AN ELECTROPHORETIC STUDY OF NEPHROTIC SERA AND URINE

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In a recent paper (1) the authors described experimental methods and results on the electrophoretic study of normal and pathological sera. This communication will deal in detail with nephrotic sera, briefly mentioned in the earlier paper, and with the proteins of nephrotic urine.

The electrophoretic pattern of a typical normal human serum, diluted 1:6 is shown in Fig. 1.

It will be recalled from the discussion in the earlier (1) paper: (*a*) that an ordinate of this diagram is proportional to the gradient of refractive index of a layer of solution, and the corresponding abscissa indicates the position of the layer in the electrophoresis cell; (*b*) that the area under each peak is proportional to the concentration of the corresponding component (or components) having a given electrophoretic mobility; and (*c*) that the mobility may be deduced from the observed displacement of the peak from the initial position of the boundary as formed between the protein and buffer solutions.

The mobilities and concentrations of the four components of normal serum have been estimated from the pattern shown in Fig. 1, as described fully in the earlier paper (1), and are recorded, along with other data, in Table I. The components have been identified by Stenhagen (2) as albumin, A, and α, β, and γ globulins respectively, as indicated in the figure. In marked contrast are the patterns of two nephrotic sera, Figs. 2 and 3. These were diluted 1:4 after the suspended fat had been removed by high speed centrifugation. Fig. 6 is the pattern of the same serum as that of Fig. 3 except that the fats had been removed by cold ether extraction.[1]

[1] The scale of ordinates for the patterns of Figs. 3 and 6 is twice that of the other patterns. Also in the experiment represented by Fig. 6, the initial boundary was not displaced from behind the horizontal glass plates of the cell before application of a potential, so that the peak due to the very slowly migrating γ globulin was obscured. In addition, a sodium phosphate buffer, ionic strength 0.1, was used in this experiment, whereas a lithium barbiturate-lithium chloride buffer, 0.025 normal in each, was the solvent in all other experiments reported in this paper. It has been our experience that the electrophoretic analysis of a serum is independent of the buffer employed but that protein mobilities vary greatly with the buffer solution used as solvent. The mobilities computed from Fig. 6 are therefore not comparable with the others and have not been included in Table I.

The difference between the patterns of nephrotic and normal sera makes the identification of components from the mobilities somewhat uncertain. In Table I they have been listed in the order of their mobilities. The occurrence, however, of the very characteristic "β globulin disturbance"

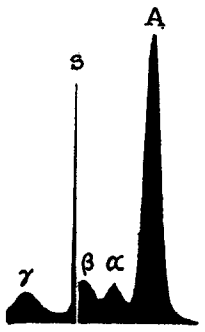

FIG. 1. Normal serum.

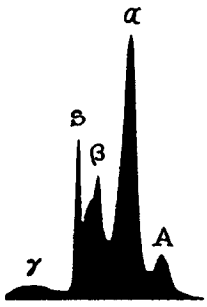

FIG. 2. Nephrotic serum (S.G.).

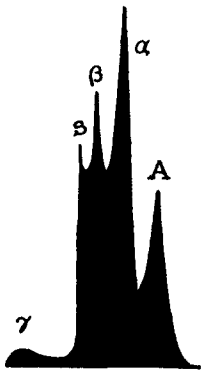

FIG. 3. Nephrotic serum (G.B.).

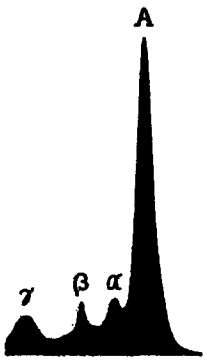

FIG. 4. Nephrotic urine (G.B.).

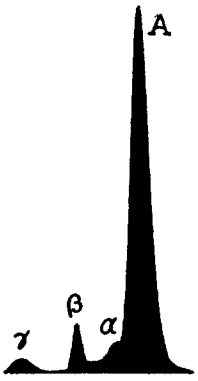

FIG. 5. Nephrotic urine (S.G.).

