

XXXIV. THE VISCOSITY OF PROTEIN SOLUTIONS. II. PSEUDOGLOBULIN AND EUGLOBULIN (HORSE).

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The following experiments form a continuation of those published recently by Lubrzynska and the author [1914], on the influence of concentration of protein upon the viscosity of horse serum and solutions of crystallised egg and serum albumin (horse). In the present case the investigation has been extended to the pseudoglobulin and euglobulin of horse serum. The significance of both sets of results is discussed at the end of the present paper.

PREPARATION OF THE MATERIAL.

By pseudoglobulin is signified the protein, soluble in distilled water at its iso-electric point, which is separated from serum by one-half saturation with ammonium sulphate¹ (addition of an equal volume of saturated ammonium sulphate solution to the diluted serum). The term euglobulin is used to indicate the protein insoluble in distilled water at its iso-electric point and therefore precipitated by dilution of the serum and acidification until the reaction is adjusted to the iso-electric point of the protein.

The protein precipitated by one-third saturation with ammonium sulphate (addition of one volume of saturated ammonium sulphate to two volumes diluted serum) is frequently considered to be euglobulin, and this method is a standard one for separating this protein from pseudoglobulin. The precipitate obtained, however, is a mixture of the two, and the total amount is considerably more than is obtained by dilution and acidification. Further, it does not include all the euglobulin, for the precipitate of pseudoglobulin, obtained when the concentration of ammonium sulphate in the filtrate is

¹ There is no reason to suppose that this method sharply separates pseudoglobulin from albumin [see Chick and Martin, 1913].

raised to one-half saturation, is found to contain more protein insoluble in water (euglobulin), than if the latter had previously been removed by dilution and acidification.

Euglobulin. In the present case the euglobulin was always prepared by the last named method. Serum was diluted ten times and the reaction adjusted to the iso-electric point of the euglobulin by means of the addition of a small amount of acetic acid, about 2-3 cc. normal acetic acid being, as a rule, found necessary for each litre of diluted serum. The optimum amount was determined by making preliminary trials with small samples, and was easily recognised by the ease and rapidity with which the precipitate settled. After one to two days' standing the precipitate was separated by centrifuging the deposit, after decanting the top liquor. It was purified three or four times by redissolving (dispersing) in a minimum of caustic soda and precipitating with an exact equivalent of hydrochloric acid. The stronger acid is here employed in place of acetic, because a smaller quantity is found to be necessary and a lower concentration of salt results on neutralising. The final precipitate was washed once with slightly acidified water. For this purpose water, saturated with carbon dioxide and diluted about 30 times with ordinary distilled water, has been found to yield the convenient, slight, acidity.

Pseudoglobulin. The pseudoglobulin, used for the experiment given in Table V, was prepared from diluted horse serum by one-half saturation with ammonium sulphate, after removal of the precipitate given by one-third saturation. The crude precipitate was dissolved in water and twice reprecipitated, the final material being dialysed for two days against running water (tap) and for sixteen days against distilled water, changed daily, in presence of toluene.

The method of precipitation by one-third saturated ammonium sulphate, as stated above, does not remove all the euglobulin. The small proportion remaining can be detected even before the dialysis is commenced, by diluting the material and acidifying with minute amounts of dilute acetic acid, a method by which euglobulin can readily be detected in presence of a small concentration of salt [see Chick, 1913]. After dialysis for nine days, as much as one-tenth of the total protein was found to be insoluble in distilled water. At the completion of the process the material contained about 14% protein, of which about one-eighth was found to be in this condition. This insoluble material, the presence of which must, I think, be attributed to a gradual degradation of the pseudoglobulin to a water-insoluble condition, shows very remarkable analogies with euglobulin and has been made the

subject of a separate investigation, the result of which will be published later. When the dialysis was complete¹, the insoluble material could be completely separated by diluting and centrifuging. The top liquor was found to be free from insoluble protein and was concentrated over sulphuric acid in a vacuum in order to obtain material of the right strength for the viscosity measurements.

In order to investigate material which had suffered no degradation, dialysis was dispensed with in preparing that used for the experiments in Table VI. The euglobulin was removed in the ordinary way from the serum by dilution (1 in 10) and acidification and a further precipitate separated by addition of ammonium sulphate to one-third saturation (19.8 g. $(\text{NH}_4)_2\text{SO}_4$ to 100 cc.); ammonium sulphate was then added to the filtrate until one-half saturation was reached (11.3 g. $(\text{NH}_4)_2\text{SO}_4$ to 100 cc., final density 1.138). The pseudoglobulin was purified as follows. The precipitate was pressed between filter paper to free it from mother liquor, redissolved in water and an equal volume of saturated ammonium sulphate was added. The resulting mixture contained an excess of ammonium sulphate, over one-half saturation, owing to the salt contained in the precipitate. This extra amount was ascertained by boiling a small sample of the mixture, filtering the protein and determining the density of the filtrate. The requisite amount of water could be then calculated and added to the mixture. The precipitate was filtered, freed from mother liquor as far as possible by pressing between filter paper and finally dissolved in a little water. The solution was found to have the following composition: protein 13.75 %, ammonium sulphate 10.46 %. It contained only the faintest trace of insoluble protein (tested by dilution and acidification).

EUGLOBULIN.

Influence of concentration of protein.

