VII. THE VISCOSITY OF SOME PROTEIN SOLUTIONS.

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(2 figures.)

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In the following work the same general methods were employed as in that already published by Martin and Chick [1912] upon the viscosity of solutions of caseinogen.

In the present instance an investigation was made of the influence of (1) concentration of protein, and (2) temperature, upon the viscosity of solutions of pure crystallised egg- and (horse) serum-albumin. For purposes of comparison, a few experiments were also made with whole serum, in some cases concentrated in vacuo in order to yield material of high protein content. The viscosity, as in the previous work, was determined by measuring the time of flow in an Ostwald viscosimeter, the figures obtained being relative to the time taken under similar conditions by pure water, which is expressed by unity. The concentrations throughout are expressed as grams per 100 grams of solution.

EGG-ALBUMIN.

The crystals of egg-albumin were obtained from egg-white by the method of Hopkins and Pinkus [1898]. The material was twice recrystallised, and then dialysed for two or three weeks against distilled water, till it contained an insignificant trace of ammonium sulphate. As considerable dilution took place during dialysis, it was necessary to concentrate the material in order to obtain the high concentrations necessary for the experiments. This was done by allowing the solution to evaporate at room temperature in vacuo over sulphuric acid, material being thus obtained which contained 28% by weight of egg-albumin. The protein-content in this and other cases was determined by boiling a weighed quantity of the solution, after dilution and acidification, and weighing the coagulum on a weighed filter, after drying at 110°.

TABLE I.

Influence of concentration of the protein upon the viscosity of solutions of pure egg-albumin (crystallised).

Temperature 25.2° C. Time of flow in viscosimeter for water 47.8 seconds.

Concentration of protein, %	Mean time of flow in viscosimeter, secs.	Density of the solution (H ₂ O at 25° C. = 1)	Coefficient of viscosity (H ₂ O = 1)
28.15	441.8	1.0805	9.99
26.83	367.4	1.0775	8.30
24.33	257.2	1.0693	5.81
20.12	159.2	1.0566	3.60
14.53	97.9	1.0402	2.21
8.877	69.4	1.0242	1.57
3.016	53.8	1.0083	1.22

Influence of concentration of Protein. In Table I are given the details of an experiment showing the influence of concentration upon the viscosity coefficient in the case of this protein. The same results are shown graphically in curve (a) of Fig. 1. Solutions containing different proportions by weight of protein were obtained by dilution of the concentrated material described above. In order to apply the necessary correction in calculating the coefficient of viscosity, the density of the solutions was directly determined by weighing a known volume in a pycnometer.

In the case of the weaker solutions, the viscosity remained low, increasing but slightly with increasing concentration of protein and only reaching a value equal to twice that of distilled water at a concentration of protein equal to about 13%. This is well seen in the curve in Fig. 1, where, up to a concentration of about 9%, the slope is very slight, in fact the relation between viscosity and concentration of protein approximates to that obtaining in solutions of non-colloidal material, and might satisfactorily be expressed by a straight line. At higher concentrations the curvature becomes increasingly greater until, at a concentration of 28% protein, the viscosity reaches the figure of nearly 10.

The curve expressing the relation between protein concentration and viscosity in case of solutions of egg-albumin is of the same general type as that obtained for caseinogen [Chick and Martin 1912]; in the case of the latter, however, a comparatively low concentration (7 to 8%) produced a viscosity equal to the value obtained with 28% egg-albumin. According to the hypothesis of Hatschek [1910, 1911, 1912] the difference between these two proteins in regard to their viscosity should be attributed to the fact that in a solution of egg-albumin much less water is appropriated.

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

- Curve (a) —○— = pure egg-albumin.
 „ (b) —●— = pure serum-albumin (horse).
 „ (c) —+— = whole serum, at protein-concentrations above and below the normal; original serum contained 7.3% protein.

by the protein phase than is the case with caseinogen. Consequently, in the former case, much higher concentration of protein is necessary to yield the high viscosity characteristic of the condition in which the aggregates of the disperse phase are approaching contact with one another. In a later communication it is proposed to discuss this theory as regards both egg-albumin and other proteins in greater detail.

Influence of Temperature. The variation of temperature was necessarily limited, the maximum range being from 0° to about 40°. Higher temperatures could not be employed without danger of the protein becoming "denaturated."

In order to make the necessary comparison with the time of flow taken by water, experiments were made over the same range of temperature with an equal total volume of distilled water in each of the two viscosimeters employed in Exp. 1 and Exps. 2, 3 and 4 respectively. Smoothed curves

TABLE II.

