CLXXIX. A COMPARATIVE INVESTIGATION OF URINE- AND SERUM-PROTEINS IN NEPHRITIS.

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(Received June 30th, 1933.)

WHILST various attempts have been made to solve the problem as to whether the passage of protein through the kidney involves structural alteration of the molecule, no complete investigation on the subject has been carried out.

Hynd [1925] found close agreement in specific rotation between the serum- and urine-albumin in the albuminuria of pregnancy except for certain cases of eclampsia in which the urinary albumin was apparently identical with cow's lactalbumin. His results, however, are not reliable since his proteins were not entirely lipid-free, and the solutions which he used for the optical rotation measurements were so dilute that in some cases his experimental error was probably nearly 30 %.

Hewitt [1927, 1; 1929] observed that albumins from the serum and urine of patients with chronic nephritis and albuminuria were identical in optical rotatory power and concluded that serum-albumin probably leaks through the kidney unchanged. He could not confirm the results of Hynd [1925]. Hewitt's method of separation of the albumins from serum and urine appears to be satisfactory, but his investigation of these proteins was confined to observations of their specific rotatory powers. Also, this worker makes no attempt to isolate his albumins in the solid form, but relies upon determinations of nitrogen in their solutions for the calculation of the protein concentration, apparently assuming that the proteins are sufficiently pure for the nitrogen percentage in them to be taken as equal to the theoretical value.

A study of the physical properties of proteins as a means of their identification has been carried out by various workers.

Hardy and Gardiner [1910] and Young [1922] have shown that the optical rotation of proteins differs with variations in the p_H of the solution, and Woodman [1921] has demonstrated that the optical rotation of a protein in alkaline solution falls for about 2 weeks. If the rotation is plotted against time, a definite curve is obtained. This worker suggests that this racemisation curve is a most satisfactory means of identifying a protein.

Cavett and Gibson [1931] have shown that the racemisation curves given by albumins and pseudoglobulins from nephritic urines are similar to those of the corresponding serum-proteins. These workers, however, appear to have assumed that a single fractional precipitation is sufficient completely to separate the albumin and the globulin in the mixed protein. Experience has shown that at least three or four fractional precipitations with ammonium sulphate are necessary if the proteins are to be obtained in a purified form, and it is of interest to note that all proteins relied upon by Woodman for his work on racemisation were fractionally precipitated seven times, while Hewitt [1927, 1, 2; 1929], who investigated the optical rotation of urinary albumin, precipitated his protein three times with ammonium sulphate.

Govaerts [1924; 1927; 1929] and Verney [1926] have investigated the osmotic pressures of the serum-proteins from nephritic patients. Both these workers, however, appear to have confined their attention to a study of the osmotic pressure of whole serum, and no evidence of an attempt to determine the osmotic pressure of solutions of the separated serum-proteins could be found in the literature.

Schretter's [1926] survey of previous work on the specific refractions of proteins in normal and pathological sera shows that the results obtained by various workers differ widely one from another, probably owing to the impure proteins employed by the different workers. Adair and Robinson [1930] have carried out a very careful study of the specific refraction increments of recrystallised albumin and globulin from horse-serum, and they have shown that the value is a constant for any given protein.

It was thought that it would form a most interesting study to investigate the identity of the urine- and serum-proteins of nephritic patients by means of the Van Slyke [1911] nitrogen distribution method and further to examine these proteins by a study of their racemisation curves, osmotic pressures and specific refractions. It was hoped that by combining the results obtained by chemical and physical means to obtain definite information as to whether the protein is excreted as a foreign body or whether it leaks through the kidney unchanged.

Source of material.

A normal healthy man was selected as a source of material for the normal plasma-proteins, whilst the blood and urine in cases of proteinuria were obtained from in-patients of the Middlesex Hospital.

S. B. This patient was a man, aged 57, who had been under observation for some 3 years. He was a typical case of nephrosis with marked oedema, a high blood-cholesterol and massive proteinuria. During the period that the specimens were collected there was no evidence of interstitial changes in the kidney. The patient subsequently developed these, and at autopsy the kidney showed severe parenchymatous degeneration with superadded interstitial changes.

