LXIX. THE ACTION OF HYPOCHLORITES ON AMINO-ACIDS AND PROTEINS.

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DAKIN [1916] has shown that, if increasing quantities of blood serum are added to a constant volume of a solution of hypochlorite, the resulting reduction in available chlorine does not take place quantitatively, but that large quantities of serum may destroy *less* hypochlorite than small quantities.

In an investigation of the reactions which take place between sodium hypochlorite and milk, I have obtained similar anomalous results, and further work on the individual constituents of the milk has shown that these results are due to the presence of caseinogen. The object of this paper is to bring forward certain evidence which points to the nature of the reactions taking place, and which indicates the explanation of the anomalous results.

TECHNIQUE.

The technique used throughout the experiments was as follows. Varying quantities of the protein or amino-acid under investigation were made up to such dilutions that, after the addition of the requisite volume of hypochlorite solution, the initial available chlorine in each solution was 0.014 % (0.004 *N*). At definite intervals after the addition of the hypochlorite, the percentage of available chlorine remaining in the solution was determined by liberation of iodine from an acid solution of potassium iodide, and back-titration with thiosulphate.

From these determinations, the percentage loss in available chlorine, due to the interaction of the hypochlorite with the protein or amino-acid under investigation, has been calculated: the results have been expressed graphically by plotting the concentration of the protein or amino-acid against the percentage of initial available chlorine (*i.e.* 100 % is equivalent to 0.014 % available chlorine). All the experiments were carried out at room temperature and the solutions were protected from direct light during the reactions.

EXPERIMENTS WITH PROTEINS.

Fig. 1 shows the effect of neutral sodium caseinogenate in reducing the strength of the hypochlorite. With the addition of small quantities of caseinogen (up to 0.05 g. per 100 cc.), the available chlorine falls to about one-third of its initial value; with larger quantities of the protein, the destruction is less,

the available chlorine only falling with 0.2 g. per 100 cc. to one-half of its initial value; with still larger quantities destruction again becomes greater, until with a concentration of 1.6 g. of caseinogen per 100 cc. there is no longer any available chlorine left in the solution. This type of reaction is not specific for caseinogen. Fig. 2 shows the corresponding curve obtained with neutral gelatin, and demonstrates the general similarity of the two reactions (*i.e.* preliminary fall, secondary rise, secondary fall). The quantitative results are however different: whereas with caseinogen the secondary rise reaches only 50 %, with gelatin it reaches over 80 %; and while the secondary fall with caseinogen reaches zero at a concentration of 1.6 % of the protein, with gelatin of an equal concentration the value is still above 50 %. The possible significance of these quantitative differences will be referred to later.

Fig. 1. Effect of neutral sodium caseinogenate in reducing the available chlorine of a solution of hypochlorite (initial available chlorine, 0.014 %). Duration of reaction, 5 hrs.

Fig. 2. Effect of neutral gelatin in reducing the available chlorine of a solution of hypochlorite (initial available chlorine, 0.014 %). Duration of reaction, 5 hrs.

EXPERIMENTS WITH INDIVIDUAL AMINO-ACIDS.

It became apparent that, in order to obtain any adequate explanation of these results, it would be necessary to work with substances of more simple constitution than the proteins. A series of experiments was therefore carried out on certain individual amino-acids, using the same technique as that noted above.

At the outset, difficulty was encountered in obtaining consistent results owing to the very marked effects produced by differences in the alkalinity of the solutions. This point is illustrated in Fig. 3, which shows the effect of addition of alkali on the reactions between hypochlorite and alanine. It will be seen that, with increasing alkalinity, destruction of the hypochlorite becomes more rapid and more complete. This corresponds with the known fact that acidity increases *chlorination*, and *alkalinity* oxidation, of substances by hypochlorites. Such an explanation does not hold for results obtained in neutral and acid solutions, a subject on which further work is in progress.

Fig. 3. Effect of solutions of alanine of varying alkalinity in reducing the available chlorine of a solution of hypochlorite (initial available chlorine, 0.014 %). Duration of reaction, 5 hrs. The figures in brackets indicate the normality of NaOH in the 0.1 % solutions of alanine which were used to make up the concentrations noted in the figure.

However, by using slightly alkaline solutions of amino-acids (containing 1 to 2 cc. of $N/10$ NaOH per 100 cc.), it was found possible to obtain concordant results.

