XLV. ELECTROPHORESIS OF SERUM GLOBULIN. I

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DESPITE the very large amount of work done on serum globulin, comparatively few investigations have dealt with the electrophoretic properties of this important protein, although, as has been shown in several papers from this and other laboratories, migration data are of very great importance for characterization of proteins and biocolloids in general.

As far as the author has been able to find, the only quantitative investigation of the *p*H-mobility relationship for this protein so far has been made by Reiner [1927], using a U-tube apparatus with two stopcocks in each limb, by which arrangement samples could be taken out for analytical determination of the migration. A series of mobilities in buffer solutions of *p*H from 3·6 to 9·5 were obtained. The isoelectric point was at *p*H 5·4. In a previous work the author [1930] has measured the electrophoresis of serum "pseudo-" and "eu-globulin" as well as unfractionated globulin, but only at one single *p*H value of 7·22. All three preparations showed heterogeneous migration, and the mobilities were different.

In connexion with some research on proteins of immune sera, the author needed data on the mobility of normal serum globulin and its fractions. Therefore a new investigation was made, which gave results deviating in certain points from those obtained by Reiner.

The procedure for measurement of electrophoresis used in this work has been described in earlier papers [Tiselius, 1930; Pedersen, 1933]. The movement of the boundaries in the U tube is followed by ultraviolet photography, a method especially valuable for obtaining information regarding electrochemical homogeneity of the substance studied.

The serum globulin was prepared by saturating horse serum to 55% with ammonium sulphate and washing the precipitate with the same solution.[1] After dissolving in 5% NaCl, the globulin was reprecipitated 3 times in the same way (serum globulin II). Treatment with NaCl left an undissolved residue. This was dissolved with 0·1*M* phosphate buffer of *p*H 7 (serum globulin I). Some of the first fraction (serum globulin II) was dialysed against distilled water and then electrodialysed between parchment membranes until the current no longer fell. The remaining solution ("pseudoglobulin") formed the third fraction studied (serum globulin III). The three fractions obtained thus differ in their solubilities quite considerably: the first being only partially soluble in 5% NaCl, whilst the last is soluble even in distilled water. The different globulin preparations were investigated in the ultracentrifuge and showed the sedimentation constant $=7\times10^{-13}$ characteristic for serum globulin in unfractionated serum. No appreciable amount of albumin ($s=4{\cdot}5\times10^{-13}$) could be detected, but a certain quantity of heavier fractions also found in serum ($s=$ about 18×10^{-13} and higher) was present.

[1] The preparation was made by Mr N. Gralén.

For the electrophoresis experiment the necessary amount of stock solution was diluted with the buffer to be used as supernatant, and the solutions were dialysed against each other overnight.

As has been shown by the author in the work referred to above, not only hydrogen ions but also other ions influence the mobility. If the effects of the hydrogen ions only are to be studied separately, all other factors except the *p*H must be kept constant. This is not possible, as the composition of the buffer solutions must necessarily vary. The simplest case is offered by buffer solutions of the type acetic acid-sodium acetate, where the sodium acetate concentration and therefore very nearly all of the electrolyte present is kept constant, the variation in *p*H being brought about by changing the amount of acid. Buffer solutions like the phosphate mixtures are more difficult, since the valency type of the electrolytes present also varies in the buffering range. In such cases the author has made use of buffer solutions of constant ionic strength for each series of determinations at varying *p*H. This is a generalization of the rule followed for the acetate buffer system.

Especially when working in wide *p*H ranges, with one buffer system following upon the other, it is absolutely necessary to follow some rule regarding the choice of the total buffer concentration or else sharp changes depending upon ions other than H^+ may occur in the mobility in the *p*H range where two buffer systems meet. The choice of the ionic strength for this purpose can be theoretically justified if the modification in mobility caused by changes in the ionic medium at constant *p*H is a non-specific electrostatic effect, as in the ionic interaction theory of Debye-Hückel. An experimental justification was found by Pedersen in some unpublished electrophoresis measurements on haemoglobin in different media. He found that the mobilities in phosphate and borate buffers, at *p*H values covered by both, were the same, provided that the ionic strength was the same. In each buffer system, however, variation in the buffer concentration at constant *p*H had a very marked effect on the mobility. Naturally, this rule cannot be expected to hold for ions which react chemically (that is not only by electrostatic effects) with the proteins. Citrate ions for example exert a very strong effect on serum globulin, increasing its negative charge.