Influence of temperature upon the viscosity of solutions of pure egg-albumin (crystallised) of varying concentration.

Exp. No.	Protein content, %	Density at 25° C. (H ₂ O at 25° C. = 1)	Temperature, °C.	Mean time of flow in viscosimeter, seconds		Coefficient of viscosity (H ₂ O at the same temperature = 1)
				Albumin solution	Distilled water (from curve)	
1	7.04	1.0192	2.8	136.8	101.8	1.37
			8.3	115.0	86.0	1.36
			15.2	95.0	70.3	1.38
			25.0	74.2	55.5	1.36
			32.3	63.0	47.9	1.34
			42.1	52.1	40.5	1.31
2	14.6	1.0404	2.8	179.2	86.2	2.16
			8.6	147.4	72.1	2.13
			14.7	123.4	61.5	2.09
			15.1	123.0	61.0	2.10
			25.0	94.0	48.0	2.04
			33.1	79.5	41.4	2.00
3	20.1	1.0566	42.9	64.2	34.5	1.94
			0	368.3	96.4	4.04
			8.0	269.7	73.3	3.89
			17.0	202.3	58.0	3.68
			25.4	161.4	47.6	3.58
			33.0	132.1	41.4	3.37
4	28.15	1.0805	41.6	109.5	35.4	3.27
			0.6	1170.2	93.8	13.48
			7.8	828.6	73.8	12.13
			15.6	614.1	60.1	11.04
			25.4	440.9	47.6	10.01
			33.9	344.9	40.8	9.13
			41.9	283.7	35.1	8.73

were drawn through the experimental points and from these the time of flow corresponding to any intermediate temperature could be read off. The values given in the 5th column of Table II were obtained in this way and, with the help of these, the coefficients of viscosity in the 7th column were calculated.

In case of the weaker solutions (from 7% up to 20%) the influence of temperature upon the viscosity was comparatively trivial, that is to say, the phenomenon was of the same order as that obtaining in the case of distilled water. A 7% solution of egg-albumin, with viscosity of 1.3, behaved on heating as pure water containing a crystalloid in solution. Even with a 20% solution, the viscosity relative to water was only about 20% greater

Fig. 2. Influence of temperature upon the viscosity of solutions of various proteins with differing protein content.

- = egg-albumin: curve a, protein content = 7.0 %.
- " b, " " = 14.6 "
- " c, " " = 20.1 "
- " d, " " = 28.1 "
- = serum protein: curve e, " " = 20.6 "
- +— = whole serum: curve f, " " = 7.0 "
- " g, " " = 18.1 "

at 0° than at 41.6°, and not until the concentration of albumin reached the high figure of 28 %, was temperature found to have any specially marked influence.

These facts are well expressed in the four curves *a*, *b*, *c* and *d* in Fig. 2, where viscosity (relative to water at the same temperature) is plotted as ordinate against temperature as abscissa.

Influence of Ammonium Sulphate upon Viscosity of Crystallised Egg-albumin. It has been shown [Chick and Martin 1913, 2; Spiro 1904] that "precipitation" of egg-albumin by ammonium sulphate is a phase-separation and that a definite proportion of both salt and water is associated with the egg-albumin in the "precipitate." Arguing from analogy, it is exceedingly probable that crystallisation of proteins from strong solutions of ammonium sulphate is of a similar character.

In order to ascertain whether such hypothetical association with ammonium sulphate had any influence on the viscosity of solutions, strictly comparable experiments were made, both before and after dialysis. Dabrowski [1912], on the basis of a comparison between the different rates of diffusion obtained with the two kinds of material, came to the conclusion that aggregates formed in solution by undialysed crystals of egg-albumin were much smaller, nearly one-sixth the size of those of dialysed albumin. It therefore seemed possible that such a fundamental difference in the type of solution might also be expressed by some significant change in viscosity.

The results obtained were, however, entirely negative (see Table III). Solutions were made of exactly similar protein content, 6.98 %, in the one

TABLE III.

Viscosity of crystallised egg-albumin.

(a) Before, and (b) after dialysis.