The other four cases, H. P., H. F., D. J. and E. F., were suffering from what would be described as chronic parenchymatous nephritis associated with long-standing proteinuria. The patients had all given a history of acute or sub-acute nephritis, and were clinically and biochemically distinct from the case of S. B.

Preparation of proteins.

Urine-proteins. The method employed for the precipitation and purification of the urine proteins was similar to that used by Hewitt [1929]. The fresh urine was filtered and 2.3 times its volume of saturated ammonium sulphate solution was added. The p_H was adjusted to 4.7 by the addition of acetic acid, and the precipitation was allowed to continue overnight. The precipitate was then separated by means of the Sharples centrifuge, ground up with a small quantity of water and added gradually and with constant shaking to 10 times its volume of a mixture of absolute alcohol (7 volumes) and ether (3 volumes) cooled to a temperature of -5° . The mixture was allowed to stand at a low temperature overnight and the precipitated proteins filtered off on a Büchner funnel, washed thoroughly, first with alcohol and ether mixture and finally with ether alone. The precipitate was then transferred to a Soxhlet apparatus and extracted with dry ether for 24 hours, any alcohol present in the ether being removed by the presence in the flask of a small piece of sodium. Both the ether and the sodium were renewed at the end of the first 2 hours. When the extraction was complete the proteins were spread in the air to dry.

Serum-proteins. The serum, prepared by the usual laboratory method, was poured slowly into 10 times its volume of a mixture of alcohol (7 parts) and ether (3 parts) cooled to -5° as recommended by Hewitt [1927, 1, 2]. The precipitation was allowed to proceed to completion overnight, and the precipitated proteins were treated in a manner similar to that described for the urine-proteins.

Separation of albumin and globulin.

Urine-proteins. The mixed proteins were ground in a mortar with a small quantity of distilled water until they had become thoroughly moistened, the quantity of water being gradually increased to a suitable volume, and the solution was then neutralised by the addition of a few drops of ammonia. The clear solution thus obtained was filtered from the small amount of insoluble material which remained and treated with half its volume of saturated ammonium sulphate solution. After the precipitation had continued overnight the globulin was filtered off on a fluted paper and the filtrate half-saturated with ammonium sulphate. In most cases there was no further precipitate, but where a turbidity was produced the solution was filtered and the precipitate discarded. The precipitation of globulin at $\frac{1}{2}$ saturation with ammonium sulphate, instead of at the more usual $\frac{1}{3}$ saturation, lessens the possibility of contamination of the precipitate with albumin, since the dry mixed protein contains a considerable percentage of ammonium sulphate which increases the concentration of this salt beyond the calculated value.

Sufficient ammonium sulphate solution was next added to the filtrate to render the final concentration of the salt 70 %, and the p_H was adjusted to 4.7 by the addition of acetic acid. Next morning the precipitated albumin was filtered off on a fluted paper. The globulin and albumin were redissolved in water and reprecipitated 3 times at concentrations of $\frac{1}{3}$ and 70 % saturation with ammonium sulphate.

After the final filtration the precipitates were taken up with water and the solutions dialysed through cellophane bags against running water for 3 days, then against distilled water which was changed daily until the protein solution was free from ammonia. The dialysed solution was filtered and evaporated down to small bulk *in vacuo* at 40°, the solution being allowed to drop into the distilling flask, from a funnel drawn out to a capillary, as fast as the water was removed by distillation. The concentrated solution of pure protein was added drop by drop to a mixture of alcohol (7 parts) and ether (3 parts) cooled to -5° as before, and, after standing for some hours, the proteins were filtered off on a Büchner funnel, dried *in vacuo* over sulphuric acid and weighed.

