

CCXXXVI. THE ACTION OF HYPOCHLORITES ON AMINO-ACIDS AND PROTEINS. THE EFFECT OF ACIDITY AND ALKALINITY.

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IN a previous paper [Wright, 1926] the author recorded the results obtained in a study of the rate of reaction between solutions of sodium hypochlorite and certain amino-acids and proteins. It was suggested that the rate of utilisation of the available chlorine was governed by at least two factors: (1) the possibility of the hypochlorite acting either as an oxidizing or as a chlorinating agent, and (2) the variable stability of the chloroamino-derivatives formed by the latter reaction. Brief reference was made in the paper to the very marked effect of alterations in the acidity of the solutions, alterations which changed completely the type of reaction curve obtained. Subsequently a number of experiments were carried out to determine more exactly the effect of varying *pH* values on the rate of reaction. The results of this additional work were not, however, published.

Quite recently Norman [1936] has described a detailed study of the action of sodium hypochlorite on glycine. As a result of her work she expresses doubt as to whether chlorination does, in fact, take place as an intermediate step in the oxidation of glycine by hypochlorite. Since the additional work carried out by the present author has a definite bearing on this question it has now been felt desirable to publish the results, although these are in an admittedly incomplete form.

Technique.

The experiments were carried out as described in the previous paper, except that the acidity or alkalinity of each system was controlled by the use of strongly buffered salt solutions. The solutions employed were:

- for *pH* 1.0 to 2.0, HCl—KCl mixtures;
- „ *pH* 5.0 to 8.0, KH_2PO_4 —NaOH mixtures;
- „ *pH* 8.5 to 10.0, borate—NaOH mixtures;
- „ *pH* 12.5, *N*/10 NaOH solution.

The reactions were carried out for 5-hour periods, the percentage of available chlorine remaining at the end of this time being determined by liberation of iodine from an acid solution of KI and back-titration with thiosulphate. The initial strength of the available chlorine was 0.014% in all experiments.

Experiments with glycine and cystine.

Glycine. Experiments were carried out with glycine over a wide *pH* range. It will be seen from Figs. 1, 2 and 3 that on either side of *pH* 8.8 the solutions showed more or less uniform gradations in the extent to which they reduced the available chlorine. All the solutions, with the exception of those at extreme *pH* values (i.e. 1.0–2.0 and 12.5), gave with low concentrations of glycine a typical straight line fall towards zero, and with higher concentrations a typical straight

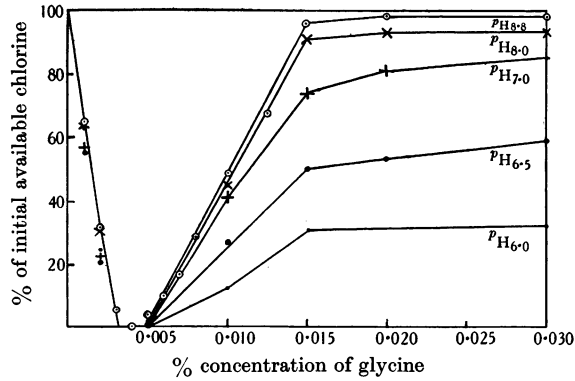


Fig. 1. Effect of glycine in reducing the available chlorine. pH range 6.0-8.8 (initial available chlorine 0.014 %).

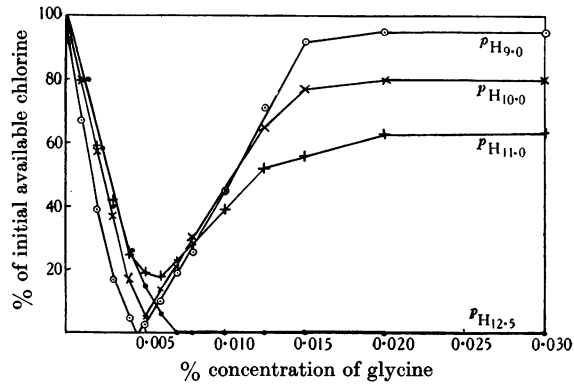


Fig. 2. Effect of glycine in reducing the available chlorine. pH range 9.0-12.5 (initial available chlorine 0.014 %).