The experiment, of which the results are detailed in Table I, was so arranged that the conditions of solution (dispersion) should approximate to those obtaining in normal serum. The euglobulin was dispersed with a small amount of alkali, the concentration of hydrogen ions, 10^{-8} N, approximated to that of serum, and in addition a small amount of sodium chloride was present, about 1 gram per 6 grams protein. The results are shown graphically

¹ During the dialysis of proteins, the reaction gradually approximates to the slight acidity characteristic of the iso-electric point, and as a consequence any euglobulin or "denatured" protein is gradually precipitated.

in curve *c*, Figure 1, where the results of similar experiments with pseudoglobulin are also plotted, together with those for serum albumin and whole serum [see Chick and Lubrzynska, 1914, p. 61] for purposes of comparison. The much greater viscosity of euglobulin is at once apparent. A solution containing 6.6 % protein has a viscosity of 3.49, i.e. more than twice that shown by solutions of serum albumin or by whole serum of equal protein-content. With higher concentration of protein (12.95 %), the viscosity of euglobulin reaches the high figure of 21.7, a value not approached by the strongest solutions obtained of serum albumin (20.6 % protein, coefficient of viscosity 7.54) or of the proteins of whole serum (18.1 % protein, coefficient of viscosity 6.38).

TABLE I.

Influence of concentration of the protein upon the viscosity of solutions (dispersions) of euglobulin (horse) in dilute sodium chloride solution and alkali.

* Concentration of sodium chloride 2.2 % to 0.5 %.

*, ,, ,, hydrogen ions $10^{-8.0}$ to $10^{-8.2}$ normal.

Temperature 25°. Time of flow in viscosimeter for water = 18.5 seconds.

Concentration of protein, %	Mean time of flow in viscosimeter, secs.	Density of solution (H ₂ O at 25° = 1)	Coefficient of viscosity, H ₂ O = 1
12.95	379.3	1.0582	21.69
10.81	191.7	1.0479†	10.86
9.84	144.9	1.0435	8.17
6.60	62.7	1.0290	3.49
3.27	31.2	1.0141	1.71

* Concentration of salt and alkali was adjusted to give results comparable with those obtained for whole horse serum [see Chick and Lubrzynska, 1914, Table VI, p. 67].

† Interpolated value.

Influence of hydrogen ion concentration and of salt content.

Hardy [1905] found that the viscosity of euglobulin, when dissolved by salt, was much lower than when dispersed by alkali. When small amounts of alkali are used, this is undoubtedly the case, the opalescent material obtained being much more viscous than the clear solution formed when salt is used to dissolve the protein. If, however, the concentration of alkali is increased, the viscosity falls rapidly. This is seen in Table II, where, in Experiment I, the result is given of three determinations of viscosity in solutions containing 5.6 % protein, but with concentration of hydrogen-ions falling from $10^{-7.46}$ N to $10^{-10.02}$ N. Corresponding to this change in reaction, there is a fall in the coefficient of viscosity from 12.17 to 3.62. The last

result is not greatly in excess of that obtained in Experiments II and III which deal with solutions of salt-globulin of the same protein concentration. The reaction in these two instances is not far removed from that of the iso-electric point which was determined by Michaelis and Rona [1910] to be at a concentration of hydrogen-ions equal to about 10^{-6} N. The direct influence of salt in lowering viscosity¹ is seen by a comparison of Experiment IV with Experiment I. In the former case, a small concentration of salt, 1 %, reduced the coefficient of viscosity from about 12.2 to 2.4 in presence of, roughly, the same concentration of hydrogen-ions. The figures for Experiments II and IV are interpolated values obtained from curves *b* and *c* respectively, in Fig. 1.

TABLE II.

Influence of concentration of hydrogen-ions upon the viscosity of euglobulin (horse) (a) dispersed by alkali, Exp. I; (b) by NaCl, Exps. II and III, and (c) by alkali + NaCl, Exp. IV.

Concentration of protein 5.68 %.					
Temperature 25°.					
Exp.	Concentration of NaCl, %	Concentration of alkali, NaOH, in terms of normality	Concentration of hydrogen-ions, in terms of normality	Density of the solution (H ₂ O at 25° = 1)	Coefficient of viscosity (H ₂ O, or salt solution, = 1)
I	—	—	$10^{-7.46}$	1.0164	12.17
	—	0.01	$10^{-9.34}$	„	6.84
	—	0.02	$10^{-10.02}$	„	3.62
II	3.6	—	$10^{-5.7}$	—	2.65*
III	3.5	—	$10^{-6.2}$	—	2.77+
IV	1.0	—	$10^{-8.1}$	—	2.39*

* Interpolated values from curves *b* and *c*, Fig. 1, see also Table I and Table III (Exp. II).

† See Exp. I, Table III.

In Table III are given the results of two experiments showing the influence of protein concentration when the euglobulin, at or about its iso-electric point, is dispersed by means of salt alone. It is seen that, when the concentration of protein is sufficiently high, very high values are obtained for the coefficient of viscosity. For example, in Experiment II, when the protein concentration was 13.2 %, the coefficient of viscosity was 29.56.

From a comparison of Tables II and III with Table I, which deals with an experiment in which the conditions obtaining in serum were closely imitated, it is evident that the euglobulin in serum must be regarded as salt-globulin.

¹ This influence would appear even more marked if, in calculating the concentration of protein in the solutions of salt-globulin, any allowance were made for the water appropriated by the salt.

