CXLVII. THE BEHAVIOUR OF URINARY PROTEINS IN THE ULTRACENTRIFUGE.

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THE question whether the proteins found in the urine in certain diseases have the same composition as the proteins in the blood is one of great importance. With the probable exception of two conditions, *viz.* orthostatic and Bence Jones proteinuria, it is believed that the presence of protein in the urine may be taken as a sign of impaired renal function. If the urinary protein is the same as the plasma protein it is reasonable to assume that the disease has damaged the kidneys in such a way as to give rise to a form of leakage of plasma into the urinary tract. If the protein is different, leakage of a gross type can be excluded and it is not at all clear how the protein finds its way into the urine. We may assume that the composition of the plasma protein is changed in the renal cells by the disease process at the same time as the protein is excreted; or we may postulate that the altered urinary proteins arise in remote parts of the circulation and are excreted by normal kidneys. Since the presence of Bence Jones protein in the blood has been demonstrated in certain cases, the group of diseases with which this protein is characteristically associated has been regarded as unique (if we except the orthostatic albuminurias) in conforming to the latter hypothesis, although a few observers [Forbes *et al.*, 1933] identify renal changes with Bence Jones proteinuria. Kerridge and Bayliss [1932] have shown that Bence Jones protein readily passes through the kidneys of the cat while normal human serum proteins do not. Kerridge [1934] has shown also that the urinary proteins from ten cases of chronic nephritis did not pass through the kidneys of the cat. The inference from this (which incidentally Kerridge avoids making) is that the proteins in the urine in chronic nephritis are the same as the proteins in the plasma, and that the theory of leakage of plasma protein through the kidneys holds. The view of the identity of urinary and plasma proteins is supported by many observers. Hewitt [1927; 1929] has shown that urinary and serum albumins from cases of chronic nephritis have the same optical rotatory powers. Smith, quoted by Hewitt [1929], has shown that they have the same absorption spectra. Cavett and Gibson [1931] have shown that they have similar racemisation curves. Widdowson [1933] has investigated the optical rotation, racemisation, osmotic pressure and specific refraction of urinary and serum protein fractions in nephritis and nephrosis and concludes that the proteins from urine and serum are identical. Kozawa *et al.* [1933] claim to have shown that the amino-acid composition, albumin/globulin ratio and isoelectric(?) point of urinary proteins are the same as those of serum proteins.

Nevertheless, there are certain points which are not explained. The fibrinogen of plasma is never found in the urine. The urinary proteins seldom have the same albumin/globulin ratio as the serum proteins, and not infrequently globulin is absent altogether from the urine. It thus becomes necessary to postulate a selective permeability of the leaking kidneys to the different protein fractions of

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serum. Kerridge and Bayliss [1932] have put forward evidence in support of the view that the renal glomeruli function as graded filters capable of allowing molecules smaller than serum albumin to pass and withholding all others. This theory fails to explain the ready passage of haemoglobin (which has approximately the same molecular weight as serum albumin) through the kidneys, and we shall show in due course that molecules of greater dimensions than serum albumin are undoubtedly found in the urine. A major criticism of theories based on the dimensions of protein molecules is that one can never be sure of the physico-chemical conditions in the renal cells across which the proteins must pass from blood stream to urine. The proteins as a class are rather prone to molecular dimensional changes in unusual environmental circumstances, particularly when the hydrogen ion concentration is abnormal.

We have described in a previous paper [McFarlane, 1935, 3] an unusual phenomenon which appeared when serum protein from certain patients passing protein in the urine was examined in the ultracentrifuge. This consisted in an abnormal degree of spreading of the *A* boundary which suggests that the "albumin" fraction is wholly or in part heterogeneous in respect of the dimensions of the constituent molecules. A less likely possibility that the molecules of the fraction are homogeneous but are endowed with an abnormal power of diffusion cannot, however, be excluded. Whatever may be the interpretation of the phenomenon the appearance denotes a fundamental change in the serum protein and it is clearly of interest to find if any similar appearance can be detected in the urinary protein. We shall report in this paper the ultracentrifugal analysis of five specimens of urinary protein obtained from patients whose sera had all been examined and reported on in the previous paper [1935, 3]. The clinical notes relating to these patients will be found appended to that paper.

The general procedure adopted was to dialyse a sample of the fresh urine in a collodion sac against 1 % sodium chloride and under pressure until equilibrium between protein solution and dialysate was reached. The refractive indices of solution and dialysate were then measured, and the solution was examined in the ultracentrifuge. The subsequent procedure was exactly as detailed in previous papers [1935, 1, 2, 3] for serum proteins.