In preliminary experiments, the loss in available chlorine caused by various quantities of amino-acids was measured after a period of 5 hours: later work showed that a complete explanation of the results could only be obtained by including in the experiments a study of the *rate* of reaction. Experiments were therefore carried out in which both the concentration of the reacting substances and the time of the reactions were varied. The results of these experiments are shown in Figs. 4 to 7.

Fig. 4 shows the course of the reaction between sodium hypochlorite and glycine. If the results obtained after a period of 5 hours are examined, it will be seen that the reaction is represented graphically (1) by a straight line fall to zero (AB) with 0.0036 % glycine, (2) by a straight line rise to 100 %

(BC) with 0.0163 % glycine, and (3) with larger quantities of glycine, by a horizontal line remaining at 100 %.

Fig. 4. Effect of glycine in reducing the available chlorine of a solution of hypochlorite (initial available chlorine, 0.014 %).

Assuming that a fall to zero corresponds to the complete reduction of the hypochlorite, and that a rise to 100 % represents the use of the whole of the hypochlorite in chlorination of the glycine (the chloroamino-derivative so formed having the property of liberating iodine from potassium iodide), the end-points on these curves will correspond to (1) oxidation of the glycine, in the ratio of one molecule of glycine to eight atoms of oxygen, and (2) chlorination of the glycine in the ratio of one molecule of glycine to two atoms of chlorine.

$$\frac{1 \text{ molecule of glycine}}{8 \text{ atoms of oxygen}} = \frac{75}{128} \text{ (theoretical), } \frac{75}{137} \text{ (experimental).}$$

$$\frac{1 \text{ molecule of glycine}}{2 \text{ atoms of chlorine}} = \frac{75}{70} \text{ (theoretical), } \frac{75}{65} \text{ (experimental).}$$

At point *C* complete chlorination of the amino-group of the glycine therefore takes place, with the formation of dichloroaminoacetic acid. If this point is joined to the point of origin (*O*), the theoretical curve of chlorination (*CO*) is given. Since *CB* falls below *CO*, it is apparent that the former is the resultant of two reactions, chlorination and oxidation: at *B* there is complete oxidation, at *C* there is complete chlorination, and at intermediate points the two reactions take place in varying proportions. It should be possible to calculate from *AB* and *CO* the proportions of glycine oxidised and chlorinated at any point on *BC*. The results of these calculations are shown in Table I, where it will be seen that the theoretical and experimental figures show excellent agreement.

Table I. *Action of sodium hypochlorite on glycine.*

Total quantity oxidised and chlorinated (from Curve <i>CB</i>)	Quantity oxidised (from Curve <i>AB</i>)	Quantity chlorinated (from Curve <i>CO</i>)	Total quantity oxidised and chlorinated (from Curves <i>AB</i> and <i>CO</i>)
(Concentrations in g. of glycine per 100 cc.)			
0.0048	0.0031	0.0016	0.0047
0.0061	0.0028	0.0032	0.0060
0.0074	0.0024	0.0048	0.0072
0.0087	0.0021	0.0065	0.0086
0.0100	0.0017	0.0080	0.0097
0.0112	0.0014	0.0096	0.0110
0.0125	0.0010	0.0113	0.0123
0.0139	0.0007	0.0130	0.0137
0.0151	0.0003	0.0146	0.0149

One other point of importance is apparent from the experiments on glycine, namely the very stable nature of the dichloroaminoacetic acid. This is shown by the fact that after 10 hours the concentration of available chlorine had not fallen below 96 % of its initial value: in this respect glycine differs markedly from the other amino-acids which were studied.

Fig. 5. Effect of alanine in reducing the available chlorine of a solution of hypochlorite (initial available chlorine, 0.014 %).

Fig. 5 shows the corresponding curves obtained with alanine. These curves exhibit a general similarity to those obtained with glycine, namely a preliminary fall, followed by a secondary rise, after which the percentage of available chlorine remains at a constant level. They differ from those obtained with glycine in that (1) the preliminary fall (denoting oxidation) is more rapid in its initial stages, but is not carried to completion; (2) the secondary rise (denoting chlorination) never actually reaches 100 %, though the shorter the duration of the reaction, the nearer does the curve approach the 100 % line: the probable point at which it would meet this line (point *C*) corresponds with the formation of dichloroaminopropionic acid; and (3) the final portions

of the curves, beyond point *C*, fall horizontally at a constant rate: this would be expected if the fall in available chlorine in this part of the curves is due to the spontaneous decomposition of the dichloroaminopropionic acid, the maximum quantity of which is formed at point *C*. Dichloroaminopropionic acid is therefore far less stable than dichloroaminoacetic acid.