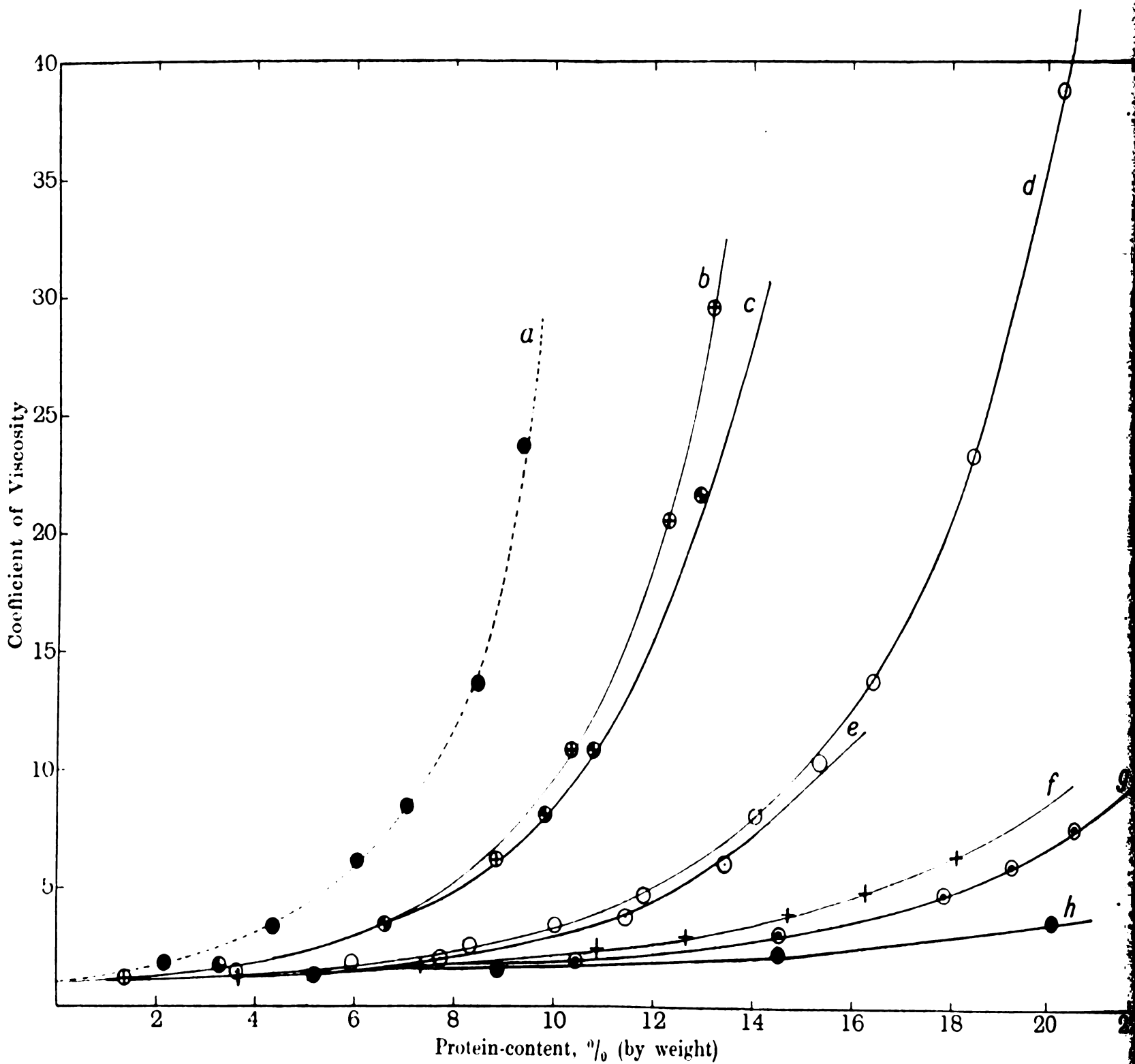


Fig. 1. Influence of protein concentration upon the viscosity of various protein solutions.

- Curve *a* - - - ● - - - , sodium caseinogenate [Chick and Martin, 1913].
 b — ⊕ — , euglobulin (salt). (See Exp. II, Table III.)
 c — ● — , euglobulin (salt + alkali). (See Table I.)
 d — ○ — , pseudoglobulin. (See Table V.)
 e — ⊖ — , pseudoglobulin (salt). (See Table VI.)
 f — + — , whole serum (horse). [Chick and Lubrzynska, 1914, Table VI.]
 g — ⊕ — , serum albumin. [„ „ „ „ „ IV.]
 h — ● — , egg albumin. [„ „ „ „ „ I.]

TABLE III.

Influence of concentration of protein upon the viscosity of euglobulin (horse) dissolved (dispersed) in sodium chloride solutions.

Exp. I. Concentration of hydrogen-ions = $10^{-6.2}$ normal.

Exp. II. " " " = $10^{-5.5}$ to $10^{-6.0}$ normal.

Temperature = 25° .

Mean time of flow for water = 55.4 seconds.

Exp.	Concentration of NaCl, %	Concentration of protein, %	Mean time of flow in viscosimeter, secs.		Density (H_2O at $25^{\circ}=1$)		Coefficient of viscosity, H_2O containing 3% NaCl = 1
			Euglobulin solution	Salt solution (3.0% NaCl)	Euglobulin solution	Salt solution (3.0% NaCl)	
I	3.0	8.02	241.1	57.6	1.045	1.0211	4.28
	3.7	5.82	142.3	"	1.038*	"	2.48
	4.3	3.62	95.2	"	1.032	"	1.62
	3.5	1.44	68.6	"	1.025	"	1.18
II	3.6	13.20	1654.1	(3.6% NaCl) 58.1	1.0650	(3.6% NaCl) 1.0257	H_2O containing 3.6% NaCl = 1 29.56
	"	12.32	1153.3	"	1.0626*	"	20.56
	"	10.57	612.3	"	1.0574*	"	10.87
	"	8.86	352.8	"	1.0522*	"	6.23
	"	6.58	189.4	"	1.0451	"	3.32
	"	1.36	69.1	"	1.0298*	"	1.19

* Interpolated values.

Influence of Temperature.

The solution used to investigate the influence of temperature contained 10.81 % protein and 1.82 % sodium chloride, and had a hydrogen-ion concentration equal to 10^{-8} N, the conditions being so arranged as to approximate to those obtaining in normal serum. The viscosity at various temperatures is expressed in relation to that of a 1.82 % solution of sodium chloride, estimations of the latter being made in the same viscosimeter at points over the same range of temperature. The results were expressed in a smoothed curve, from which the values in the 3rd column of Table IV were obtained. With a rise of temperature from 2.1° to 41.3° , the coefficient of viscosity was decreased to less than one quarter (see Table IV), an effect very much greater than that obtained with the other serum proteins investigated. A solution of serum albumin containing 20.65 % protein had its viscosity reduced by only about 30 % for a similar change of temperature, and while, for the whole proteins of serum, the effect of temperature was somewhat greater, it was insignificant compared to the result obtained for euglobulin.