Serum-proteins. The precipitated serum-proteins were separated by a method similar to that employed for the urine-proteins, but all precipitations were carried out in a 100 cc. centrifuge-tube, and the precipitates were centrifuged instead of being removed by filtration. Globulin was precipitated 4 times at $\frac{1}{3}$ saturation with ammonium sulphate. After each precipitation of the globulin, the concentration of ammonium sulphate was increased to 50 %. In some cases a very small precipitate was formed but never in sufficient quantity to warrant further investigation. It was therefore removed by filtration, the ammonium sulphate concentration was increased to 70 % and the albumin precipitate centrifuged off, taken up with water and reprecipitated 3 times. The solutions of the separated proteins were dialysed as before, then filtered, and the water was removed by leaving the solutions in a vacuum desiccator over sulphuric acid for about a week.

It will be observed that the above method of isolation of the proteins from serum or from urine makes no attempt to separate the two globulins, euglobulin and pseudoglobulin, by fractional precipitation. According to various workers [see Hartley, 1914; Woodman, 1921; Cavett and Gibson, 1931] euglobulin is precipitated at $\frac{1}{3}$ saturation with ammonium sulphate, and pseudoglobulin at $\frac{1}{2}$ saturation with this salt. It has already been pointed out, however, that it is difficult to ascertain exactly the ammonium sulphate concentration of the solution during the process of fractional precipitation, and it was thought that it would be more satisfactory to carry out analyses on the total globulin rather than to attempt a separation of the two globulins which would certainly be most unreliable. Hartley [1914] has shown that analyses of euglobulin and pseudoglobulin failed to reveal any striking differences in the chemical composition of these two substances.

Analysis of urine- and serum-proteins by the Van Slyke nitrogen distribution method.

The small amounts of serum-albumin and globulin available for analysis necessitated the use of a micro-method for the determination of the Van Slyke [1911] nitrogen distribution. The procedure adopted was that of Narayana and Sreenivasaya [1928] with the modifications that the ammonia-N was determined by the method of Plimmer and Rosedale [1925] and the arginine-N by a micro-adaptation of the method of Koehler [1920].

Table I. *Comparative analyses of urine-albumin by the micro- and macro-methods of determination of the nitrogen distribution.*

(Results expressed as percentage of total N.)

	Duplicate analyses by the micro-method		Analyses by Plimmer and Rosedale's modification of Van Slyke's method
	1	2	
Ammonia-N	6.63	6.28	6.40
Humin-N	1.12	1.25	1.49
Arginine-N	7.99	7.82	7.54
Histidine-N	7.01	6.53	5.09
Cystine + lysine-N	14.44	14.77	20.50
Amino-N of filtrate	60.34	61.16	58.29
Non-amino-N of filtrate	2.02	1.88	1.53
Total N recovered	99.55	99.69	100.84

The results of these determinations are shown in Table I and it will be observed that the agreement between the results for the duplicate analyses by the micro-method is fairly close. Apart from the histidine, which is a calculated percentage and which is therefore most liable to error, the variation is always less than 0.5 % of the total nitrogen. Certain differences between the results of the analyses by the micro- and macro-methods are, however, apparent. The micro-method gives results for the cystine- and lysine-nitrogen which are about 6 % lower than those obtained by the macro-method, while the value for the non-basic nitrogen is definitely higher. This difference is explained by the fact that the precipitation of the bases in the micro-method is carried out in a more dilute solution than when the experiment is performed on a macro-scale. Hence a larger correction for the solubility of the bases must be applied in the case of the small scale experiment. Since no figures are available for this correction, and since it did not fall within the scope of this work to determine them, it was decided that in order to obtain comparative results for the analyses of the urine- and serum-proteins it would be necessary to carry out all the analyses by the micro-method.

The results of these analyses are shown in Tables II and III, being expressed as percentages of the total nitrogen in the protein.

It will be observed that there are no very striking differences between the values obtained for urine-protein and those of the corresponding serum-protein in patients suffering from nephritis or nephrosis. From this it may be concluded that there is no chemical alteration in the structure of the protein during its passage through the kidney.