Most of the author's previous measurements were made at an ionic strength of 0·02, which is usually a sufficiently high concentration to depress the boundary disturbances. Serum globulin requires a much higher salt concentration to remain in solution. All determinations were thus made at an ionic strength of 0·1. Acetate and phosphate mixtures were used. The protein concentration was 0·2–0·3 %.

As the conductivity was about 5 times higher than in the dilute buffer solution used previously for water-soluble proteins, the voltage was reduced to between half or one third, in order not to exceed the maximum allowable load on the apparatus.

The results of the measurements on horse serum globulin are given in Table I below and in Fig. 1. A curve has been drawn only for whole serum globulin (preparation II above).

For comparison some measurements were also made on unfractionated serum globulin, prepared from rabbit serum (last column in Table I, and Fig. 2).

The accuracy in these experiments was not as high as usual for several reasons. The high conductivity, the low mobility, and above all the very marked heterogeneity of migration all tend to increase the error of measurement. In the procedure followed, heterogeneity of migration can be detected, as has been shown by the author in the work already referred to above. If several

Table I. *Electrophoretic mobilities of the different serum globulin preparations at 20° in acetate and phosphate buffer solutions of varying* p*H and constant ionic strength 0·1. + and − refer to the charge on the protein*

		Mobility in cm.^{2} volt^{-1} sec.$^{-1}$ × 10^5			
		Horse serum globulin			
Buffer	*p*H	I	II	III	Rabbit serum globulin
Acetate	4·08	+6·3	+5·6	+5·8	—
	4·63	+3·9	—	+3·1	+3·2
	4·69	—	+2·4	—	—
Phosphate	5·28	+0·3	−0·3	−0·1	−0·1
	5·63	−1·7	—	—	—
	6·02	−1·9	−3·1	−2·1	−3·4
	6·87	—	—	−4·2	—
	6·98	−3·7	−5·3	—	−5·8
	8·03	−6·8	−8·0	−6·3	—

Isoelectric points $(pH)_0$ and slope of the mobility − *p*H curve at $(pH)_0$:
Horse serum globulin, unfractionated (prep. II) $(pH)_0 = 5{\cdot}20$, $(du/dpH)_0 = 4{\cdot}3 \times 10^{-5}$.
Rabbit serum globulin, unfractionated $(pH)_0 = 5{\cdot}26$, $(du/dpH)_0 = 4{\cdot}6 \times 10^{-5}$.

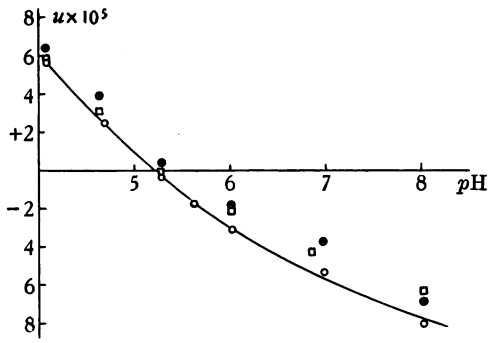

Fig. 1. The mobility of horse serum globulin at different *p*H values and 20°. □ Serum globulin I, o serum globulin II, ⊗ serum globulin III. The curve has been drawn to fit the values for serum globulin II.