Exp.	Protein content, %	Ammonium sulphate, %	Temperature, °C.	Mean time of flow in viscosimeter, seconds		Coefficient of viscosity of $(\text{NH}_4)_2\text{SO}_4$ solution in exp. (a) and H_2O in exp. (b) = 1
				Albumin solution	3.51 % $(\text{NH}_4)_2\text{SO}_4$ solution in exp. (a) and H_2O in exp. (b)	
(a)	6.98 ¹	3.51	0.6	145.9	109.9	1.35
	"	"	25.5	75.9	58.8	1.31
	"	"	45.1	51.4	39.8	1.32
(b)	6.98 ¹	—	1.0	145.4	109.0	1.36
	"	—	25.4	74.5	57.0	1.33
	"	—	45.1	49.2	38.3	1.31

¹ Density of the solution taken as 1.019 for the purpose of calculating viscosity coefficient.

case from undialysed crystals, with their ammonium sulphate (= 3.51 %), and in the other from dialysed material. In the former case viscosity was determined relative to pure water and in the latter case in relation to a 3.51 % solution of ammonium sulphate. Comparison was instituted at three different temperatures. Temperature was not found to have any unusual influence on viscosity, nor was any difference traced between the two types of solution. At the same time it must be admitted that the concentration of protein employed (7 %) was rather low, and it would be well to repeat the experiment with stronger solutions.

SERUM-ALBUMIN (HORSE).

A series of experiments similar to those with egg-albumin were also carried out with pure serum-albumin. The protein in this case was also crystallised in presence of ammonium sulphate according to the method of Hopkins and Pinkus [1898] and, after recrystallisation, dialysed against distilled water in presence of toluene for some weeks. A solution was obtained, containing 20.65 % protein and traces only of ammonium sulphate.

Influence of Concentration of Protein. The density of serum-albumin solutions has been shown to bear a linear relation to the concentration of protein [Chick and Martin 1913, 1]. In order, therefore, to apply the appropriate correction in calculating the coefficient of viscosity it was only necessary to make direct determinations in a few cases. The straight line could then be drawn expressing the relation of density to protein content and the other values of density required be obtained by interpolation; such values are marked with an asterisk in Table IV.

TABLE IV.

Influence of concentration of the protein upon the viscosity of pure serum-albumin (crystallised).

Temperature 25.4° C. Time of flow in viscosimeter for water 55 seconds.

Concentration of protein, %	Mean time of flow in viscosi- meter, secs.	Density of the solution (H ₂ O at 25° C. = 1)	Coefficient of viscosity, H ₂ O = 1
20.65	391.4	1.0593	7.54
19.24	310.2	1.0549*	5.95
17.85	249.2	1.0509*	4.76
14.54	159.8	1.0412	3.02
10.45	104.3	1.0296	1.95
5.19	71.3	1.0153	1.32
2.59	61.6	1.0075	1.13

* Interpolated values.

The experiments showing the influence of protein content on viscosity are detailed in Table IV and graphically expressed in Fig. 1, curve (b), where viscosity as ordinate is plotted against concentration of protein as abscissa.

The results show great similarity with those obtained for egg-albumin. In Fig. 1, curves (a) and (b) run closely together at first. As the concentration of protein increases, however, it is seen that serum-albumin has a much higher viscosity than egg-albumin.

Influence of Temperature. The influence of temperature was investigated in one experiment only, of which details are given in Table V, and graphically expressed in curve (e) Fig. 2. This experiment was made with the most concentrated solution available, containing 20.1 % protein by weight. The viscosity relative to water was not only much greater than that of egg-albumin of similar strength (see Exp. 3, Table II), but showed a much greater change with alteration of temperature, viz. from 7.3 at 43° to 10.3 at 1.6° (compare Table V with Exp. 3, Table II).

TABLE V.

Influence of temperature upon the viscosity of pure serum-albumin (crystallised).

Protein-content = 20.65 %. Density = 1.0593 (at 25° C.).

Temperature, °C.	Mean time of flow in viscosimeter, seconds		Coefficient of viscosity (H ₂ O at the same temperature = 1)
	Albumin solution	Distilled water (from curve)	
1.6	1026.0	105.6	10.29
3.95	906.2	98.2	9.77
8.8	713.2	84.7	8.92
14.8	553.8	71.0	8.26
25.4	391.4	54.8	7.56
33.4	325.8	47.0	7.34
43.0	275.1	40.0	7.28

WHOLE SERUM (HORSE).

The sample of horse-serum selected for these experiments contained 7 % total protein. In order to obtain solutions of protein-content comparable to those employed above, some concentration of the serum proteins was necessary. By placing in shallow dishes for 48 hours in vacuo at room temperature material was readily prepared, which contained 18 % protein. The constituents of the serum did not appear to have been affected by the process, for, after diluting the concentrated material to obtain a solution whose protein-content was equal to that of the original serum, the viscosity was also found to be the same as that previously determined (see Table VI).