Table II. *Analyses of urine- and serum-albumins by the Van Slyke nitrogen distribution method.*

(Results expressed as percentage of total N.)

	S. B.		H. P.		H. F.		D. J.		E. F.	Normal
	Urine- albumin	Serum- albumin	Urine- albumin	Serum- albumin	Urine- albumin	Serum- albumin	Urine- albumin	Serum- albumin	Urine- albumin	serum- albumin
Ammonia-N	6.63	6.25	7.66	7.08	6.97	7.13	7.40	7.37	7.38	7.31
Humin-N	1.12	1.48	0.70	1.50	0.34	0.73	0.40	0.83	0.66	0.47
Arginine-N	7.99	28.93	7.80	8.01	8.33	7.83	8.64	8.57	8.00	8.22
Histidine-N	7.01		7.52	7.68	6.32	6.56	6.34	5.76	7.66	6.83
Cystine + lysine-N	14.44		11.75	11.25	13.44	12.18	11.93	11.93	11.76	12.38
Amino-N of filtrate	60.34	60.86	61.08	61.88	60.97	61.57	61.33	61.93	59.89	60.60
Non-amino-N of filtrate	2.02	2.97	3.16	2.43	2.86	3.37	2.94	3.04	3.54	3.55
Total N recovered	99.55	100.49	99.67	99.83	99.23	99.37	98.98	99.43	98.89	99.36

Table III. *Analyses of urine- and serum-globulins by the Van Slyke nitrogen distribution method.*

(Results expressed as percentage of total N.)

	S. B.		H. P.		H. F.		D. J.		E. F.	Normal
	Urine-globulin	Serum-globulin	Urine-globulin	Serum-globulin	Urine-globulin	Serum-globulin	Urine-globulin	Serum-globulin	Urine-globulin	serum-globulin
Ammonia-N	11.46	11.89	10.42	8.65	9.80	9.27	9.02	9.04	9.79	9.25
Humin-N	0.96	1.52	1.70	0.41	1.10	0.56	0.82	0.74	1.62	0.53
Arginine-N	9.70	10.07	10.22	10.01	10.20	10.49	11.46	11.57	10.98	10.67
Histidine-N	4.72	5.18	5.21	4.52	5.24	4.23	2.54	2.80	2.96	4.21
Cystine+lysine-N	7.16	7.08	7.78	9.63	8.31	7.46	9.86	10.33	9.28	9.78
Amino-N of filtrate	63.37	61.46	58.70	60.88	60.82	63.82	63.52	61.93	58.22	61.32
Non-amino-N of filtrate	3.08	3.16	5.43	5.84	3.58	3.71	2.76	2.76	6.70	3.57
Total N recovered	100.45	100.36	99.46	99.94	99.05	99.54	99.98	99.17	99.55	99.33

A comparison of the analyses of the proteins from different patients shows some very interesting results. The albumins and globulins in the urine and serum of patients suffering from nephritis are apparently identical with those of normal human serum. In the case of nephrosis, however, certain slight differences are apparent. The corresponding urine- and serum-proteins are identical, but the analyses of these differ in certain respects from those of the proteins from normal serum or from the urine or serum of patients who are suffering from nephritis. The most marked difference is seen in the percentage of ammonia-N. This in the case of the urine- and serum-albumin from a patient suffering from nephrosis is about 1 % lower than the mean value obtained from normal serum-albumin and nephritic urine- and serum-albumin. The corresponding value for globulin in nephrosis is about 2 % higher than the normal value. Slight differences are also noted in the percentage of nitrogen in the basic and non-basic fractions. The basic nitrogen for albumin has a value 2 % higher in the case of nephrosis, while the basic nitrogen in the globulin is about 2 % lower than in the normal protein. These differences are small, and since only one case of nephrosis was studied, no definite statement can be made regarding this apparent slight alteration in the chemical structure of the urine- and serum-proteins in nephrosis.

The racemisation curves of urine- and serum-proteins.

The racemisation of the proteins was investigated by a method similar to that employed by Cavett and Gibson [1931].