TABLE IV.

Influence of temperature upon the viscosity of solutions (dispersions) of euglobulin (horse) in dilute sodium chloride and alkali.

Concentration of sodium chloride = 1.82 %.			
,, ,, hydrogen-ions = $10^{-8.03}$ normal.			
,, ,, protein = 10.81 %.			
Density of protein solution = 1.0479.			
,, ,, 1.82 % NaCl solution = 1.0127.			
Temperature, °C.	Mean time of flow in viscosimeter, secs.		Coefficient of viscosity (1.82 % NaCl solution at the same temperature = 1)
	Euglobulin solution	1.82 % solution of NaCl (from curve)	
2.1	939.8	36.3	26.79
9.1	532.4	29.5	18.67
17.4	288.7	24.1	12.39
25.0	198.1	20.8	9.85
32.1	138.6	18.6	7.71
41.3	93.6	16.2	5.97

The great influence of rise of temperature in lowering viscosity is characteristic of emulsoid colloids, perhaps the most striking example being that of solutions of gelatin. The difficulty experienced in filtering serum and other solutions containing proteins is also lessened to a surprising extent on raising the temperature owing to the fall in viscosity. With dilute solutions, of low viscosity, the effect may not be much in excess of that observed for water itself, or for solutions of crystalloids, but it becomes progressively greater with increase in concentration of the colloids and consequent rise in viscosity. For example, a solution of egg-albumin containing 7.04 % protein (coefficient of viscosity = 1.36 at 25°) showed a change of viscosity only about 5 % greater than that of distilled water when the temperature was raised nearly 40 degrees centigrade. When the strength of protein was increased to 20.1 % (coefficient of viscosity = 3.58 at 25.4°) and 28.15 % (coefficient of viscosity at 25.4° = 10.01) the change in the value of the coefficient was about 23 % and 54 % respectively for a similar change in temperature [see Chick and Lubrzenska, 1914, Table II]. In the case of whole serum (protein = 7.68 %; coefficient of viscosity = 1.87 at 25°), the coefficient of viscosity was decreased nearly 14 %, and with concentrated serum (18.1 % protein, coefficient of viscosity = 6.34 at 25°) the fall in value was about 51 % on raising the temperature about 40° [Chick and Lubrzenska, 1914, Table VII].

It is therefore quite consistent that solutions of pure euglobulin, with their very high viscosity, should suffer an even greater change in this respect with alteration of temperature. An interpretation of the phenomenon will be suggested later (see p. 277).

PSEUDOGLOBULIN.

Influence of concentration of protein.

The results of viscosity measurements with solutions of pure dialysed pseudoglobulin are set forth in Table V. The method by means of which it was freed from the water-insoluble material, formed during its lengthy preparation, has already been described (p. 263). The results are also graphically shown in curve *d* of Figure 1. It will be seen that, as regards viscosity, solutions of pseudoglobulin occupy a position intermediate between those of euglobulin and of serum albumin. For solutions of equal protein content, the viscosity of those of pseudoglobulin falls far below those of euglobulin and this is especially marked in the stronger solutions. For example, a solution containing 10% protein, exhibits a viscosity less than one-third of that of a solution of euglobulin of equal strength. At the same time, the viscosity is considerably in excess of that of serum albumin, a 10% solution being nearly twice and a 20% solution about five times as viscous [see Chick and Lubrzynska, 1914, p. 65].

TABLE V.

Influence of concentration of the protein upon the viscosity of solutions of pseudoglobulin (horse) dialysed.

Temperature 25°.

Time of flow in viscosimeter for water = 48 secs.

Concentration of hydrogen-ions = $10^{-6.8}$ to $10^{-6.9}$ normal.

Concentration of protein, %	Mean time of flow in viscosimeter, secs.	Density of the solution (H ₂ O at 25° = 1)	Coefficient of viscosity (H ₂ O = 1)
20.37	1753.6	1.0619	38.79
18.45	1059.7	1.0558*	23.31
16.43	628.8	1.0490	13.74
14.06	370.8	1.0425	8.05
11.82	218.0	1.0341	4.70
10.04	160.1	1.0289*	3.43
8.32	121.5	1.0239*	2.59
5.95	87.5	1.0169	1.85
3.61	66.4	1.0102	1.40

* Interpolated values.

Influence of ammonium sulphate.

In Table VI are given the results of an experiment with un-dialysed material containing the salt which separated with the protein phase during precipitation by half-saturated ammonium sulphate. The insoluble protein present amounted only to a slight trace. The concentration of ammonium sulphate was equal to 10.4 %, and, in order to compare the results with those given in Table IV, the percentage concentration of protein is reckoned, not as grams per 100 grams of the system but as grams per 100 grams protein and water, see 3rd column Table VI.

TABLE VI.

Influence of concentration of protein upon the viscosity of solutions of pseudoglobulin (horse) containing ammonium sulphate.

Temperature 25°.

Time of flow in viscosimeter for 10.3 % $(\text{NH}_4)_2\text{SO}_4$ = 53.9 seconds.

Time of flow for water = 48 seconds.

Concentration of hydrogen-ions = $10^{-6.8}$ normal.

Concentration of $(\text{NH}_4)_2\text{SO}_4$ = 10.4 %.

Concentration of protein		Mean time of flow in viscosimeter, secs.	Density of solution (H_2O at 25° = 1)	Density of H_2O containing 10.3 % $(\text{NH}_4)_2\text{SO}_4$	Coefficient of viscosity, H_2O containing 10.3 % $(\text{NH}_4)_2\text{SO}_4$ = 1
G. in 100 g. total system	G. in 100 g. water + protein				
13.75	15.35	535.2	1.1025	1.0601	10.33
12.05	13.45	312.6	1.0972*	,,	6.00
10.26	11.45	198.8	1.0917*	,,	3.80
6.93	7.73	106.8	1.0814	,,	2.02
4.16	4.65	75.3	1.0729*	,,	1.41

* Interpolated values.

