Studies on Proteins, III.
Action of Superheated Water on Proteins, II.[1]

By

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It has been shown in the previous article[2] on this subject, that proteins when subjected to the action of superheated water, were transformed into soluble and insoluble substances which were regarded as the result of reverse reactions—polymerisation or condensation and hydrolysis—which take place simultaneously in the reaction system, and that these reaction products were different with each individual protein from which they were derived, from the study of the elementary analysis and the distribution of nitrogen, which have no definite chemical compositions, according to the various external conditions under which the proteins were submitted to the chemical action.

For the verification of the above conclusions, the present research on the determination of the P_H value and the buffer action of the individual reaction products from protein at the various stages of the reaction, was undertaken.

Table I.

Edestin.

Time (in hours) (t)	0	1	2	4	20
Diss. subst. (%) (Q)	0	5·7	15·5	24·7	63·4
$\Delta Q/\Delta t$ [3]		5·7	9·8	4·6	2·4
P_H	6·3	7·3	—	—	6·9

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1. The expenses of this investigation were shared by the Imperial Academy of Japan.

2. These memoris, A, **10**, 163 (1927).

3. ΔQ and Δt denote the difference of Q and t respectively.

Time (in hours) (t)	0	3	6	9
Quantity of diss. amide nitrogen (Q) (%)	0	25.	49.	83.
$\Delta Q/\Delta t$	8·3	8	11·3	

Gliadin.

Time (in hours) (t)	0	2	6	15·5
Diss. subst. (%) (Q)	0	5·5	9·9	28·8
$\Delta Q/\Delta t$	2·75	1·1	2.	
P_H	5·7	—	6·37	6·44

Casein.

Time (in hours) (t)	0	2	6	17	20
Diss. subst. (%) (Q)	0	1·34	5·85	14.	
$\Delta Q/\Delta t$	0·67	1·1	0·74		
P_H	4·6	—	4·7	—	5 0

For the determination of the P_H value of the reaction products—the residue and solution,—each protein was heated with water in a stoppered bottle at 120° for one, six, and twenty hours, respectively, and the hydrogen ion concentration of the products was studied by electrometric method as will be seen in the above table.

The P_H value of the solution from edestin, as may be seen in the table, was increased at the beginning of the reaction and then decreased, whilst that from casein shows a constant increase in the progress of reaction. Although the P_H value in case of gliadin was on the increase with reaction time, which seems apparently to be changed in an analogous manner with that in the case of casein, it should be proper to consider, when we study it precisely, that gliadin behaves toward superheated water partly like edestin and partly like casein, since the rate of increment in the P_H value of the solution from gliadin for a first certain time is greater than that for latter part of the reaction period.

When the P_H value of the reaction products of the proteins was compared with the distribution of nitrogen and especially with their amide nitrogen content (Table I) it was noticed that some parallelism existed between them, and the vicissitude of the P_H value of the solution with lapse of the reaction time, will be explained well by our hypothes mentioned in the previous article that the compounds containing diamino

nitrogen would be transformed partly into those containing the amide nitrogen during the process, and moreover, that the manner in which the protein would be decomposed into fragmentary complex groups, would be a characteristic of the individual protein.

The solution obtained from edestin with water at 120° for twenty hours, having the value of P_H of 6·9, will become cloudy when the P_H of the solution reaches 6 by the addition of acetic acid and the precipitation of a compound vividly took place while the P_H was climbing from 5·3 to 5·0 and completed at P_H of 4·8, accordingly the isoelectric point of the substance was assumed to be $P_H=5·2$, since at that point the affinity of a compound for water is in a relative minimum.[1] The same was noticed with the solution ($P_H=6·4$) which obtained by digestion of gliadin with water for twenty hours; when acetic acid was added gradually to the solution, and the precipitation of a compound occurs when the acidity of the solution has reached to $P_H=6·4$, the vicissitude of its isoelectric point. No flocculation, however, has occurred of the solution of the P_H of 5·0, which was prepared from casein under the same condition as the other proteins, owing to the P_H of the solution being already on the acid side of the isoelectric point of compounds which were regarded as being formed as cleavage products of the casein molecule, and consequently the so-called residue was considered partly of these cleavage products which remained there, without entering into the solution.