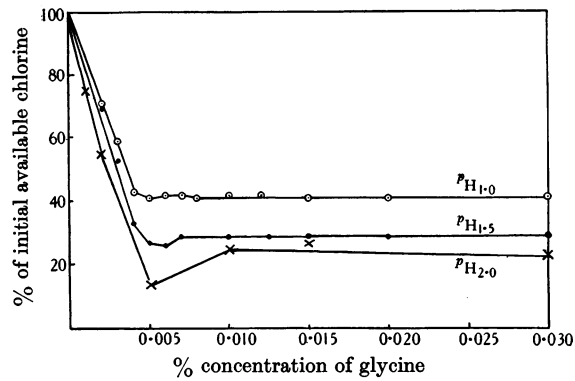


Fig. 3. Effect of glycine in reducing the available chlorine. pH range 1.0-2.0 (initial available chlorine 0.014 %).

line rise towards 100%. This confirms the previous work. It will, however, be noted that the slope of the initial fall is steepest with the more acid solutions. That is to say it requires less glycine to use up a given quantity of available chlorine in the acid solutions than in the more alkaline solutions. The whole of the available chlorine in the solution is, for example, removed by less than 0.003% glycine at pH between 6.0 and 8.8, whilst at pH 12.5 double this quantity of glycine is required. The Cl/glycine ratios at these extremes are 4.6 and 2.3 respectively. This difference can be most readily explained on the assumption that in acid solution the glycine is first chlorinated and that the resulting chloro-amino-acid is then oxidized by further quantities of hypochlorite, whilst in alkaline solution the destruction of available chlorine is due to oxidation alone.

Turning to the secondary rise, it will be seen that at pH 8.8 there is no appreciable reduction in available chlorine when the quantity of glycine present exceeds 0.015%. In the previous paper this fact was explained as being due to the quantitative formation of the relatively stable chloroaminoacetic acid which would be capable of liberating iodine from KI in the same quantities as the hypochlorite initially present. If this explanation is correct, the lower levels attained at pH on either side of 8.8 must be due to a loss of stability in the more acid and the more alkaline solutions. At pH 12.5, where presumably no chlorination takes place, there is of course no secondary rise.

The curves for pH 1.0–1.5 (Fig. 3) show entirely different characteristics from the remaining curves on the acid side of pH 8.8. There is no preliminary fall to zero and no secondary rise, but the curves fall to a constant value at between 25 and 40% of the initial available chlorine. At pH 2.0 the type of curve is intermediate. It will be shown later that similar anomalous curves are given in extremely acid solutions by both caseinogen and gelatin.

Cystine. Only three pH values were used in studying this amino-acid (Fig. 4). At pH 8.8 the curve is similar to that obtained in the previous work. There is no secondary rise with this amino-acid, the chloroamino-derivative

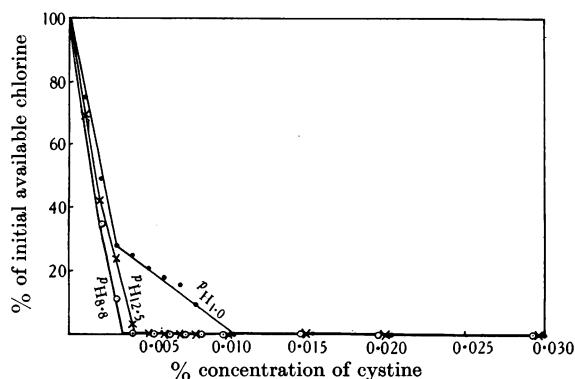


Fig. 4. Effect of cystine in reducing the available chlorine. pH range 1.0–12.5 (initial available chlorine 0.014%).

being very unstable and decomposing, with complete loss of available chlorine, within 5 hours at room temperature. The curve at pH 12.5 shows a steeper slope than that obtained at pH 8.8, indicating (as noted with glycine) the probable double nature of the reaction at the latter acidity. At pH 1.0 the results are again anomalous, the fall to zero taking place along two consecutive straight lines, one with a steep slope and one with a more gradual slope.

With this amino-acid, one marked difference was observed between the reactions taking place in acid and alkaline solutions. With acid solutions the liquid remained colourless, except for a slight initial coloration due to liberation of chlorine in the solution. With alkaline solutions the liquid rapidly acquired a pale straw-yellow colour, the intensity of the colour depending on the concentration of cystine. This colour was found to be due to the formation of polysulphides which could be decomposed by addition of acid, with liberation of hydrogen sulphide.

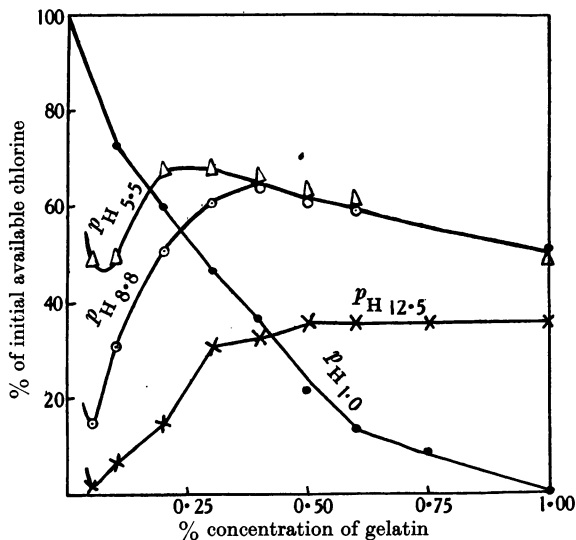


Fig. 5. Effect of gelatin in reducing the available chlorine. pH range 1.0–12.5 (initial available chlorine 0.014 %).

