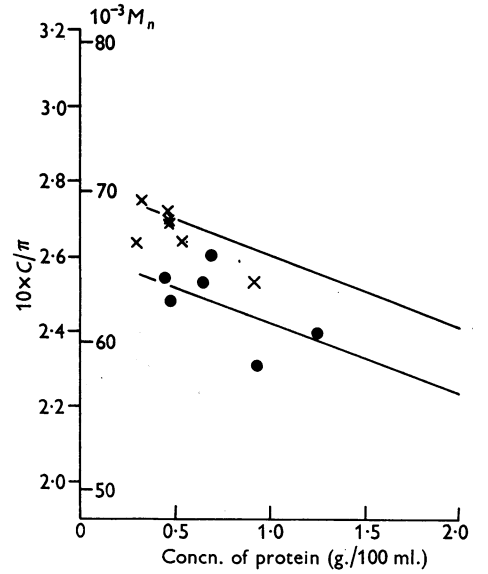


Fig. 9. C/π versus C for albumin prepared from three specimens (two cases) of nephrotic serum, shown in relationship to the 95% confidence limits for normal serum albumin. ×, Albumin fractions in which no impurity could be detected; ●, albumin contaminated with α-globulin.

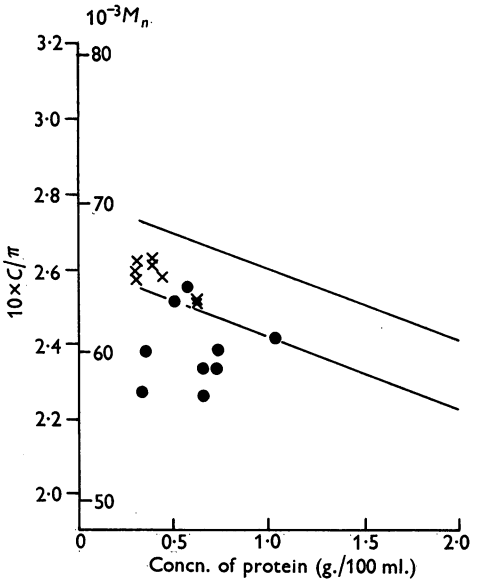


Fig. 10. The same as Fig. 9 except that the points refer to albumin prepared from three specimens (two cases) of nephrotic urine.

Average molecular weights of the serum and urine globulins

Normal and nephrotic serum proteins have average molecular weights higher than that of albumin, whereas nephrotic-urine proteins have lower average molecular weights. Hence the average molecular weight of the globulins in serum is greater and in urine less than that of albumin. As Figs. 3–7 show, it was not possible completely to separate the entire globulins from albumin by zone electrophoresis. Since direct measurements were therefore impossible an indirect method has been used. Adair & Robinson (1930) assumed that the osmotic pressures of mixtures of albumin and globulin obeyed Dalton's law of partial pressures.

$$\pi \text{ total} = \pi \text{ albumin} + \pi \text{ globulin}.$$

Since at infinite dilution

$$\pi = (C/M_n)RT,$$

$$\frac{C \text{ (total)}}{M_n \text{ (total)}} = \frac{C \text{ (albumin)}}{M_n \text{ (albumin)}} + \frac{C \text{ (globulin)}}{M_n \text{ (globulin)}}.$$

Table 5. *Number-average molecular weights of the globulins in serum and urine as derived from the average molecular weight of the total protein and the percentage of albumin present*

Protein		$10^{-3} M_n$ total protein	Albumin percentage	$10^{-3} M_n$ globulin
Normal serum I		78	59	100
Normal serum II		77	67	106
Normal serum III		78	56	95
Nephrotic serum				
Case I	A	177	24	354
	B	162	18	233
	C	99	36	132
	D	94	41	129
	E	95	50	158
Case II	A	102	40	153
	B	126	31	202
Nephrotic urine				
Case I	A	64	63	58
	B	57	59	46
	C	67	70	65
Case II	A	56	76	36
	B	59	62	49
	C	63	66	55
	D	58	65	45
Ultrafiltrates				
Normal serum II				
(1)		64	73	56
(2)		68	75	68
Nephrotic serum				
Case III		68	70	68
Nephrotic urine				
Case II	C	47	73	26
	D	53	67	36