From these facts, we concluded that the constituents of the solutions resulting from the action of the superheated water on proteins, would be as different essentially from each other, as the difference of the individual proteins in the chemical constituents, which has already been admitted by chemists.[2] Accordingly, the following statement will be borne in mind that the constituents of the insoluble residue, from the study of the P_H value of the soluble substances, would be different from each other, as we stated in the previous article, since the hydrogen ion concentration of a solvent is one of the most important factors controlling the solubility of proteins or amphotelytes of complex nature.[3]

A solution from 2 grams of edestin, by digesting with 40 c.c. of water at 120°, for one hour, and made up to 50 c.c., which was found

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1. W. M. Clark: The Determ. of Hydorogen Ion Conc., 2nd. ed. (1923), p. 342.
2. T. B. Osborne: The Vegetable Proteins, (1909), p. 59.
3. Robertson: Physical Chemistry of Proteins, (1918), pp. 275, 248.
   J. Loeb: Proteins and the Theory of Colloidal Behavior, pp. 243, 266.
   L. Michaelis: Die Wasserstoffionen-Konzentration, (1919), p. 44.
   R. H. Bogue: Colloidal Behavior, (1924), p. 35.

to have a value of P_H of 7.4. To the solution were added a successively increasing amount of N/25 acetic acid on the one hand, and N/50 sodium hydroxide solution on the other, and the resulting P_H was measured in each case. The P_H values were then plotted as usual and the results were as may be seen in Curve III, in Fig. 1 and Fig. 2. Curve II′, in these figures, was constructed analogously with data obtained with the solution prepared from the protein after digesting for twenty hours under the same conditions. Curve II of the figure were obtained in a similar way of ten times diluted solution of the latter solution. The results show that the buffer action of the solution as we expected from other examples, depended upon the concentration of the constituents. The solution obtained by digestion for twenty hours is much more resistant to change in the P_H than the solution of the same concentration, obtained by digestion for only one hour. These relations were observed to be true also of casein, as will be seen in Fig. 5 and Fig. 6, but of the solution from gliadin, these stochiometrical relations do not hold; the solutions of the same concentration show the same buffer action though they were prepared under different conditions (Fig. 3 and Fig. 4). That the P_H value of the solution from edestin was decreased from 6·96 to 6·78 when diluted ten times, whilst of its hundred times diluted solution the P_H value was inversely increased to 8·07, are facts worth discussing.

For obtaining more definite knowledge of the occurrence of compounds of complex nature such as albumose or peptone, in the solution which was resulted by digestion of protein, the buffer value of the solution was investigated.

As was supposed from the study of the buffer values (dB/dP_H) of the solutions, differentiated graphically from their titration curve above-mentioned, following the method proposed by D.D. van Slyke,[1] there occur at least two compounds of complex nature in each solution, as will be seen in Fig. 7, 8, 9, 10, 11 and 12, obtained by the action of superheated water on proteins. Such indication of the buffer value for the existence of the compounds will be proved experimentally in the next article by isolating them from the solution.

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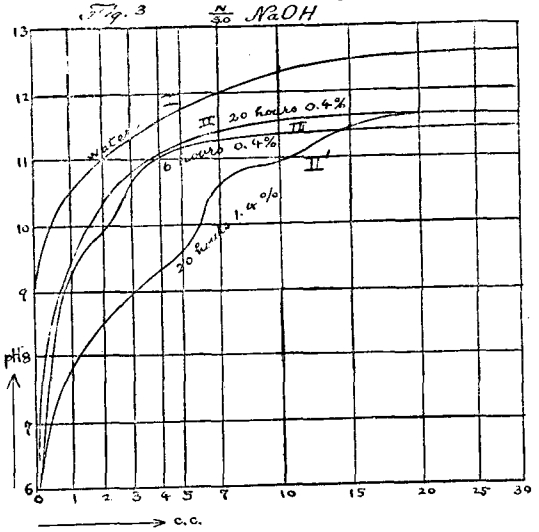
1. J. Biol. Chem., **52**, 524 (1922).