Approximate values for the concentrations of albumin and globulin fractions in the urinary proteins were obtained by the following procedure. The dialysed and concentrated urinary protein solution was diluted with ten times its volume of 1 % sodium chloride and an equal volume of saturated ammonium sulphate added. Precipitation was given 2–4 hours for completion and then the mixture was centrifuged until the globulin precipitate was well packed down on the bottom of the tube. The supernatant albumin solution was poured off and the precipitate drained carefully and redissolved in water. The globulin solution was then dialysed against 1 % sodium chloride and the concentration of globulin measured refractometrically. Only in the cases of two of the urines (K.J.K. and L.E.) was a significant globulin precipitate obtained. The other urines appeared to contain only albumin.

Exp. 1. Urinary protein from A.S.L. (pneumonia and nephrosis) and A.B. (myeloma).

Neither urine in this experiment gave a globulin precipitate but the urinary protein of A.B. had all the characteristics of Bence Jones protein, precipitating at 50° and redissolving completely at 100°. We isolated the protein from this urine by the following procedure. To 1 volume of urine 2 volumes of saturated ammonium sulphate were added, and the precipitate was centrifuged off after

4 hours and washed with 66 % saturated ammonium sulphate. The precipitate was redissolved in a small volume of water, the turbid solution filtered and the protein reprecipitated with a large excess of 66 % saturated ammonium sulphate. The precipitate was redissolved and second and third reprecipitations carried out in the same manner. The final solution was dialysed until free of sulphate ions and electrodyalysed for 4 hours at 440 volts. The liquid was pale brown and clear. Before examining a portion of this in the ultracentrifuge, sufficient solid sodium chloride was added to it to make a 0.9 % solution.

Table I shows the collected results for experiments on the two specimens of dialysed whole urinary protein and on the preparation of Bence Jones protein. Fig. 1 *a* shows the curve of an exposure taken 54½ minutes after reaching full

Fig. 1 *a*. Fig. 1 *b*.

Table I. *Results obtained in the investigation of urinary proteins from A.S.L. (pneumonia and nephrosis), and A.B. (myeloma).*

	Concentrated urinary protein from A.S.L.	Concentrated urinary protein from A.B.	Bence Jones protein A.B.
Cell thickness (mm.)	2.00	2.00	6.00
Optical scale distance (cm.)	3.18	2.58	7.64
Cell temperature (first exposure)	33.5°	35.5°	26°
Cell temperature (last exposure)	35.3°	40.4°	29°
Approximate duration of run (hours)	2½	3½	2½
Mean centrifugal force used (times gravity)	250,000	240,000	210,000
Total $n_1 - n_0$ of concentrated urine, by refractometer $\lambda = 589 m\mu$	0.00812	0.0114	—
Corresponding total protein concentration (g./100 ml.)	4.02	5.64	—
Total $n_1 - n_0$ corrected to $\lambda = 366 m\mu$	0.00877	0.0123	—
Average $S_{w, 20} \cdot 10^{13}$ of protein	3.34	3.26	3.74
Average $n_1 - n_0$ of protein calculated from area of curve ($\lambda = 366 m\mu$)	0.00788	0.0116	0.00295
"% difference" total $n_1 - n_0$	-10 %	-5.7 %	—
Spreading coefficient of <i>A</i> boundary (cm. ² /sec. $\times 10^7$)	3.5	9.3	22.6

speed in the experiment on the urinary protein of A.S.L. The corresponding curve in the case of A.B. shows no significant points of difference from this curve. Fig. 1 *b* shows the curve of an exposure taken 81 minutes after reaching full speed in the experiment on Bence Jones protein. The curves of these proteins show no signs of a globulin fraction, thus differing markedly from the curves of the parent sera [*cf.* 1935, 3, Fig. 9 *b*]. They also show no signs of the presence of an

X protein, which is a point of similarity with the parent serum in the case of A.S.L. The sedimentation constants of the urinary proteins appear to be slightly higher than corresponds to the *A* fractions in the parent sera. The most important point, however, is the high values of the boundary spreading coefficients. These are much higher than correspond to homogeneous boundaries of the same sedimentation constants. Figs. 1 *a* and 1 *b* show the high ratio of breadth at the base to height in these curves. The urinary proteins in these cases therefore show essentially the same phenomenon as the *A* fractions in the parent sera. In the case of A.S.L. the spreading coefficient of the urinary protein boundary is in good agreement with the value for the *A* boundary of the serum. This is not so with the serum and urine of A.B. It must be realised, however, that the serum *A* fraction may contain both homogeneous and polydisperse protein, whereas the urinary protein may only contain the latter and would in that case have a larger boundary spreading coefficient.