As a matter of fact, for accurate comparison, some allowance should also be made for the water appropriated by so large a proportion of salt, but that is impossible to estimate. While the effect of salt in lowering viscosity is much less than that obtaining in the case of euglobulin, its influence is evident from a comparison of Tables V and VI, or of curves *d* and *e*, Figure 1.

SIGNIFICANCE AND INTERPRETATION OF RESULTS.

From a survey of the foregoing results, it is seen that, as regards their viscosity, solutions of the three proteins of serum, albumin, pseudoglobulin and euglobulin, form a series varying from comparatively labile fluids, resembling those of crystalloids, in case of albumin, to liquids of considerable

viscosity, in the case of euglobulin. A concentration of protein of at least 10 % is necessary to reveal the colloidal nature of serum albumin and leads to excessively high viscosities with the other two proteins.

The interpretation of the characteristic phenomena displayed by solutions of these proteins may be found in the two-phase nature of the system. Proteins are "hydrophile emulsoids" and the actual volume of the disperse phase may be assumed to be much greater¹ than that indicated by the solution volume of the protein, as determined from the density of the solution.

Hatschek [1910, 1911] has developed a theory of the viscosity of two-phase systems based upon his own observations of the viscosity of oil-water emulsions. He has shown that when the volume of the oil approaches 70 % of the total volume, at which point the oil particles touch one another, there is an enormous increase in viscosity. This, in his opinion, is due to the inability of the oil particles to roll upon one another under the influence of a shearing force; as a result they suffer deformation. From mechanical considerations he has found that, when the volume of the oil is more than one-half the total volume, the viscosity of such a two-phase system can be expressed in terms of the phase ratio as follows:

$$\eta = \eta' \frac{\sqrt[3]{A}}{\sqrt[3]{A}-1} \dots\dots\dots(1),$$

where

η = the viscosity of the system,

η' = the viscosity of the continuous phase,

V = volume of the system,

v = volume of the disperse phase,

and

$A = \frac{V}{v}$ = the phase ratio.

If the viscosity of the continuous phase be taken equal to 1.0

$$\eta = \frac{\sqrt[3]{A}}{\sqrt[3]{A}-1} \dots\dots\dots(2),$$

or

$$A = \left(\frac{\eta}{\eta-1} \right)^3 \dots\dots\dots(3).$$

According to this expression, the viscosity of the system is independent of the viscosity of the disperse phase and of the size of its particles, but depends upon the relative volume of the two phases.

The formula was tested by Hatschek [1911, 1913] in the case of oil-water emulsions, of which the composition was accurately known. When

¹ In this connection, it is of interest to note that Findlay and Creighton [1911] found that the solubility of oxygen, at atmospheric pressure, in de-aerated serum was only about one-fifth as great as in water; in the case of nitrogen, the difference was even greater.

the emulsion was sufficiently concentrated, the known volume of oil emulsified was found to be in close agreement with the value calculated by means of the above formula.

Hatschek [1912] has found the above formula also applicable to colloidal solutions of the emulsoïd type, in cases where the disperse phase occupies more than one-half the total volume of the system, i.e. where A is less than 2. The two instances selected by him are solutions of glycogen, using the viscosity measurements of Bottazzi and d'Errico [1906], and those of caseinogen, using the results of Chick and Martin [1912]. The value of $A \left(= \frac{V}{v} \right)$ was reckoned by means of the formula (3) given above, V being the volume occupied by 100 grams of the system. The value of $A' \left(= \frac{V}{c} \right)$, where c is the weight of dissolved substance, could be calculated from the measured density of the system and the known concentration of the colloid. The ratio $\frac{A'}{A} \left(= \frac{v}{c} \right)$, expressing the volume occupied by unit weight of the dissolved substance, was then calculated and, in case both of glycogen and caseinogen, Hatschek found a very fair constancy in value, when A was less than 2.

One of the most important results of Hatschek's expression is that, given the viscosity, not only is a method afforded of determining the phase ratio, but from the latter, given the concentration of the colloid and the density of the system, a calculation can, for the first time, be made of (a) the volume occupied, and (b) the amount of water taken up, by unit weight of a hydrophile colloid.

In the case of caseinogen, the value of (a) is equal to 9.3¹, that is to say each gram of caseinogen in colloidal solution occupies a volume equal to 9.3 cc. having taken up 8.6 cc. water (solution volume of caseinogen = 0.70). It follows therefore that even in so comparatively dilute a solution as 6% (100 grams of the system occupying 98 cc.) the caseinogen phase would occupy 56 cc. or 57% of the total volume.

Using Hatschek's formula the values of $\frac{v}{c}$ for all the serum proteins used in the present investigation have been calculated and the results for euglobulin and pseudoglobulin are given in Tables VII and VIII and IX respectively. Table X contains a similar set of results for serum albumin, using the viscosity measurements published previously [Chick and Lubrzenska, 1913].

¹ The mean value given by Hatschek, 9.52, is slightly too high, the density of the system being neglected in evaluating A' .

TABLE VII.

Volume occupied by the euglobulin phase in solution at 25° C.

(a) Dispersed with salt and alkali (see Table I).

(b) Dispersed with alkali (see Table II).