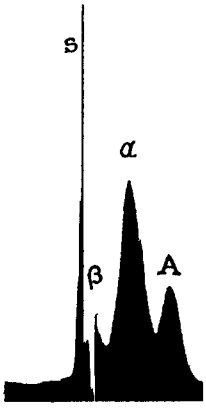

FIG. 6. Nephrotic serum (G.B.), ether-extracted.

in the third peak of Figs. 2 and 3 suggests that this boundary is due, in part at least, to the same β globulin as in normal serum. As mentioned in our previous paper (1) the β globulin disturbance, which produces a very sharp spike, s, is due to convection, resulting from reaction in the neighborhood of the boundary following electrophoretic separation of the constituents. It has been observed only in the patterns of descending boundaries and is, apparently, an indication of the complexity of the β component.

It is of interest that the ether extraction of the nephrotic serum, Fig. 6, reduced the concentration of the β component much more than that of the other constituents without, however, eliminating the β globulin disturbance. It should be recalled that the determination of concentrations from the electrophoretic patterns is a refractometric method and both proteins and dissolved lipoids affect the refractive index. The difference given in Table I, of 1.39 per cent, in the total solute content of the centrifuged and ether-

TABLE I

Concentrations and Mobilities of the Components of Normal and Nephrotic Sera and Nephrotic Urine

Fig. No.	Material	pH	Dilution	Concentration					Mobilities × 10^5			
				Total per cent	1	2	3	4				
					Albumin	α	β	γ	Albumin	α	β	γ
1	Normal serum, T.S.	7.84	6	6.92	4.43	0.61	1.02	0.86	6.2_4	4.5	3.0	0.3
2	Nephrotic serum, S.G.	7.82	4	5.21	0.53	2.58	1.77	0.33	6.4_0	4.9	3.5	0.6
3	Nephrotic serum, G.B.	7.83	4	4.53	0.88	1.71	1.71	0.23	6.3_6	4.9	3.6	0.3
6	Nephrotic serum, G.B. (ether-extracted)	7.73	4	3.14	0.74	1.46	0.74	0.20‡				
5	Nephrotic urine, S.G.	7.78	0	1.29*	1.08	0.05_6	0.09	0.06_5	5.9_5	4.7	3.0	0.3
4	Nephrotic urine, G.B. Feb. 7, 1939	7.81	0	1.39*	0.94	0.13	0.15	0.17	5.9_7	4.5	2.9	0.3
	Nephrotic urine,† G.B. Feb. 28, 1939	7.77	0	2.02	1.39	0.19	0.22	0.23	5.4_9	4.1	2.6	0.2

* Nitrogen determinations of these solutions by Dr. Bourdillon gave protein concentrations of 1.33 per cent (S.G.) and 1.43 per cent (G.B.).

† A second sample of urine from patient G.B. taken 3 weeks after the first sample. It may be noted that the relative concentrations of the components of the two samples agree very closely.

‡ Estimated by assuming the ratio γ/A to have the same value as in the centrifuged serum of G.B.

extracted sera of patient G. B. (Figs. 3 and 6) appears to be due to lipids in clear solution by virtue of an association with the β component of the nephrotic serum. The following observation supports this conclusion. A determination of lipoid carbon on the clear centrifuged serum of patient S. G. (Fig. 2), by Dr. Jordi Folch-Pi of this Institute, gave a value of 1.61 per cent computed as phospholipid or 1.27 per cent computed as cholesterol. A total nitrogen determination on this same serum by Dr. Jaques Bourdillon, also of this Institute, indicated the presence of 3.64 per cent of protein. The sum of the protein and lipo-protein concentration in this serum is thus between 4.91 and 5.25 per cent, in satisfactory agreement with the value

of 5.21 per cent obtained from Fig. 2. It has been provisionally necessary to assume the same specific refractive increment for dissolved lipoid as for serum globulins.[2]

In order, therefore, to account for the effect of ether extraction on the electrophoretic patterns of nephrotic sera it seems necessary to assume that there are associating forces between lipoids and a β globulin. However, these forces are so weak that they may be broken by cold ether extraction.