The boundary spreading coefficient in the case of the purified Bence Jones protein is very high. The total protein concentration however is low, and the experiment was carried out with a lower mean centrifugal force than usual. The value may not therefore be compared with the others, but it is higher than that of horse serum albumin in the same circumstances. The $S_{W,20}$ value corresponds with the value for horse serum albumin in the same concentration. It is definitely higher than the values obtained by Svedberg and Sjögren [1929] in the examination of a specimen of Bence Jones protein which had the same mean molecular weight as egg albumin, *viz.* 35,000. It is also interesting to find that the isoelectric point of this specimen of Bence Jones protein as measured by Tiselius is at p_H 5.20, whereas the isoelectric point of our protein, measured by Pedersen, is at p_H 6.56. It appears that the term Bence Jones protein does not always refer to the same substance, but that urinary protein of varying composition can give the characteristic Bence Jones reactions. Some specimens of Bence Jones protein for instance crystallise easily while others cannot be crystallised. Svedberg and Eriksson-Quensel (private communication) have had a sample of Bence Jones protein which proved to be quite polydisperse. Mainzer [1932, 1, 2, 3], by investigating the electrometric titration values and transport cataphoresis of Bence Jones protein, also comes to the conclusion that it is not a chemical individual.

Exp. 2. Urinary protein from K.J.K. (chronic nephritis), L.E. (albuminuria of pregnancy), and H.A.A. (metastatic tumour of bone).

In the precipitational analysis of two of these sera (K.J.K. and L.E.) substantial globulin precipitates were obtained while the urinary protein of H.A.A. gave only a faint opalescence. The results of the analysis were

- Proportion of globulin in urinary protein of K.J.K., 19 %
- Proportion of globulin in urinary protein of L.E., 14 %
- Proportion of globulin in urinary protein of H.A.A., less than 2 %

The results obtained in the investigation of one specimen of dialysed and concentrated whole urinary protein from each of the three patients are shown in Table II.

Fig. 2 *a* shows the curve of an exposure taken 115½ minutes after reaching full speed in the experiment on K.J.K.'s urinary protein, and Fig. 2 *b* shows the curve of an exposure taken 149 minutes after reaching full speed in the experiment on H.A.A.'s urinary protein. The curves in the third experiment (L.E.) are very similar to those for K.J.K., and differ significantly from the curves in

Figs. 1 *a* and 1 *b* in showing the presence of a *G* fraction. No significance attaches to the fact that the proportion of *G* fraction in the urines is not the same as in the original sera, because it is probable that in the precipitation method of analysis a globulin fraction is obtained which is contaminated with considerable amounts of albumin.

Fig. 2a.

Fig. 2b.

Table II. Results obtained in the investigation of urinary proteins from K.J.K. (nephritis), L.E. (albuminuria of pregnancy), and H.A.A. (metastatic tumour of bone).

	Concentrated urinary protein from K.J.K.	Concentrated urinary protein from L.E.	Concentrated urinary protein from H.A.A.
Cell thickness (mm.)	2.00	2.00	2.00
Optical scale distance (cm.)	1.68	1.98	6.78
Cell temperature (first exposure)	32.9°	33.3°	33.0°
Cell temperature (last exposure)	36.2°	36.9°	37.3°
Approximate duration of run (hours)	3½	2½	3
Mean centrifugal force used (times gravity)	255,000	255,000	255,000
Total $n_1 - n_0$ of calculated urinary protein, by refractometer, $\lambda = 589 m\mu$	0.0107	0.0110	0.00780
Corresponding total protein concentration (g./100 ml.)	5.30	5.45	3.86
Total $n_1 - n_0$ corrected to $\lambda = 366 m\mu$	0.0115	0.0119	0.00842
Average $S_{W,20} \cdot 10^{13}$			
<i>A</i> fraction	3.02	2.95	1.44
<i>G</i> fraction	5.41	4.77	—
Average $n_1 - n_0$, calculated from sedimentation, diagram $\lambda = 366 m\mu$			
<i>A</i> fraction	0.00971	0.00904	0.00567
<i>G</i> fraction	0.00150	0.00066	—
Total	0.01121	0.00970	0.00567
"% difference" total $n_1 - n_0$	-2.6 %	-18.5 %	-33 %
Proportion of total calculated $n_1 - n_0$ due to <i>G</i> fraction	13 %	6.8 %	—
Spreading coefficient of <i>A</i> boundary (cm. ² /sec. $\times 10^7$)	2.7	4.9	18.5

It is obvious that the urinary protein of H.A.A. is almost completely polydisperse. Considerable amounts of protein had reached the bottom of the cell before all the protein had left the region of the meniscus. The $S_{W,20}$ value has little meaning in such a case because of the asymmetry of the curve and the doubtful significance of the apex at any instant. The value recorded in Table II, viz. 1.44, is obtained from measurements of the rate of movement of the apex.