About 0.4 g. of the urine-protein was shaken with 10 cc. of distilled water and left to stand overnight. 10 cc. of *N* NaOH were then added, and the mixture was well shaken and filtered through a pad of asbestos in a small Gooch crucible. By these means about 20 cc. of a clear solution consisting of 2 % protein in *N*/2 NaOH were obtained.

Polarimeter readings were taken at intervals of a few hours during the first day, and subsequently only once every 24 hours, and the racemisation was allowed to proceed for 10 days. A Schmidt and Haentsch polarimeter was employed, and a Zeiss electric sodium vapour lamp was used as a source of light ($\lambda = 5893 \text{ \AA.}$). This lamp, whose intensity is about 50–100 times as great as that of a Bunsen burner with rock salt, enabled the polarimeter readings to be taken with ease and accuracy, and without any undue strain on the eyes.

After each reading, the 10 cc. of protein solution in the polarimeter-tube were added to the remainder of the same solution in a test-tube, which was then stoppered and incubated at 38° throughout the racemisation period. When

Fig. 2. Racemisation of serum-albumins in alkaline solution.

Fig. 4. Racemisation of serum-globulins in alkaline solution.

Fig. 1. Racemisation of urine-albumins in alkaline solution.

Fig. 3. Racemisation of urine-globulins in alkaline solution.

it was desired to take another reading, the solution was removed from the incubator, cooled in running water, and the clean dry polarimeter-tube was refilled. This procedure makes it possible to carry out a series of readings on solutions of several proteins simultaneously, even though only one polarimeter-tube is available. The 20 cc. of protein solution originally prepared were sufficient to fill the tube 10 or 11 times provided that care was taken during the filling process.

In the case of the serum-proteins, where only a small amount of material was available, a micro-polarimeter-tube was employed, 8 cm. in length and of 0.15 cc. capacity.

About 0.1 g. protein was dissolved in 5 cc. $N/2$ NaOH and the solution was filtered. The polarimeter-tube was freshly filled between each reading as before.

The specific rotatory power of the protein at each stage during the racemisation period was calculated from the mean value of 10 polarimeter readings. At the end of the experiment an aliquot portion of the protein solution was diluted to 5 times its volume and the total N determined on duplicate portions of 2 cc. by the micro-Kjeldahl method. The percentage of total N in the dry protein having been previously estimated, the concentration of protein in the solution could be calculated. From these results the specific rotatory powers could be determined, and these are shown in Figs. 1-4 where specific rotatory power is plotted against time of racemisation.

There is evidently no striking difference between the optical rotation of the urine-protein and that of the corresponding serum-protein in patients suffering from nephritis or nephrosis. These results confirm those which were obtained from the chemical investigation of the same proteins and indicate that there is no alteration in the structure of the protein during its passage through the kidney.

It will be observed from Fig. 1 that the points plotted for the racemisation of urine-albumin from five different patients suffering from nephritis or nephrosis all lie on the same curve as those for normal serum-albumin. Similar results are obtained for the serum-albumins (Fig. 2), the urine-globulins (Fig. 3) and the serum-globulins (Fig. 4). Hence the albumins and globulins in the urine and serum of patients suffering from nephritis are apparently identical with those of normal human serum, so far as their optical rotations are concerned. Certain slight differences in the chemical structure of the proteins in the case of nephrosis have already been noted. A similar difference was not apparent in the optical properties of the proteins. The albumins and globulins from the urine and serum of a patient suffering from nephrosis are apparently physically identical with those of normal human serum.

It is probable therefore that in nephrosis there is some alteration in the arrangement of the amino-acids in the serum- and urine-proteins, but this difference is not detectable by a measurement of their optical rotations.

Measurement of the osmotic pressures of urine- and serum-proteins.

The apparatus used for the measurement of the osmotic pressures of solutions of albumin and globulin from urine and serum was similar to that described by Verney [1926], except that one osmometer only was used, instead of the series employed by him. A water manometer was used to measure the osmotic pressure, and a disc of cellophane (substance 300) was employed as the semi-permeable membrane.