	Concentration of protein, % =c	Concentration of hydrogen-ions, in terms of normality	Concentration of NaCl, % =c	Density of system =δ	Coeff. of viscosity =η	A = vol. of 100 g. system vol. disperse phase (=v) = $\left(\frac{\eta}{\eta-1}\right)^3$	A' = vol. of 100 g. system weight of dissolved substance (=c) = $\frac{100}{\delta \times c}$	Volume occupied by 1 g. dissolved substance = $\frac{A'}{A} = \frac{v}{c}$	Mean value of $\frac{v}{c}$
(a)	12.95	10 ^{-8.1}	2.2	1.0582	21.69	1.152	7.298	6.335	6.51
	10.81	„	1.8	1.0479	10.86	1.336	8.828	6.608	
	9.84	„	1.7	1.0435	8.18	1.479	9.738	6.584	
	6.60	„	1.1	1.0290	3.487	2.757	14.72	5.339	
	3.27	„	0.5	1.0140	1.710	13.96	30.13	2.158	
(b)	5.68	10 ^{-7.5}	—	1.0164	12.17	1.294	17.32	13.39	
	„	10 ^{-9.3}	—	„	6.84	1.608	17.32	10.77	
	„	10 ^{-10.0}	—	„	3.62	2.638	17.32	6.57	

TABLE VIII.

Volume occupied by the euglobulin phase in salt solution at 25° C.

(See Table III, Exp. II.)

Concentration of protein, % $=c$	Density of the system $=\delta$	Coefficient of viscosity $=\eta$	$A =$ vol. of 100 g. of system	$A' =$ vol. of 100 g. of system	Volume occupied by 1 g. dissolved substance $=\frac{A'}{A} = \frac{v}{c}$	Mean value of $\frac{v}{c}$
			vol. of disperse phase ($=v$) $=\left(\frac{\eta}{\eta-1}\right)^3$	wt. of dissolved substance ($=c$) $=\frac{100}{\delta \times c}$		
13.20	1.0650	29.56	1.109	7.114	6.415	6.51
12.32	1.0626	20.56	1.162	7.640	6.574	
10.57	1.0574	10.87	1.336	8.948	6.698	
8.86	1.0522	6.23	1.690	10.73	6.346	
6.58	1.0451	3.32	2.930	14.54	4.964	
1.36	1.0298	1.19	245.5	71.40	0.291	

TABLE IX.

*Volume occupied by the pseudoglobulin phase in solution at 25° C. (dialysed).
(See Table V.)*

Concen- tration of protein, % = c	Density of the system = δ	Coefficient of viscosity = η	$A =$ Total vol. of system vol. of disperse phase (= v) $= \left(\frac{\eta}{\eta - 1} \right)^3$	$A' =$ Total vol. of system wt. of dissolved substance (= c) $= \frac{100}{\delta \times c}$	Volume occupied by 1 g. dissolved substance $= \frac{A'}{A} = \frac{v}{c}$	Mean value of $\frac{v}{c}$
20.37	1.0619	38.79	1.082	4.623	4.272	4.50
18.45	1.0558	23.31	1.141	5.133	4.499	
16.43	1.0490	13.74	1.254	5.803	4.627	
14.06	1.0425	8.05	1.488	6.822	4.585	
11.82	1.0341	4.70	2.050	8.180	3.990	
10.04	1.0289	3.43	2.813	9.682	3.441	
8.32	1.0239	2.59	4.322	11.74	2.716	
5.95	1.0169	1.85	10.31	16.53	1.603	
3.61	1.0102	1.40	42.86	27.42	0.640	

TABLE X.

*Volume occupied by the serum albumin phase in solution at 25° C.
[See Chick and Lubrzenska, 1914, p. 65.]*

Concen- tration of protein, % = c	Density of the system = δ	Coefficient of viscosity = η	$A =$ vol. of 100 g. of system vol. of disperse phase (= v) $= \left(\frac{\eta}{\eta - 1} \right)^3$	$A' =$ vol. of 100 g. of system wt. of dissolved substance (= c) $= \frac{100}{\delta \times c}$	Volume occupied by 1 g. dissolved substance $= \frac{A'}{A} = \frac{v}{c}$	Mean value of $\frac{v}{c}$
20.65	1.0593	7.538	1.534	4.572	2.980	2.81
19.24	1.0555	5.875	1.751	4.924	2.838	
17.85	1.0513	4.763	2.028	5.329	2.628	
14.54	1.0413	3.025	3.332	6.605	1.982	
10.45	1.0296	1.952	8.618	9.295	1.078	
5.19	1.0153	1.316	72.20	18.98	0.263	
2.59	1.0075	1.128	684.0	38.32	0.056	

In all cases, with the exception of the experiment set forth in Table VII *b*, which will be discussed separately later on, the value $\frac{v}{c}$ remained constant when the disperse phase occupied more than one-half the total volume. It was greatest in the case of euglobulin, one gram of which, in salt solution, was calculated to have a volume of 6.5 cc. while each gram of dissolved serum albumin was found only to occupy 2.81 cc., pseudoglobulin being intermediate between the two ($\frac{v}{c} = 4.52$). The solution volume of these

TABLE XI.

Water taken up by various proteins in the formation of colloidal solution at 25° C.

Protein	Density of the protein in solution at 25° (H ₂ O at 25° = 1)	Solution volume (as calculated from the density of the system)	Volume occupied by 1 g. protein in solution, cc.	Water associated with 1 g. protein, when in solution, cc.
Serum albumin (crystallised)	1.38	0.72	2.81	2.09
Pseudoglobulin	1.39	0.72	4.50	3.78
Euglobulin (salt)	1.42	0.70	6.51	5.81
Sodium caseinogenate	1.43	0.70	9.33	8.63
Egg-albumin (crystallised)	1.36	0.73	2.30	1.57

proteins can be calculated from the density of their solutions and hence the actual amount of water associated with each gram of protein can be calculated. These values vary from 5.8 cc. in the case of euglobulin to 3.8 cc. and 2.1 cc. for pseudoglobulin and serum-albumin respectively. These figures are all collected in Table XI, those for caseinogen and egg-albumin being added for purposes of comparison.