The association of lipoid material with *normal* serum proteins has been noted by Sørensen (3) who says " . . . the perfect clearness of such liquids as serum and plasma, in spite of their contents of lipoids, is explicable only by assuming linkage between lecithin and sterols on one hand and the proteins on the other." In addition, Tiselius (4) has noted that most of the lipoid material of normal serum moves with the β component. Unlike the lipoids of centrifugally cleared nephrotic sera, however, only inappreciable quantities of the lipoids of normal serum can be extracted by cold ether. The question as to whether the protein part of the lipo-protein complex is a normal β globulin in the case of the nephrotic serum is still open. It will be observed, however, that the disturbance characteristic of normal β globulin is present in both the centrifuged and ether-extracted nephrotic sera but, as will be seen, is absent in the patterns of nephrotic urine.

Additional evidence that at least one of the β globulins of normal serum has an affinity for lipoid material is afforded by the fact, first observed by Tiselius (5) and confirmed by us, that the opalescence of normal serum, due presumably to suspended fat globules, migrates with the β globulin. Since Abramson (6) has shown that the mobility of a particle suspended in a protein solution is due to a layer of the protein on the particle and, in general, has the same value as the mobility of the dissolved protein, we may conclude that the suspended fat is coated with a β globulin.

The electrophoretic patterns of the urinary proteins of the two nephrosis cases are shown in Figs. 4 and 5. It will be noted that there is a decided resemblance of the patterns of the proteins of nephrotic urine to that of normal serum. It would, however, be unwise to conclude on this evidence that the proteins are necessarily identical in the two cases. For example, the β globulin disturbance is lacking in the pattern of the nephrotic urine, indicating that one at least of the β globulins is missing. Moreover Bourdillon (7) has found, by an osmotic method, a molecular weight of 62,000

[2] Longsworth *et al.* (1), page 410.

for the urine albumin of patient G. B. and 72,000 for normal serum albumin. We have confirmed the ratio of the molecular weights by the measurement, at 0°C., of diffusion coefficients, D, of these two albumins. The results obtained were 3.51×10^{-7} and 3.34×10^{-7} cm.²/sec., for the urine and serum albumins respectively.[3] Since the two albumins probably have the same density, δ, and very nearly the same shape, a rough estimate of the relative molecular weights may be made with the Einstein equation (9), $D = kT/6\pi\eta r$, in which k is the Boltzmann constant, T the absolute temperature, η the viscosity, and r the radius of the molecule. Since the molecular weight $M = 4N_0\pi r^3\delta/3$ (N_0 being Avogadro's number) then $M'/M = (r'/r)^3 = (D/D')^3$ as a reasonable first approximation. The ratio thus computed from the diffusion measurements is 0.863 while that observed by Bourdillon was 0.860, indicating that the osmotic and diffusion measurements are in good agreement as to the relative molecular weights. Finally it may be noted in Table I that the mobilities of the urine albumins are consistently lower than those of either normal or nephrotic serum albumins. However, it seems probable that the nephrotic kidney is able to pass at least a portion of the components of normal serum.

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SUMMARY

The electrophoretic patterns of the sera and urine of two cases of lipoid nephrosis have been obtained and have been compared with a typical pattern of normal serum. The patterns of the pathological sera deviated widely from the normal, indicating relatively low albumin and high globulin content. The comparison of the patterns of nephrotic sera cleared by centrifugation and by ether extraction shows that a large portion of the β globulin consisted of a labile lipo-protein. The pattern of the nephrotic urine proteins resembled that of normal serum, with, however, significant differences.

[3] The method employed in the diffusion measurements was a modification of that used by Lamm (8). The schlieren scanning process was used instead of the scale method. Corrections were applied to the observed values for small amounts of globulin known, from the electrophoretic patterns, to be present in the albumin preparations. Details of the method and computations will be reported later.

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