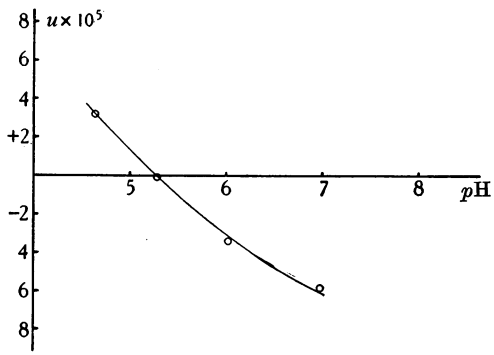

Fig. 2. The mobility of rabbit serum globulin at different *p*H values and 20°.

components are present, one may observe either several boundaries or a general diffuse blurring of the originally sharp boundary, depending upon the differences in their mobilities. The effect is reversed if the direction of the current is changed

and may thus be distinguished from the effect caused by diffusion alone. The preparations of serum globulin investigated by the author in the previous communication all showed marked heterogeneity in this way. This result could be confirmed for all preparations studied in the present paper, the heterogeneity being especially marked at large *p*H values. Here also the differences between the different fractions are most marked, I and III showing smaller mobilities than II (Fig. 1). A curve has been drawn for fraction II in Fig. 1. The two other fractions seem to be somewhat more positive: but the differences are rather slight in the isoelectric range and do not lead to more than 0·1 *p*H difference in the isoelectric point. There is no parallelism between solubility in NaCl and electrophoretic mobility: the fractions of high and low solubility are both more positive than the intermediate fraction.

The value of the isoelectric point $(p\mathrm{H})_0 = 5{\cdot}20$ is somewhat lower than the value of 5·4 obtained by Reiner [1927]. However, it is probable that our preparations are not quite comparable: Reiner's globulin had been extracted with alcohol-ether to remove lipoid. Moreover, he was not able to investigate the globulin solutions at sufficiently high salt concentrations to keep all of the protein in solution, but the greater part of the globulin precipitated and only the supernatant was used for electrophoresis determination. Thus Reiner's results should rather be compared with those obtained in the present paper with the most soluble fraction (III) which in fact gives $(p\mathrm{H})_0 = 5{\cdot}3$.

However, a very marked difference in our results is found at higher *p*H values, where Reiner finds an extremely rapid change between *p*H 5·8 and 6·0 from 1·30 to $6{\cdot}74 \times 10^{-5}$ cm.^{2} sec.$^{-1}$, the mobility at higher *p*H values being almost constant, whereas the results obtained in the present paper give a smooth curve very similar in shape to that found for other proteins. The effect is probably due to the fact that the buffer was changed (from acetate to phosphate) just as in the *p*H interval in question.

The rabbit serum globulin gives very nearly the same curve as the corresponding preparation from the horse (see Fig. 2). In fact, the differences are within the experimental errors. The migration is heterogeneous.

The electrochemical heterogeneity of serum globulin demonstrated in the present paper as well as in the preliminary experiments in the previous communication seems of interest also in view of the fact that Felton [1928], Felton & Kauffmann [1933] and Reiner & Reiner [1933] have isolated globulin fractions with somewhat different *p*H precipitation maxima from horse serum. For normal serum the fractionation obtained in this way is not very marked; both fractions have a precipitation maximum at about *p*H 5·5–6·0, but one fraction was more completely precipitated at *p*H 5·1 than at 6·0, the other more completely at *p*H 6·7 than at 5·1 (Reiner & Reiner). The most direct method, on these lines, of fractionating serum globulin is to make use of electrophoresis for the separation. This was done in a specially constructed apparatus in which serum could be subjected to prolonged electrophoresis at high potential and samples of the differently migrating fractions could be isolated. At *p*H 8 the slowest fraction consists of a protein with isoelectric point *p*H 5·6 and a mobility as low as $2{\cdot}4 \times 10^{-5}$ at *p*H 8 which showed the characteristics of serum globulin (sedimentation constant $s = 7 \times 10^{-13}$, precipitation with 50 % saturation of ammonium sulphate). Moreover, this protein gave a much more uniform migration than any of the preparations of serum globulin studied before.

The next paper will deal with the properties of fractions thus obtained by electrophoretic analysis of serum.

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