Titration Curve of Gliadine Hydrolysate

Fig. 3 $\frac{N}{50}$ NaOH



Titration Curve of Edestin Hydrolysate

Fig. 1. $\frac{N}{50}$ NaOH

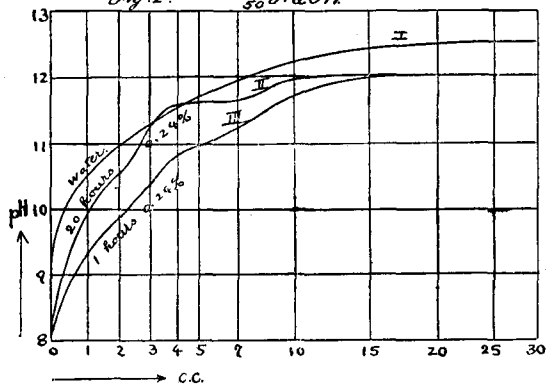


Fig 4 $\frac{N}{26}$ Acetic Acid.

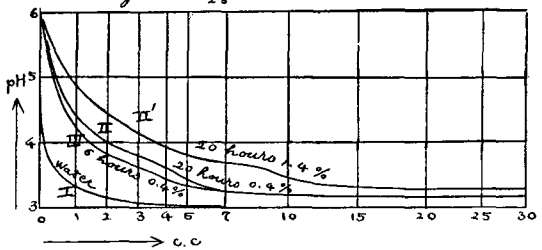
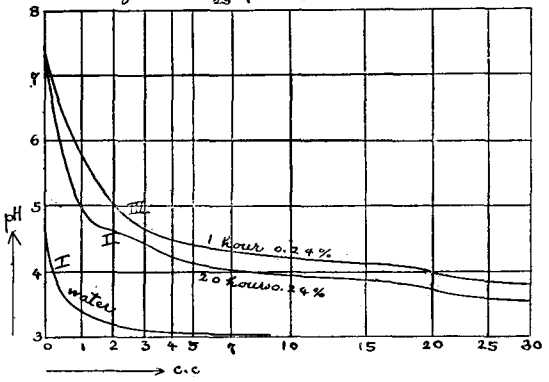
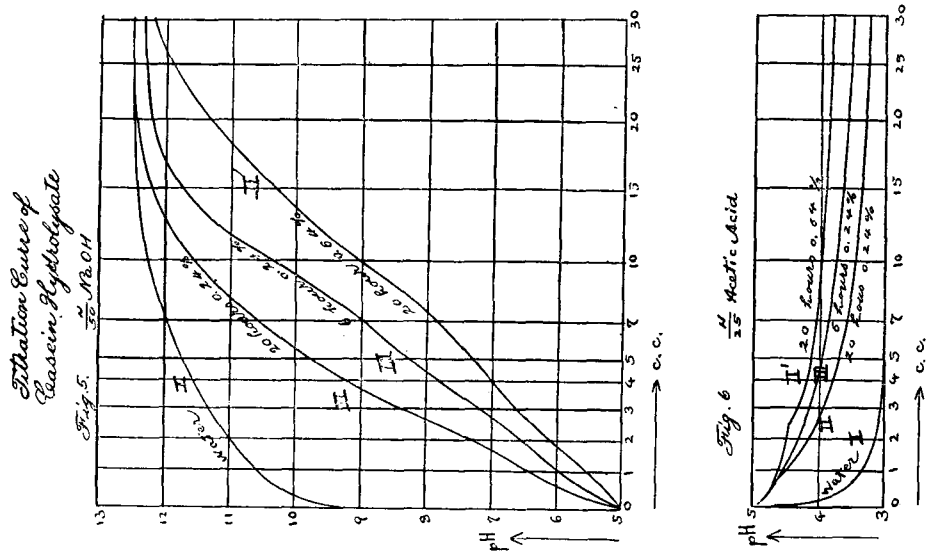
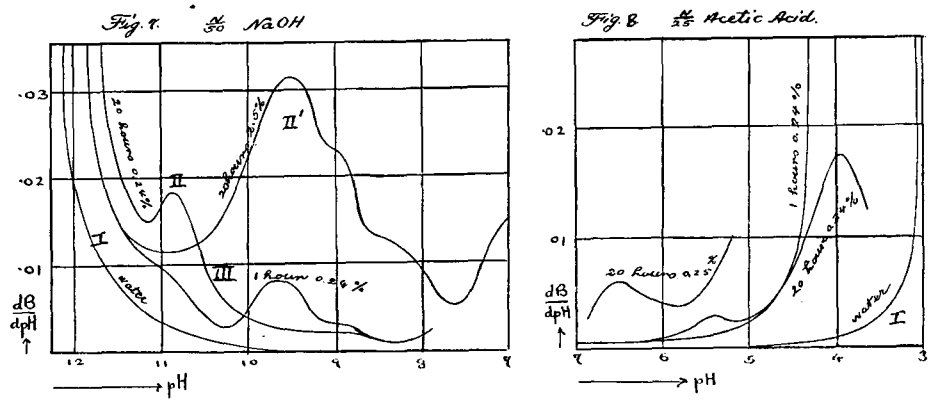