The precautions which Verney took to sterilise the parts of his apparatus

were found to be unnecessary, when, instead of serum, a solution of purified protein was used. The metal parts were coated with a thin layer of paraffin wax before the commencement of the experiment.

The albumin or globulin was dissolved in 0.9 % sodium chloride solution so that the protein concentration was approximately 6 %, and 0.9 % sodium chloride solution was used as the external liquid. Verney's procedure was followed exactly, and the osmosis was allowed to continue for 24 hours. After taking the manometer reading the protein solution was removed and the total N determined. Duplicate osmotic pressure determinations for each protein were made, and the results were expressed as osmotic pressure (cm. water) per g. protein per 100 cc. They are shown in Table IV.

Table IV. *Osmotic pressures of urine- and serum-proteins.*

(Calculated per g. protein per 100 cc.)

	O.P. of urine- albumin	Temp. ° C.	O.P. of serum- albumin	Temp. ° C.	O.P. of urine- globulin	Temp. ° C.	O.P. of serum- globulin	Temp. ° C.
S. B.	8.810	15	8.973	16	1.704	16	1.763	18
H. P.	9.400	17	—	—	1.796	16	1.754	17
H. F.	7.868	15	8.240	18	1.758	15	1.745	18
D. J.	8.596	16	8.058	17	1.719	16	1.678	17
E. F.	8.775	15	—	—	1.765	15	—	—
Normal	—	—	8.862	17	—	—	1.769	18

It will be observed from the results that the osmotic pressure per g. urine-albumin per 100 cc. is almost identical with the osmotic pressure of the corresponding serum-albumin, and similar results are obtained for the urine- and serum-globulins. The variation in the values for the osmotic pressures of the proteins from the different patients is very small. A mean value of 1.75 cm. water per g. protein per 100 cc. is obtained for the osmotic pressure of urine- and serum-globulins, while the corresponding value for the albumins is about 8.65 cm.

Govaerts [1927] obtained values for the osmotic pressures of serum-globulin and albumin of 1.95 and 7.54 cm. water respectively. His values were not obtained by direct measurement however but were calculated from a knowledge of the osmotic pressure, the protein-N content and the albumin/globulin ratio of the serum. Von Farkas [1927] obtained values of 2.51 and 6.80 cm. water per g. globulin and albumin per 100 cc. respectively.

The results shown in Table IV for the osmotic pressure per g. globulin per 100 cc. are a little lower than those obtained either by Govaerts or by von Farkas, while the corresponding values for albumin are slightly higher than those found by these other workers.

Govaerts [1927] states that the values obtained by him are merely empirical values, obtained as the result of observations, and must not be regarded as physico-chemical constants. Howe's [1924] method, which was employed by Govaerts for the determination of the albumin/globulin ratio, cannot be considered to be quantitatively accurate. A small error in the protein separation will make a considerable difference to the albumin/globulin ratio and consequently to the calculated osmotic pressure for each protein. The method employed in the present instance for the fractional precipitation of the urine- and serum-proteins and the use of solutions of the separated proteins for the osmotic pressure determinations eliminate all error due to an incomplete separation, and the osmotic pressure measurements are accurate to within 3 %. Govaerts considers his results to have an accuracy of 10 %.

The specific refractions of urine- and serum-proteins.

The specific refractions of the urine- and serum-proteins were determined by means of a Zeiss portable interferometer. The instrument was first calibrated by means of sodium light, but white light was used for all subsequent measurements. The proteins were ground up with 0.9 % sodium chloride solution, filtered through a pad of asbestos, and the clear solutions were used for the specific refraction determinations. Sodium chloride solution, of exactly the same strength as that used for dissolving the protein, was placed in the comparison chamber of the apparatus. After the interferometer readings had been taken, total N was determined on duplicate 1 cc. portions of the protein solution, and from the results the percentage of protein in the solution was calculated.