Influence of Concentration of Protein and of Temperature. The proportion of salt contained in serum was found to have an insignificant influence upon both the density and the viscosity of the system; hence the values of the coefficient of viscosity were calculated in relation to pure water as before. In Table VI are given the results of a series of experiments in which the protein-content varied from 1.8% to 18% by weight.

In obtaining values for the density of the various solutions, the same method was adopted as in the case of serum-albumin; after a direct determination had been made in case of one or two solutions, a curve was constructed from which intermediate values could be obtained. Such interpolated values are marked with an asterisk in Table VI.

TABLE VI.

Viscosity of horse-serum at concentrations above and below the normal; the normal serum contained 7.3% protein.

Temperature 25° C. Time of flow in viscosimeter for water 55.5 seconds.			
Concentration of protein, %	Time taken for flow in viscosimeter, secs.	Density of the solution (H ₂ O at 25° C. = 1)	Coefficient of viscosity (H ₂ O = 1)
1.836	62.4	1.0069*	1.13
3.665	70.3	1.0143	1.28
5.134	79.4	1.0193*	1.46
5.84	82.3	1.0220*	1.52
7.31 ¹	92.8	1.0276	1.72
7.33 ²	92.5	1.0276	1.71
9.13	110.2	1.0344*	2.05
10.89	131.3	1.0405	2.46
12.67	156.6	1.0477*	2.96
14.71	206.2	1.0553*	3.92
16.27	253.6	1.0617	4.85
18.10	331.5	1.0679*	6.38

¹ Normal serum.

² Prepared by dilution from concentrated serum.

* Interpolated and extrapolated values.

From Table VI, and more readily from curve (c) Fig. 1, it is seen that for low concentrations of total protein the viscosity of whole serum also remained low, varying from about 1.10 to 1.7 as the concentration of total protein was raised from 1.80% to that of the normal serum (7.3%). As the serum was concentrated, however, the viscosity increased rapidly with increasing concentration of total protein, and when it possessed a little more than twice the normal protein-content the viscosity relative to water reached the value of 5.0.

In comparison with serum- or egg-albumin the total proteins of serum yield much higher viscosity when in solution. For example, while 18% solutions of serum- and egg-albumin gave coefficients of viscosity equal to 4.9 and 2.8 respectively (see curves (b) and (a), Fig. 1), concentrated whole serum containing the same proportion of total protein had a viscosity of 6.38.

The influence of temperature upon viscosity was also greater with the proteins of whole serum. With the concentrated material, containing 18% protein, the coefficient of viscosity fell 34% (from 8.63 to 5.7) with increase in temperature of about 40° (see Table VII). A slightly greater rise of temperature in case of solutions containing 20% serum- and egg-albumin produced a decrease in viscosity coefficient of only about 29% and 19% respectively (see Tables V and II).

TABLE VII.

*Influence of temperature upon the viscosity of whole serum (horse),
(a) normal serum, (b) concentrated serum.*

Exp.	Protein content, %	Density at 25° C. (H ₂ O at 25° C. = 1)	Temperature, °C.	Mean time of flow in viscosimeter, seconds		Coefficient of viscosity (H ₂ O at the same temperature = 1)
				Serum	Distilled water (from curve)	
(a)	7.68	1.0290	0	218.2	111.6	2.01
	"	"	8.0	164.9	86.7	1.96
	"	"	17.0	125.5	67.1	1.92
	"	"	25.4	99.6	54.9	1.87
	"	"	33.0	88.6	47.3	1.82
	"	"	41.6	70.4	40.9	1.77
(b)	18.1	1.0679	0.2	897.2	111.0	8.63
	"	"	8.2	581.5	86.0	7.22
	"	"	14.3	462.3	71.9	6.86
	"	"	25.0	328.9	55.4	6.34
	"	"	32.2	270.3	48.0	6.01
	"	"	39.8	224.6	42.1	5.70

It is evident that one or more of the remaining proteins in horse serum must, when in solution, possess a viscosity much higher than that of the serum-albumin, and the results of some preliminary experiments suggest that the small proportion of "euglobulin" contained in the serum exercises a disproportionate influence upon the viscosity of the whole.

In a second communication it is proposed to publish the results of experiments, similar to the above, carried out with purified samples of

“euglobulin” and “pseudoglobulin,” prepared from horse serum, and to include a general discussion of the theoretical bearing of the whole series of data.

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