In order to institute useful comparison with the other proteins in serum, solutions of euglobulin in presence of salt have alone been taken into account in the preceding paragraph, because, in normal serum, it is under those conditions that this protein exists. When euglobulin is dispersed by alkali alone, its viscosity shows very remarkable variations, as may be seen from the results of Exp. I in Table II, where the euglobulin was practically salt-free. Under these circumstances the degree of viscosity depends upon the amount of alkali employed, being comparatively high at first and rapidly falling with increasing concentration of alkali and hydroxyl-ions. Increase of the latter from near the neutral point to a concentration of about 10^{-4} N (hydrogen-ion concentration 10^{-10} N) was accompanied, in the case of a 5.68 % euglobulin dispersion, by a fall in the viscosity coefficient from 12.17 to 3.62, which latter number is near the figure obtained for salt-globulin of the same protein-concentration. This decrease in viscosity is accompanied by a visual change, the globulin mixture, which remains in the form of a precipitate at concentrations of hydrogen-ions from about 10^{-5} to 10^{-6} N, changing to a thick, opalescent fluid at the neutral point (conc. $H^+ = 10^{-7}$ N) and to a thin, clear liquid at a concentration of hydrogen-ions equal to about 10^{-10} N. The observed change in size of the protein particles would, according to the theory of Hatschek, be unaccompanied by any change in viscosity unless there were a concomitant change in phase-ratio. In other

words, we must suppose that, in the more alkaline solution, the globulin phase contains less water and that the amount appropriated by the protein steadily increases as the iso-electric point is approached, with a consequent great increase in the volume of the disperse phase. In Table VII *b* the calculated value of $\frac{v}{c}$, the volume occupied by one gram protein in solutions of varying alkalinity, is seen to vary from 13.4 at the neutral point to 6.6 in the most alkaline solution employed. The latter figure, which is practically the same as that obtained for salt-globulin, is probably too low, for, in this case, the volume of the disperse phase, as ascertained by Hatschek's formula, is less than one-half the total volume and the calculation is therefore not strictly permissible.

In the presence of a small concentration of salt, the water appropriated by the globulin phase appears to be nearly independent of the reaction, the calculated value of $\frac{v}{c}$ being the same in the case of the two experiments in Table I and Table III, when the hydrogen-ion concentration was varied from 10^{-8} N to 10^{-6} N (see Tables VII *a* and VIII respectively).

The fact that the addition of alkali to a protein, with the consequent formation of protein salt and increase of protein ions, should be accompanied by a *lowering* of viscosity, and the conception that this is due to a less degree of association of the protein with water is opposed to the results of similar investigations with other proteins. Lacqueur and Sackur [1903] showed that increase in alkalinity led to an *increase* in viscosity in case of sodium caseinogenate. Pauli and Handovsky [1909 and 1910], working with the proteins of ox-serum, have shown that an addition of either acid or alkali leads to a preliminary rise in viscosity, followed by a fall as the concentration of either acid or alkali is further increased. Pauli and Falek [1912] have demonstrated the same phenomenon in case of gelatin solutions, and the work of Spiro [1904], Fischer [1910] and Chiari [1911] has shown that the water imbibed by gelatin increases rapidly as the reaction is made either more acid or more alkaline than the iso-electric point.

The general influence of salts¹ in lowering viscosity of protein solutions (and imbibition of water by gelatin), presumably due to their water-withdrawing capacity, was also demonstrated by the above observers and has been confirmed in the present work. The effect in the case of euglobulin is of great importance and has been fully discussed above; it is also well marked in the case of pseudoglobulin.

¹ What is possibly an analogous phenomenon with alcohol has been demonstrated by Brailsford Robertson [1912]. He found that the viscosity of sodium caseinogenate solutions (about 3 %) was lowered when the proportion of alcohol present was raised from 50 % to 75 %.

Influence of temperature. In the light of the theory developed above, the very marked influence of temperature upon the viscosity of protein solutions (see Table IV above, and Tables II, V and VII, Chick and Lubrzynska [1914]) may be explained by a gradual loss of water from the protein phase as temperature is raised. It is usual, with most substances of crystalloid nature, to find less water needed for the solution of each gram of the solute at a higher temperature, and the same property may also be assumed for substances of a colloidal nature. On such an assumption, the greater the initial appropriation of water, either due to the nature of the colloid or to its high concentration, the greater will be the loss of water from the disperse phase on rise of temperature and the diminution in its volume; in consequence the greater will be the fall in viscosity.

The experimental facts are in accord with this conception. With solutions of egg-albumin, where a comparatively small amount of water is assumed to enter into the composition of the disperse phase, the effect of temperature upon the viscosity coefficient is found to be negligible except in the case of very concentrated solutions (20 % and upwards). On the other hand, comparatively dilute solutions of euglobulin, the most "hydrophile" of the proteins studied, show very marked changes in viscosity with alteration of temperature (see Table IV). In the following manner, I have attempted to estimate the volume occupied by one gram of (salt) euglobulin in solution at various temperatures. From curve *c*, Fig. 1, it is possible to determine the concentration of solutions which, at 25°, would possess the same viscosity coefficients as those determined for the 10.81 % solution at the various temperatures (see the 4th column, Table IV). In the case of these solutions, at 25°, the concentration (in %) multiplied by 6.51, gives the volume (in cc.) occupied by the disperse phase. The same values for the volume of the disperse phase will apply to the 10.81 % solution at the temperatures where the viscosity corresponds, if changes in viscosity due to alteration of temperature are assumed to be the result only of changes in phase-ratio (and if the differences in density of the solutions, caused by differences in temperature and concentration, are disregarded). In this way, the volume of one gram of euglobulin, in salt solution, at 2.1° has been estimated at 8.3 cc. and that at 43° at 5.3 cc.; at 25° the volume has already been calculated as 6.51 cc.

APPLICATION OF THE ABOVE RESULTS TO THE SALTING OUT OF PROTEINS.

The results of the preceding experiments, and the conceptions derived from them, throw a very interesting and illuminating light upon the phenomena displayed in the precipitation of proteins by neutral salts. They explain very clearly the influence of increased protein concentration, both in raising the proportion of protein thrown out by a given concentration of salt, and in lowering the limit of salt concentration at which precipitation occurs.

