CXIX. THE KINETICS OF PROTEIN DENATURATION.

PART II. THE EFFECT OF VARIATION IN THE HYDROGEN ION CONCENTRATION ON THE VELOCITY OF THE HEAT DENATURATION OF EGG-ALBUMIN; THE CRITICAL INCREMENT OF THE PROCESS.

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THE first work carried out on the rate of the heat denaturation of egg-albumin was that of Chick and Martin [1910], who found that the reaction velocity slowed off more rapidly than was accounted for by the unimolecular law. They also found that the hydrogen ion concentration (in acid solution) and the hydroxyl ion concentration (in alkaline solution) were reduced during the course of the reaction¹, and to this they attributed the falling off of reaction velocity mentioned above. This alteration of the hydrogen ion concentration (or more briefly the p_H of the solution) has been confirmed by Quagliariello [1912], Sørensen and Jurgensen [1911], and more recently by Wu and Wu [1925]. In later papers, Chick and Martin [1912] show that when precautions are taken to keep the p_H of the solutions constant (by saturation with boric acid in acid solution and with magnesium oxide in alkaline solution) a steady unimolecular velocity constant is obtained for the process.

In the present work the solutions were first maintained at a constant p_H by means of buffers but it was found that while a steady unimolecular constant could be obtained in this manner, the actual velocity of denaturation was much enhanced, which is in agreement with the observation of Zoller [1921] that phthalate buffers lower the coagulation temperature of egg-albumin. Further, in view of the finding of Chick and Martin [1910] that the presence of neutral salt lowers the critical increment² of the heat denaturation of egg-albumin, the use of buffers was abandoned.

¹ Sørensen [1925] shows that this change of p_H as the reaction proceeds is due to hydrolytic scissions of the protein molecule, which, however, as they do not proceed hand in hand with coagulation, cannot be the fundamentally important processes in denaturation.

² This quantity has already been defined in Part I.

EXPERIMENTAL.

The present work has been carried out at temperatures of 65° and 70.2°, in unbuffered solutions but at varying initial p_H values in such a manner that a p_H range of approximately 5 to 8 has been covered.

The method employed was to conduct an experiment in the manner described in Part I and not only to measure the albumin content of each sample withdrawn but also the p_H thereof¹.

The logarithm of the concentration of albumin was then plotted against time. By taking tangents on the resulting curve the unimolecular constants of the reaction at the times corresponding to the determined p_H values were obtained.

By varying the initial p_H of the solution the above-mentioned range has been studied. The logarithms at a given temperature were then plotted against p_H . In this manner a continuous curve is obtained.

The egg-albumin used was prepared by the method of Hopkins and Pinkus as modified by Sørensen [1917, 1]. The estimation of the albumin content of each sample was made by the method of Devoto as given by Hopkins [1900]. This consists in the gravimetric estimation of the coagulum formed on heating a known volume of the solution for 2 hours in the presence of saturated ammonium sulphate.

In all the following experiments the solutions contained initially 1 g. of albumin and 0.5 g. of ammonium sulphate per 100 cc. The velocity constants are expressed in each case in $\log_e$ and sec^{-1} .

*Results.*SECTION 1. *Heat denaturation of egg-albumin at 70.2°.*

Table I.

Time in minutes	Residual albumin in mg. per cc.	$k_{\text{uni}} \times 10^3$	p_H
(i) p_H range 4.98 to 5.50 (during the course of the experiment)			
0	5.60	5.1	4.98
1	4.11	3.84	5.16
2	3.35	3.23	5.24
3	2.82	2.46	5.34
4	2.48	2.02	5.43
(ii) p_H range 5.34 to 5.84 (during the course of the experiment)			
0	7.59	2.56	5.34
2	6.03	2.22	5.43
5	4.43	1.62	5.54
7	3.71	1.48	5.58
10	3.24	1.15	5.66
21	1.67	0.45	5.84

¹ p_H measurements were made at 37° by means of the quinhydrone electrode following the technique of Corran and Lewis [1924].

Table I (*continued*).