The average molecular weights of the globulins have been calculated from this expression, a value of 68 000 for the molecular weight of albumin being assumed. The accuracy and validity of the results obtained depend largely upon the electrophoretic analysis. The filter-paper method had a reproducibility of ± 6 % of the total protein and so precluded high accuracy, and in addition there was the possibility that the nephrotic proteins had different dye-binding characteristics from normal proteins, thus giving systematically false results. With these objections in mind the values given in Table 5 are to be regarded as at best semi-quantitative. It is noticeable, however, that the three specimens of normal serum globulins show a small range of values, whereas nephrotic-serum globulins always show increased values which, like the average molecular weight of the whole serum proteins (Fig. 1), may be related to the severity of the condition. The urine globulins show relatively small and possibly insignificant variations, but all have lower average molecular weights than that of albumin. The globulins in the ultrafiltrates of serum are comparable with those of urine, and ultrafiltration of urine proteins also appears to effect a reduction in the average molecular weight of its globulins.

DISCUSSION

In this work protein concentrations have been derived from the estimated protein nitrogen concentration multiplied by 6·25. This is valid for albumin and γ-globulin (Tristram, 1953), but many of the α- and β-globulins include substantial carbohydrate or lipid prosthetic groups which contain little or no nitrogen. If the small proportion of prosthetic-group nitrogen be ignored, the protein concentrations and hence the average molecular weights derived here for mixtures containing α- and β-globulins refer to the polypeptide residues of these molecules.

The average molecular weights of normal serum proteins have been estimated from precise osmotic-pressure measurements by Adair, Adair & Greaves (1940) and Popják & McCarthy (1946). These investigators used a nitrogen-into-protein conversion factor, related to the albumin:globulin ratio, some 3 % higher than the factor used in this work. If their data are recalculated with the 6·25 factor the average molecular weight obtained by Adair *et al.* (1940) is 80 000 and that obtained by Popják & McCarthy is 82 000. These values compare with the results of 77 000–79 000 reported here.

Nephrotic-serum proteins. Nephrotic serum albumin to which immunological and chemical standards of purity were applied did not differ significantly in molecular weight from that of normal serum, and it appears possible that the reports of Charlwood

(1952) and Bourdillon (1939) may be explained by contamination with a globulin impurity of higher molecular weight. This result contrasts with the average molecular weights found for the whole serum proteins, which were always increased, in three instances being raised to more than twice the normal value (Fig. 1). The increases observed in different cases and during the phase of recovery in case I appeared to be related to the severity of the condition. The calculated average molecular weights of the globulins also appeared to be increased in every instance; this finding may be correlated with their altered electrophoretic distributions, which showed a preponderance of the α_2-fraction. This observation indicates the fallacy in calculating the colloid osmotic pressures of nephrotic sera from albumin and total globulin contents based on findings from normal sera.

Nephrotic-urine proteins. Precipitation occurred during the dialyses of some of the urine specimens. Subsequent work has shown that when normal serum is dialysed with urine the average molecular weight of the proteins is identical whether or not precipitation occurs. It is probable therefore that the average molecular weights of the nephrotic-urine proteins were unaffected by precipitation during dialysis. The purified urine albumin did not differ significantly in molecular weight from that of normal serum, and previously reported low values probably reflected a globulin impurity of low molecular weight. The average molecular weights of the whole urine proteins ranged from 56 000 to 67 000 and the variations could not be correlated either with simultaneous variations of the serum proteins or with the severity of the condition. Protein cleavage in the urine could not be entirely excluded, but the fact that ultrafiltrates from normal and nephrotic serum were comparable both in average molecular weights and in immunoelectrophoretic analyses with the urine proteins made it unlikely that protein breakdown in the urine is the major cause of these low values.