The values obtained, by the use of Hatschek's formula, for the amount of water presumably associated with unit weight of the various serum proteins in formation of colloidal solutions, yield a satisfactory explanation of the well-known precipitation limits of these proteins. The euglobulin, needing most water for its colloidal solution, is first driven out as water is withdrawn during any salting-out process; a higher concentration of salt is necessary to throw down the pseudoglobulin; while the albumin, which appropriates comparatively little water, is the last to be precipitated.

The current notion that albumin is less readily precipitated than the other proteins of serum, because it has a "greater affinity" for water, is thus seen to be erroneous. It is probably more consistent with the truth to affirm that the albumin, needing the exclusive use of less water for its own solution, is less liable to suffer precipitation when competition occurs.

SUMMARY.

1. The viscosity of solutions of pseudoglobulin and euglobulin from horse serum has been investigated as regards the influence of:

- (a) Concentration of protein.
- (b) Temperature.
- (c) Salt-content.
- (d) Hydrogen-ion concentration.

The results of similar experiments with serum albumin and whole serum, published previously by Chick and Lubrzynska [1914], are included in the general survey of the results.

2. In all cases, increase in protein concentration is accompanied by a disproportionately great increase in the viscosity of the solution. The effect is greatest in case of euglobulin, solutions of which exhibit a high viscosity at a comparatively low protein content. It is least in case of serum albumin, which, for strengths of protein under about 10%, behaves almost as a

crystalloid. Pseudoglobulin is intermediate between the other two proteins in this respect.

3. The viscosity of euglobulin solutions is dependent upon the manner in which solution (dispersion) of the protein is obtained. In case of "alkali globulin," where no salt is present, the viscosity of the solution depends upon the degree of alkalinity, falling rapidly with decrease in concentration of hydrogen-ions and distance from the iso-electric point. The viscosity of solutions of "salt-globulin" (NaCl) is considerably lower than that of alkali-globulin of equal protein and hydroxyl-ion content; in this case the viscosity is largely independent of the concentration both of salt (if above a small minimum, 0.5 to 1.0 %) and hydroxyl-ions. Euglobulin in serum is in the condition of salt-globulin.

4. The presence of a salt ($(\text{NH}_4)_2\text{SO}_4$) lowers the viscosity of pseudoglobulin solutions; it is without influence on the viscosity of solutions of albumin (egg) in concentrations up to 7 %.

5. The viscosity of protein solutions is decreased with rise of temperature frequently to a degree far in excess of that displayed by water or solutions of crystalloids. The greater the viscosity of the solution, the greater is the temperature effect, which is thus much enhanced in solutions of high protein concentration and most marked in case of euglobulin.

6. An interpretation of the above results is found in the two-phase nature of the systems studied. By means of Hatschek's formula, which gives an expression for phase-ratio in terms of viscosity, it is possible to calculate the relative volumes of protein- and water-phase in the more concentrated solutions employed. Hence values can be obtained for the volume occupied by one gram of the various proteins when in solution. These were found to show a satisfactory constancy and to be 2.8 cc., 4.5 cc. and 6.5 cc. for serum albumin, pseudoglobulin and salt-euglobulin respectively, at 25°. (The value obtained for alkali-euglobulin varied from 13.9 cc. to 6.6 cc. according to the alkalinity of the solution.) The solution volume of these proteins, reckoned from the density of their solutions, is equal to 0.7; the amount of water taken up by one gram of the protein at 25° would therefore be 2.1, 3.8 and 5.8 cc. respectively.

7. The conclusions given in no. 6 afford a satisfactory explanation of (a) the influence of protein concentration upon both the amount of protein precipitated, and the limit of salt concentration required to commence precipitation, in case of a pure protein; and (b) the relative order in which the above three proteins are precipitated from a mixture with increasing concentration of salt.

8. Hatschek's theory explains the observed disproportionate increase in viscosity of protein solutions with increase in concentration of the protein, the volume of the disperse phase being increased at the expense of the continuous phase. The relative magnitude of the phenomenon in case of the three proteins investigated is also interpreted.

9. The influence of temperature upon the viscosity of protein solutions would be explained by assuming a less degree of hydration of the colloid at higher temperature (an analogous phenomenon is encountered with crystalloids). A calculation has been made of the volume occupied by one gram of the protein at various temperatures in case of euglobulin. These volumes vary from 8.3 cc. at 2° to 5.3 cc. at 41°.

REFERENCES.

- Bottazzi and d'Errico (1906), *Pflüger's Archiv*, **115**, 359.
Chick (1913), *Biochem. J.* **7**, 318.
— and Lubrzynska (1914), *Biochem. J.* **8**, 59.
— and Martin (1912), *Zeitsch. Chem. Ind. Koll.* **11**, 102.
— and Martin (1913), *Biochem. J.* **7**, 59.
Chiari (1911), *Biochem. Zeitsch.* **33**, 167.
Findlay and Creighton (1911), *Biochem. J.* **5**, 294.
Fischer (1910), *Das Oedem*, Dresden.
Hardy (1905), *J. Physiol.* **33**, 251.
Hatschek (1910), *Zeitsch. Chem. Ind. Koll.* **7**, 301.
— (1911), *Zeitsch. Chem. Ind. Koll.* **8**, 34.
— (1912), *Zeitsch. Chem. Ind. Koll.* **11**, 284.
— (1913), *Trans. Faraday Society*.
Michaelis and Rona (1910), *Biochem. Zeitsch.* **28**, 193.
Lacqueur and Sackur (1903), *Beiträge*, **3**, 103.
Pauli and Falek (1912), *Biochem. Zeitsch.* **47**, 269.
Pauli and Handovsky (1909), *Biochem. Zeitsch.* **18**, 340.
— (1910), *Biochem. Zeitsch.* **24**, 239.
Robertson (1912), *Die physikalische Chemie der Proteine*, Dresden.
Spiro (1904), *Beiträge*, **5**, 276.