Time in minutes	Residual albumin in mg. per cc.	$k_{\text{uni}} \times 10^4$	p_{H}
(iii) p_{H} range 6.20 to 6.48 (during the course of the experiment)			
0	7.27	2.07	6.20
10	6.47	1.79	6.26
30	5.22	1.60	6.32
50	4.42	1.48	6.35
80	3.34	1.33	6.42
125	2.47	0.96	6.48
(iv) p_{H} range 6.10 to 6.34 (during the course of the experiment)			
0	7.64	3.07	6.10
10	6.57	2.69	6.14
30	4.77	2.27	6.20
60	3.24	2.00	6.24
92	2.25	1.75	6.28
120	1.74	1.29	6.34
(v) p_{H} range 7.34 to 6.89 (during the course of the experiment)			
0	6.86	2.07	7.34
10	6.05	1.92	7.29
30	4.86	1.60	7.21
50	3.98	1.24	7.12
80	3.43	0.85	7.02
120	2.96	0.55	6.89
(vi) p_{H} range 7.72 to 7.23 (during the course of the experiment)			
0	6.46	3.84	7.72
10	5.18	3.20	7.65
20	4.42	2.74	7.56
40	3.28	2.13	7.38
60	2.58	1.75	7.26
80	2.09	1.60	7.23

Table II. *Summarising table.*

Variation of k_{uni} with p_{H} at 70.2°		Variation of k_{uni} with p_{H} at 70.2°	
p_{H}	$k_{\text{uni}} \times 10^5$	p_{H}	$k_{\text{uni}} \times 10^5$
4.98	510.0	6.34	12.9
5.16	384.0	6.35	14.8
5.24	323.0	6.42	13.3
5.34	250.0	6.48	9.6
5.43	212.0	6.89	5.5
5.50	134.0	7.02	8.5
5.54	162.0	7.12	12.4
5.58	148.0	7.21	16.0
5.66	115.0	7.23	16.0
5.84	45.0	7.26	17.5
6.10	30.7	7.29	19.2
6.14	26.9	7.34	20.7
6.20	21.7	7.38	21.3
6.24	20.0	7.56	27.4
6.26	17.9	7.65	32.0
6.28	17.5	7.72	38.4
6.32	16.0		

The data of this table are plotted in Fig. 1.

It will be seen from these results that the velocity of the heat denaturation of egg-albumin passes through a minimum in the neighbourhood of the point at which the concentration of hydrogen ion is equal to that of hydroxyl ion, namely, at p_{H} 6.76 measured at 37°.

Fig. 1. Heat denaturation of egg-albumin. Variation of k_{uni} with p_H at 70.2° .

It will also be noticed in this case that the asymmetry of the curve is not so pronounced as in the case of oxyhaemoglobin (see Part I). This is attributed to the fact that the minimum is now further removed from the isoelectric point of the protein and hence the hydration of the molecule is varying with p_H to a smaller extent (compare the discussion of results given in Part I).

SECTION 2. Variation with p_H of the velocity of heat denaturation of egg-albumin at 65° .

The following table summarises the results of several experiments analogous to those described in the previous section. Fuller details are omitted for the sake of brevity.

Table III. Summarising table.

p_H	$k_{uni} \times 10^6$	p_H	$k_{uni} \times 10^6$
4.93	311.0	7.21	5.9
5.02	254.0	7.36	9.7
5.08	205.0	7.42	11.4
5.17	184.0	7.58	16.8
5.30	125.0	7.68	17.3
5.48	56.0	7.73	18.8
5.78	26.7	7.79	20.0
5.89	19.5	7.81	21.3
6.03	14.9	7.83	23.7
6.07	13.7	7.89	24.6
6.13	12.1	7.94	26.7
6.23	9.2	7.99	32.0

The data of this table, when plotted, yield a graph similar to Fig. 1.

Here again the velocity of denaturation passes through a minimum in the neighbourhood of "neutrality." It might therefore be reasonably expected that, if p_H variation were the only cause of the falling off of the velocity, a solution initially at p_H 6.76 (measured at 37°) would remain sensibly steady

in p_H and also give a unimolecular velocity constant. The necessary measurements have been made; the results are recorded in the following section.

SECTION 3. *Heat denaturation of egg-albumin at p_H 6.76.*

Table IV.

Temp.	Time in minutes	Residual albumin in mg. per cc.	$k_{uni} \times 10^5$	
70.2°	0	6.75	—	Mean k_{uni} 5.2×10^{-5}
	30	6.13	5.4	Mean k_{uni} of duplicate ex-
	80	5.25	5.2	periment 5.1×10^{-5}
	120	4.68	5.1	Average k_{uni} 5.15×10^{-5}
	200	3.72	5.0	This value has been inserted
	300	2.76	5.0	in Fig. 1

Table V.