Also, the apparent area of the curve is not constant but diminishes steadily as the experiment progresses. The values for $n_1 - n_0$ are all much lower than the measured total $n_1 - n_0$ value of the original preparation. The $n_1 - n_0$ value in Table II corresponds to the mean area. The boundary spreading coefficient is calculated from the mean area and from the height of the apex above the base line in two exposures.

The A and G fractions in the other two urines have sedimentation constants corresponding approximately to those of the A and G fractions in the sera from the same patients. The A boundary spreading coefficients are lower in the urinary proteins but this is not inconsistent with the much higher concentrations in which they were examined. The values are greater than those for horse serum albumin in corresponding concentrations, and a comparison of the general dimensions of the curves with those of A curves of normal human serum of the same, or slightly smaller, total protein concentration [*e.g.* 1935, 2, Fig. 15] confirms that abnormal spreading of the A boundaries is present.

DISCUSSION.

We conclude from the results of these experiments that in the cases examined there is a difference between urinary protein and normal serum protein. The difference consists in an abnormal degree of spreading in the centrifugal field of the lighter fraction in the urinary protein. There is evidence that the same abnormal spreading characterises the A fractions in the sera of the patients. But the total urinary and serum proteins from the same patients have not identical compositions. In three cases no globulin fraction is present in the urine although in two of the three there is an abnormally high concentration of globulin in the serum.

There are various theories which fit these facts. An attractive one is that there is present in the sera effete polydisperse protein which the kidney is able to excrete by virtue of some vital activity. The existence of a vital activity which enables the kidneys to discriminate between homogeneous and heterogeneous protein would not be surprising in view of the fact that nature appears only to elaborate monodisperse proteins and these readily become polydisperse when subjected to artificial treatment. Denaturation and insolubility appear to be terminal stages in a state of progressively increasing molecular polydispersity. It would clearly be of importance therefore for the organism to be able to rid itself of polydisperse protein arising as the result of disease processes in the tissues.

Another explanation depends on the fact that many proteins are irreversibly altered by treatment with urea. Anson and Mirsky [1929], Wu [1929], Hopkins [1930], Pauli and Weiss [1931], and Burk [1932] have all investigated the effect of urea on proteins. A denaturation of the protein takes place which is apparent as an insolubility at the isoelectric point. Hopkins [1930] also obtains a positive nitroprusside reaction indicating constitutional changes in the protein molecule. Saturated or very concentrated solutions of urea are used to produce these effects. Hopkins finds that the proteins of diluted serum or of solutions of serum albumin are rendered insoluble much less readily than those of solutions of egg albumin, although they give the nitroprusside reaction at once after treatment with high concentrations of urea. This fact suggests that insolubility is a gross terminal stage of the effect and is preceded by less obvious chemical structural changes in the molecule. It may be therefore that lower concentrations of urea, such as are present in urine and in some pathological sera, are capable of causing an

early partial denaturation of serum proteins which is only detectable by ultracentrifugal analysis. If this is the case it is possible that the polydispersity of the urinary and serum proteins which we have found is unrelated to the mechanism of protein excretion. Further ultracentrifugal investigation of the effect of moderate concentrations of urea on the serum proteins is required before we can come to a conclusion regarding this possibility.

In the two cases of our group which had globulin in the urine, *viz.* K.J.K. and L.E., the first was a characteristic case of chronic nephritis and the other was in an advanced state of toxæmia with a high probability of renal damage. In the other three which had no globulin in the urine there were no clinical grounds for suspecting renal disease. These facts suggest that when there is globulin in the urine the kidneys are damaged, and when the urinary protein gives only an albumin precipitation reaction we are concerned with polydisperse protein excreted by normal kidneys.

SUMMARY.

1. Five specimens of urinary proteins have been investigated in the ultracentrifuge. Two of these contain albumin and globulin fractions while three contain only albumin.

2. The albumin in all five specimens has an abnormally high power of boundary spreading, which suggests that the protein is wholly or partly polydisperse. The sera from the same five patients show similar signs of a polydisperse albumin fraction.

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