The average molecular weights calculated for the urine globulins were invariably less than those of serum albumin. The probable low molecular weight of the fractionated material in the albumin α_1-region (Table 5 and Fig. 10) suggests that the urine α_1-globulin is comprised of proteins of low molecular weight. Several α_1-glycoproteins having high carbohydrate contents and molecular weights based on dry weights and sedimentation coefficients of about 45 000 have been isolated from normal serum by Schultze, Göllner, Heide, Schönenberger & Schwicke (1955). Had the contribution of the carbohydrate been ignored, as in the results reported here, the derived molecular weights would have been about 30 000, and it seems likely that these proteins form a high proportion of the α_1-globulin fraction of the urine proteins. The urinary α_2- and β-globulins may likewise represent only the lower molecular-weight members of the groups of proteins having these mobilities in the serum. The immunoelectrophoretic analyses were consistent with this hypothesis by showing fewer antigenic components with the mobilities of α- and β-globulins in the urine than in the serum (Fig. 2).

Certain conclusions may be drawn concerning the mechanism of proteinuria in these cases. The identity in terms of molecular weight of albumin from nephrotic serum and urine with albumin from normal serum implies that the increased excretion of this protein is not the result of a reduction of its molecular dimensions, but rather that the abnormality resides in the kidney. The nephrotic kidney, however, even during heavy proteinuria, retains to a remarkable degree its characteristic of selective molecular filtration. This selectivity may be regarded as a function of the glomerular membrane, and since the ultrafiltration experiments showed that it may be reproduced by a suitable membrane *in vitro*, it is not necessary to invoke tubular activity to account for the composition of the urine protein mixture. That the nephrotic glomeruli retain a high degree of selectivity does not necessarily imply that this aspect of their function is entirely normal, especially since the selectivity of the normal glomerulus as defined by the relative transmissibility of various sized proteins into its filtrate is as yet unknown.

SUMMARY

1. The number-average molecular weights of the proteins of three normal human sera lay between 77 000 and 79 000.

2. The number-average molecular weights of the serum proteins from four cases of the nephrotic syndrome ranged between 177 000 and 90 000 and the increase could be related to the severity of the clinical condition.

3. The number-average molecular weights of the urine proteins from the same cases ranged between 54 000 and 67 000, and showed no systematic relation to the clinical condition.

4. Protein mixtures resembling those of nephrotic urine in electrophoretic and immunoelectrophoretic analyses and in number-average molecular weight were prepared from normal and nephrotic sera by ultrafiltration through membranes of appropriate permeability.

5. Purified albumin prepared from nephrotic serum and urine had the same molecular weight as that from normal serum.

I am indebted to Dr P. G. H. Gell for the immunoelectrophoretic analyses, to Dr J. Hardwicke for help with the quantitative filter-paper electrophoretic analyses, and to

Dr D. R. Stanworth for the carbohydrate stains and for an analysis in the ultracentrifuge. It is a pleasure to acknowledge the advice and encouragement of Professor J. R. Squire. This work was done during the tenure of a grant from the Medical Research Council.