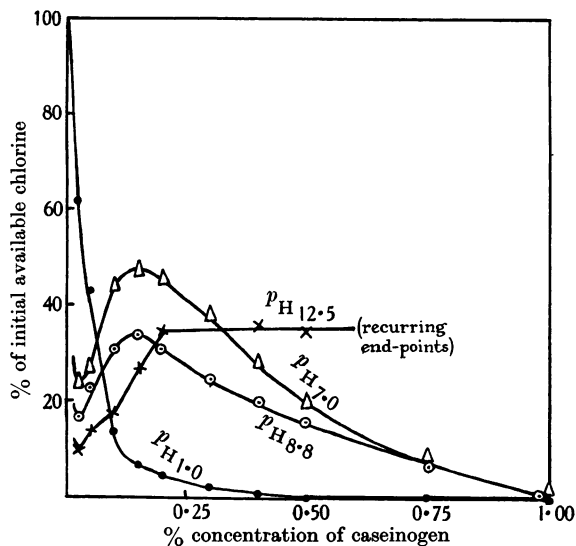


Fig. 6. Effect of caseinogen in reducing the available chlorine. pH range 1.0–12.5 (initial available chlorine 0.014 %).

Experiments with gelatin and caseinogen.

Similar experiments have been carried out with gelatin and caseinogen (Figs. 5 and 6). At pH between 5.0 and 9.0 the curves show a general similarity to those obtained in the earlier work, i.e. an initial fall and secondary rise, and a final but slower fall in the percentage of available chlorine. The secondary rise is smaller and the final fall is sharper with caseinogen than with gelatin, a fact which was attributed in the previous work to variations in the stability of the chloroamino-derivatives formed as a result of the hypochlorite action. It may be noted that with both proteins the highest secondary rise was obtained with pH on the acid side of 8.8 and not (as with the individual amino-acids) at pH 8.8.

The curves for pH 1.0 have an entirely different form from those obtained in slightly acid, neutral or alkaline solution. They show, however, a marked similarity to that obtained with a glycine-cystine mixture (Fig. 7).

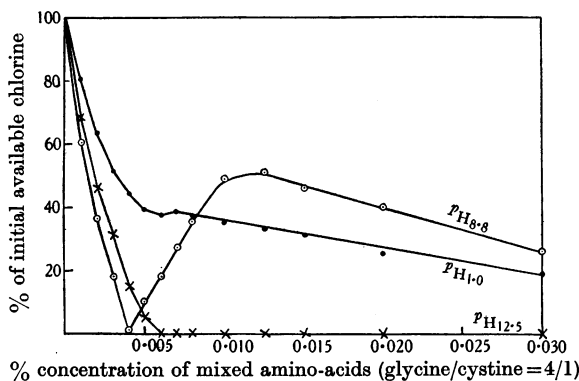


Fig. 7. Effect of a glycine/cystine mixture in reducing the available chlorine. pH range 1.0–12.5 (initial available chlorine 0.014 %).

At pH 12.5 the proteins fail to show the same type of curve as those given with either the individual or the mixed amino-acids. The curves for both proteins show an initial fall to a relatively low figure, the available chlorine being reduced to a very small fraction of its initial strength. Thereafter the curves rise to a constant level of roughly 35%—the titrations tending to give recurring end-points. No explanation can be given for this phenomenon.

One further point may be mentioned. In carrying out a number of supplementary experiments with gelatin and caseinogen it was found that, using a ratio of protein/available chlorine of 0.050%/0.014% and in solutions of HCl varying between 0.1 and 0.02 *N*, a precipitate flocculated out within a few minutes. This precipitate was slightly yellow and was capable of liberating iodine from KI. It was, however, decomposed by the addition of alkali, with the formation of a light greenish yellow solution. The precipitate was not formed at other acidities and only appeared when the ratio of protein/available chlorine was approximately that quoted.

DISCUSSION.

In a recent paper Norman [1936] cites experiments which, she states, indicate that hypochlorites do not normally react with amino-acids to form chloroamino-derivatives, but that "the theory of direct oxidation to cyanide seems at least as well founded as that of the formation of chloroamino-acids". This is in direct opposition to the views expressed by the present author [Wright, 1926] that

hypochlorite may act either as an oxidizing or as a chlorinating agent, the nature of the reaction depending on the relative quantities of the amino-acid and of the available chlorine present.