Fig. 2. $\frac{N}{2.5}$ Acetic Acid.





Buffer value curve of Edestin Hydrolysate



Buffervalue curve of Gliadine Hydrolysate

Fig. 9. $\frac{n}{50}$ NaOH

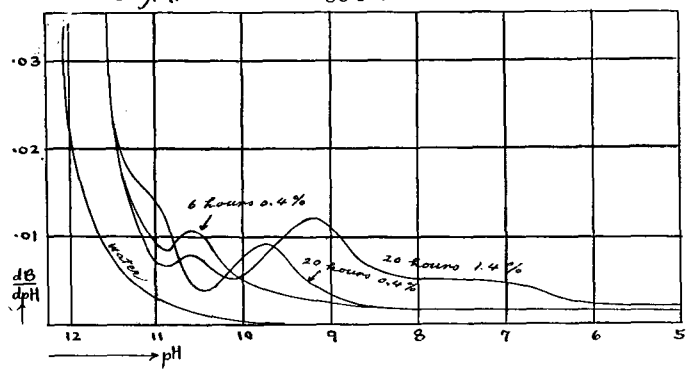
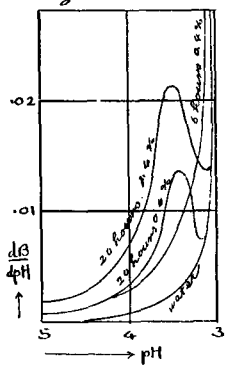


Fig 10. Acetic Acid.



Buffervalue curve of Casein Hydrolysate.

Fig. 11. $\frac{n}{50}$ NaOH

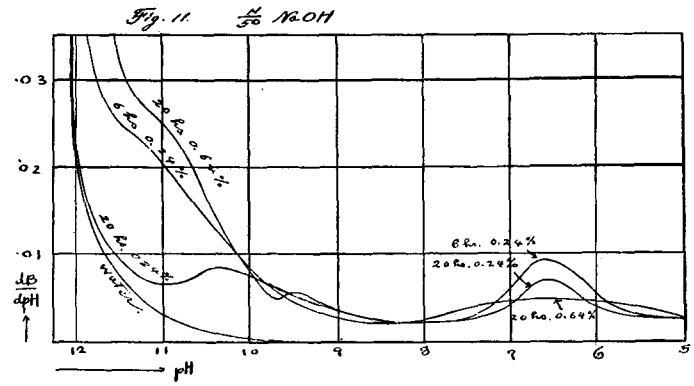


Fig 12. Acetic Acid.