Fig. 6 shows the corresponding curves obtained with cystine. These curves again exhibit a general similarity to those obtained with glycine. The reactions are, in this case, extremely rapid, and the tetrachloroamino-derivative, which is formed by chlorination of the cystine, exists for only a very short time. The general direction of the curves indicates that it is undoubtedly formed, though as much as 40 % is decomposed within 5 minutes of its formation. At the end of 5 hours there is, in fact, no indication that chlorination has taken place, since the curve of the reaction falls in a straight line to zero, indicating oxidation alone. It is only by a study of the rate of reaction that the intermediate formation of di-tetrachloroaminothiopropionic acid is indicated.

Fig. 6. Effect of cystine in reducing the available chlorine of a solution of hypochlorite (initial available chlorine, 0.014 %).

* Denotes theoretical point of formation of di-tetrachloroaminothiopropionic acid.

It has been impracticable to make a detailed study of other amino-acids, but 5-hour reactions have been carried out with a few of them (Fig. 7). The reactions are represented graphically by an initial straight line fall, either to a constant level or to a very slowly falling curve, the rate of fall depending on the individual amino-acid. Such curves are of the same type as that given by alanine at the end of 5 hours' reaction, and it appears probable that intermediate formation of chloroamino-derivatives takes place with these amino-acids as it does with alanine.

Fig. 7. Effect of various amino-acids in reducing the available chlorine of a solution of hypochlorite (initial available chlorine, 0.014 %).

EXPERIMENTS WITH MIXED AMINO-ACIDS.

From the work described above, it is possible to select two amino-acids, one of which yields a very stable chloroamino-derivative (glycine), and the other an unstable chloroamino-derivative (cystine). It was felt that interesting results might be obtained by a study of the reactions of sodium hypochlorite with mixtures of these two amino-acids.

Fig. 8. Effect of a mixture of seven parts of glycine to three parts of cystine in reducing the available chlorine of a solution of hypochlorite (initial available chlorine, 0.014 %).

Fig. 8 shows the effects of a mixture of seven parts of glycine to three parts of cystine in reducing the available chlorine of a solution of hypochlorite. At the end of half-an-hour the curve shows the typical preliminary fall and secondary rise obtained with individual amino-acids, followed by a straight line remaining at a roughly constant level. As the reaction progresses, however, this final portion of the curve tends to fall off, until, at the end of 5 hours, the whole curve approximates to the protein-type referred to at the

beginning of the paper (i.e. preliminary fall, secondary rise, secondary fall). The secondary fall in the curve is due to a reaction which takes place between the cystine and the dichloroaminoacetic acid which is formed initially by chlorination of the glycine. This reaction may be demonstrated by adding quantities of cystine to a solution of dichloroaminoacetic acid, previously prepared by the interaction of theoretical quantities of glycine and sodium hypochlorite. The results are shown in Fig. 9, and demonstrate the correspondence of the curves so obtained with those given by the mixture of amino-acids.

Fig. 9. Effect of cystine in reducing the available chlorine of a solution of dichloroaminoacetic acid (initial available chlorine, 0.014 %).

THE EXPLANATION OF THE ANOMALOUS RESULTS OBTAINED WITH PROTEINS.

To return to the original problem, namely, the explanation of the anomalous results obtained with proteins, it appears from the above experiments that these are due to two factors, (1) the possibility of sodium hypochlorite acting either as an oxidising or as a chlorinating agent, and (2) the variable stability of the chloroamino-derivatives formed by the latter reaction.

By taking mixtures of two amino-acids, one of which has a stable, and the other an unstable, chloroamino-derivative, it is possible to reproduce the protein-type of curve: further, by altering the ratio of the one amino-acid to the other, the quantitative nature of the curves may be altered. Fig. 10 shows the effect of alterations in the proportions of glycine/cystine in causing alterations in the resultant curves. As the proportion of glycine/cystine is raised, the curves exhibit a higher secondary rise, and a slower secondary fall.

In this connection it is suggestive that caseinogen contains very little glycine and relatively large amounts of cystine, and gives a *low* secondary rise and a *quick* secondary fall; while gelatin contains large amounts of glycine

and only minute quantities of sulphur-containing amino-acids, and gives a *high* secondary rise, and a *slow* secondary fall.

Fig. 10. Effect of mixtures of glycine and cystine in reducing the available chlorine of a solution of hypochlorite (initial available chlorine, 0.014 %).

REFERENCE.

Dakin (1916). *Brit. Med. J.* i, 852.