The specific refraction of each solution was calculated and hence the specific refraction of a 1 % solution of protein in 0.9 % sodium chloride solution could be determined.

Table V. *Specific refractions of urine- and serum-proteins.*

(Calculated for a 1 % solution.)

	Urine-albumin	Serum-albumin	Urine-globulin	Serum-globulin
S. B.	0.001965	—	0.002053	0.002132
H. P.	0.001982	—	0.001923	0.002067
H. F.	0.002000	0.001977	0.001923	0.001905
D. J.	0.001992	0.002071	0.002261	0.002198
E. F.	0.001914	—	0.002155	—
Normal	—	0.001968	—	0.002053

The results of these measurements are shown in Table V, and it will be observed that there are no striking differences between the specific refractions of the urine- and serum-albumins, or of the urine- and serum-globulins. The values for the albumins vary over a very small range, from 0.00191 to 0.00207. The variation in the values for the urine- and serum-globulins is somewhat greater, the figures ranging from 0.00191 to 0.00226. The values obtained for the urine- and serum-proteins of any one patient, however, are in close agreement.

Adair and Robinson [1930] obtained a mean value of 0.00181 for the specific refraction increment of recrystallised horse serum-albumin, while their corresponding value for globulin was 0.00186. Other workers [Starlinger and Hartl, 1925; Schretter, 1926] have investigated human serum-proteins and have obtained a mean value of 0.00200 for the specific refraction of albumin and 0.00219 for human serum-globulin.

Thus, the results obtained from the present experiments agree fairly closely with those obtained by other workers.

The variation in the specific refraction of the proteins studied is sufficiently small to warrant the conclusion that there is no alteration in the physical structure of the serum-proteins in nephritis or in nephrosis so far as can be determined by measurements of their specific refractions.

DISCUSSION.

The results of the investigations which have been made on the nitrogen distribution, racemisation, osmotic pressure and specific refraction of urine- and serum-proteins from nephritic patients indicate that the corresponding urine- and serum-proteins from any one patient are identical.

In ordinary nephritis and in prolonged proteinuria there appears to be no alteration in the structure of the proteins. This fact seems to decide once and for all that there is no defect in protein synthesis in these cases.

As judged by physical methods the proteins from patients suffering from nephritis and nephrosis appear to be the same. Chemically, however, they are distinct, though the differences noted are not very great. Since only one case of nephrosis was studied, no great emphasis can be laid on this slight difference in chemical structure.

Since the physical characteristics of the proteins are the same in all cases studied, it appears unlikely that these slight chemical differences in the case of nephrosis are of any clinical importance.

SUMMARY.

1. The albumins and globulins from the urine and serum of nephritic patients, and from normal human serum, have been separated by fractional precipitation with ammonium sulphate.

2. The separated albumins and globulins were analysed by a micro-modification of the Van Slyke nitrogen distribution method. Measurements of the optical rotation and racemisation, osmotic pressure and specific refraction of the proteins have also been made.

3. The results of these analyses indicate that the proteins isolated from the urine of patients suffering from nephritis or nephrosis are identical with the corresponding serum-proteins and there is no evidence of any alteration in the chemical or physical structure of the proteins during their passage through the kidney.

4. The proteins isolated from the urine and serum of patients suffering from nephritis appear to be identical in chemical and in physical structure with those of normal human serum.

5. The urine- and serum-proteins of a patient suffering from nephrosis exhibit certain slight differences in chemical structure from the normal serum-proteins. The racemisation curves, osmotic pressures and specific refractions of the urine- and serum-proteins of a patient suffering from nephrosis, however, appear to be identical with those of the proteins from normal human serum.

The author wishes to thank Prof. E. C. Dodds for his advice and encouragement, the Council of the Middlesex Hospital Medical School for the hospitality afforded and Mr M. E. H. Fitzgerald for his assistance with the practical work.

Part of the expense of this work was defrayed by a grant from the Thomas Smythe Hughes Medical Research Fund.

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