Temp.	Time in hours	Residual albumin in mg. per cc.	$k_{uni} \times 10^6$	
65°	0	7.77	—	Mean k_{uni} 2.86×10^{-6}
	5	7.37	2.93	Mean k_{uni} of duplicate ex-
	10	7.00	2.90	periment 2.74×10^{-6}
	20	6.32	2.87	Average k_{uni} 2.80×10^{-6}
	30	5.72	2.84	
	60	4.22	2.82	

The average value of k_{uni} of Table V lies on the curve given by plotting the data of Table III.

The velocity constants obtained above are quite good. The slight fall which is noticed is in all probability due to the p_H employed not being the exact position of the minimum. It seems to be a fair inference from these experiments that denaturation *per se* does not necessarily involve an alteration in p_H as the reaction proceeds.

The important point brought out by these results is that the velocity of the heat denaturation of egg albumin passes through a minimum under conditions where the concentration of hydrogen ion is sensibly equal to that of hydroxyl ion¹. This is the more interesting in view of the results of Part I of this series, in which it was shown that the heat denaturation of oxyhaemoglobin showed the same behaviour. In the present instance there can be no question of the minimum occurring at the isoelectric point of the protein which is at p_H 4.84 at room temperature [Sørensen, 1917]. In the case of haemoglobin the isoelectric point (p_H 6.5 at 37°) is so slightly removed from neutrality (p_H 6.76 at 37°) that it might have been suggested that the solution which had p_H 6.76 at 37° might be isoelectric at the higher temperature. In view of the present results, however, it may be taken that the conclusions arrived at in Part I are substantially correct.

¹ As the curve (Fig. 1) does not cover the region of the isoelectric point the possibility of there being a *maximum* velocity in the neighbourhood of that point suggests itself. However, experiments at other temperatures (not quoted) show that the velocity of heat denaturation passes continuously through this region without inflexion or change of direction.

The observed critical increment of the heat denaturation of egg-albumin.

For the purpose of calculating the critical increment (cf. Part I) the values used are the velocity constants corresponding to the minimum points on the p_H/k_{unit} curves. From these it is calculated that the apparent critical increment of the process is 130,000 calories. For the same process Chick and Martin [1910] obtained values varying from 128,000 to 141,000 calories. These authors used as a measure of the rate of the reaction the time taken for the concentration of albumin to fall between two definite limits. This method is not so accurate as that employed in the present work and in view of this fact the agreement between the values of the critical increment obtained in the two investigations is excellent.

In Part I of this series it was suggested that the mechanism of the heat denaturation of proteins consisted of a localised hydrolysis of certain links (nature unspecified) situated at various points on a large, heavily hydrated molecular unit. It was shown that the observed critical increment of the heat denaturation of oxyhaemoglobin (78,000 calories) corresponded to the hydrolysis of three or four linkages of a type corresponding to a critical increment of 20,000 to 30,000 calories. It will be seen that the apparent critical increment of the heat denaturation of egg-albumin corresponds to five or six such hydrolysable linkages.

Further, the work of Lüers and Landauer [1922] must be considered. These authors find that for the heat denaturation of leucosin the observed critical increment is 82,500 calories. This also fits in quite well with the magnitudes considered here.

SUMMARY.

The effect of the variation of the hydrogen ion concentration on the velocity of the heat denaturation of egg-albumin has been studied in unbuffered solutions at 65° and 70.2°. It is found that the rate of reaction passes through a minimum at a p_H 6.76 where the concentration of hydrogen ion is sensibly equal to that of hydroxyl ion. It is pointed out that in this instance the asymmetry of the curve is not so pronounced as in the case of the heat denaturation of oxyhaemoglobin and that this lends support to the idea that the water of hydration of the micelle is in some way involved in the reaction.

The critical increment of the heat denaturation of egg-albumin has been found to be 130,000 calories.

It is also shown that the results of Lüers and Landauer for the heat denaturation of leucosin are in general agreement with the results recorded in this paper.

In conclusion the writer desires to express his thanks to the Department of Scientific and Industrial Research for a maintenance grant which enabled this investigation to be carried out.

REFERENCES.

The references will be found at the end of Part III, p. 992.