REFERENCES

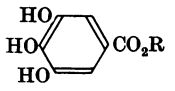
Adair, G. S., Adair, M. E. & Greaves, R. I. N. (1940). *J. Hyg., Camb.*, **40**, 548.
Adair, G. S. & Robinson, M. E. (1930). *Biochem. J.* **24**, 1864.
Bayliss, L. E., Kerridge, M. T. & Russell, D. S. (1933). *J. Physiol.* **77**, 386.
Bourdillon, J. (1939). *J. exp. Med.* **69**, 819.
Brewer, D. B. (1951). *Proc. R. Soc. Med.* **44**, 557.
Burtin, P. & Grabar, P. (1954). *Sem. Hôp. Paris*, **30**, 1.
Campbell, P. N. & Stone, N. E. (1956). *Biochem. J.* **62**, 9P.
Charlwood, P. A. (1952). *Biochem. J.* **52**, 279.
Flodin, P. & Porath, J. (1954). *Biochim. biophys. Acta*, **13**, 175.
Gell, P. G. H. (1955). *J. clin. Path.* **8**, 269.
Grabar, P. & Williams, C. A. (1953). *Biochim. biophys. Acta*, **10**, 193.
Hardwicke, J. (1954*a*). *Proc. R. Soc. Med.* **47**, 832.
Hardwicke, J. (1954*b*). *Biochem. J.* **57**, 166.
Kiöw, E. & Grönwall, A. (1952). *Scand. J. clin. Lab. Invest.* **4**, 244.
Ma, T. S. & Zuazaga, G. (1942). *Industr. Engng Chem. (Anal.)*, **14**, 280.
Popják, G. & McCarthy, E. F. (1946). *Biochem. J.* **40**, 789.
Rowe, D. S. (1956). *J. Physiol.* **134**, 1P.
Rowe, D. S. & Abrams, M. E. (1957). *Biochem. J.* **67**, 431.
Schultze, H. E., Göllner, I., Heide, K., Schönenberger, M. & Schwicke, J. (1955). *Z. Naturf.* **106**, 463.
Squire, J. R., Blainey, J. D. & Hardwicke, J. (1957). *Brit. med. Bull.* **13**, 43.
Tristram, G. R. (1953). In *The Proteins*, vol. 1A, p. 215. Ed. by Neurath, H. & Bailey, K. New York: Academic Press.
Wallenius, G. (1954). *Acta Soc. Med., Upsalien*, suppl. 4.
Wells, H. S. (1932). *Amer. J. Physiol.* **101**, 409.
Widdowson, E. M. (1933). *Biochem. J.* **27**, 1321.

The Transformation of Gallates into Ellagate

By D. E. HATHWAY
The British Leather Manufacturers' Research Association, Milton Park, Egham, Surrey

(*Received* 1 *April* 1957)

Herzig, Pollak & Bronneck (1908) demonstrated that aeration of an ammoniacal solution of ethyl gallate (I) yielded ellagic acid (V), and recently humic acid and a trace of hydrogen peroxide were also found (Hathway, 1957) amongst the ultimate products of reaction. In this study, 4:5:6:4′:5′:6′-hexahydroxydiphenic acid was shown to be the chemical precursor of ellagic acid, and gallic acid the chemical precursor of the humic acid by-product. It is now suggested that the principal reaction may

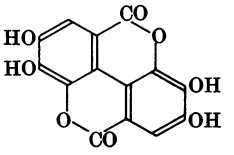


Gallic acid (R = H)

(I), where R = Et.

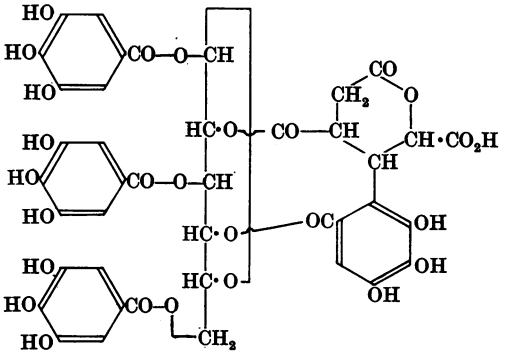
(II), where R = Me.

(III), where R = β-D-glucose-1-.



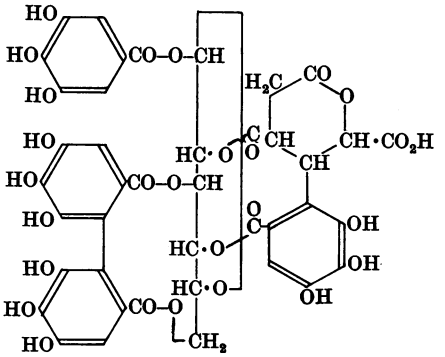
Ellagic acid

(V)



Chebulinic acid

(IV)



Chebulagic acid

(VI)