In her preliminary experiments with glycine Norman obtained curves which were in general similar to those obtained in the author's earlier work, discrepancies in the rate of reaction probably being due (as she suggests) to differences in the alkalinity of the solutions. In her later experiments, however, Norman only employed solutions in which there was "sufficient excess of chlorine to complete the reaction", that is to say a Cl/glycine ratio greater than 4.25. Her experiments were therefore limited to a study of the reactions which take place during the initial fall in available chlorine which is shown clearly in Figs. 1 and 2 of the present paper, and which was largely attributed to oxidation in the author's earlier paper. Norman's conclusions cannot be applied to the remaining portion of the curves (i.e. the secondary rise towards 100%), where the Cl/glycine ratio falls below 1.0. Only in one instance does she give the results of an experiment in which this ratio was approached, namely in a study of the interaction of 15 mg. glycine with 14.3 mg. Cl. In an un-neutralized solution she found a reduction of about 10% in the available chlorine within 10 hours, the equivalent figure recorded by the author for a slightly more alkaline solution being 4%. In a neutralized solution she found a reduction of about 30% within the same period. Commenting on these facts she states that "these results may be more reasonably explained on the basis of a slow oxidation rather than as the breakdown of an unstable dichloro-compound, which would be expected to be more stable in the neutralized experiment than in the untreated experiment". Such an assumption is quite unwarranted without experimental evidence regarding the relative stability of dichloro-amino-derivatives in solutions of varying pH. From the results obtained in the present paper it would, in fact, appear that the dichloro-amino-derivative of glycine (if formed) is more stable at pH 8.8 than at any pH on either side of this value.

In view of Norman's criticisms of the conclusions set out in the author's earlier paper, it appears desirable to summarize briefly the main facts which indicate that hypochlorites normally react with amino-acids and proteins to form (if only as unstable intermediate products) typical chloro-amino-compounds.

(1) It does not appear possible to reconcile a purely oxidation theory with the fact that large quantities of amino-acids or proteins cause a smaller reduction in available chlorine than small quantities.

(2) Such anomalous behaviour can be readily explained on the assumption that hypochlorite normally acts as a chlorinating agent, and that it is only in the presence of a relative excess of hypochlorite that oxidation takes place.

(3) The fact that the secondary rise in each of the amino-acid-hypochlorite curves reaches a maximum at the theoretical point at which a definite chloro-amino-derivative would be formed provides strong indirect evidence for the existence of such derivatives, though these may vary markedly in their stability [Wright, 1926].

(4) The evidence presented in the present paper confirms and amplifies these conclusions. In particular the variations observed in the quantities of glycine required to destroy the available chlorine at different pH show clearly that in neutral and moderately acid solutions some intermediate reaction accompanies oxidation.

(5) With cystine such differences were accompanied by visible changes in the solutions, chlorine being evolved in acid solution and polysulphides being formed in extreme alkaline solution.

(6) Under certain conditions it was found possible to isolate a yellow precipitate from the hypochlorite-protein solutions which was capable of liberating iodine from KI, presumably through the presence of chloroamino-groups in the precipitated material. In this connexion Norman gives the details of an experiment designed to determine the extent of formation of chloroamino-groups in treated gelatin. She immersed 0.2 g. of solid gelatin in a solution containing 60 mg. of available chlorine and determined the available chlorine in the gelatin after washing until the washings gave no test for chlorine. Such a technique is open to numerous errors. It is somewhat surprising to find that in the present work the ratio of gelatin/glycine used by Norman (i.e. 3.6) was precisely that at which the yellow precipitate referred to above separated out (i.e. 0.050/0.014, or 3.3). It seems probable that Norman's solutions were too alkaline, since the curves shown in Fig. 5 of this paper show that in alkaline solutions the reduction in available chlorine is at a maximum when the gelatin/chlorine ratio is about 3.3.

(7) The views put forward by the author are in conformity with those of Dakin [1915], and of Rideal [1910] who concludes that in ordinary disinfection hypochlorites leave behind them, as substitution compounds which maintain the germicidal powers, the chloroamines and chloro-proteins.

Conclusions based on indirect methods of investigation, such as reaction velocity studies, are admittedly open to criticism. The relative instability of the chloroamino-derivatives of amino-acids and proteins makes their isolation and study a matter of the utmost difficulty. Until such studies have been undertaken any final interpretation of reaction velocities will not be possible. Nevertheless the evidence summarized above does appear to provide strong grounds for postulating the formation of chloroamino-derivatives as intermediate products in the oxidation of amino-acids and proteins by hypochlorites, except in solutions of extreme alkalinity.

SUMMARY.

1. The reactions occurring between sodium hypochlorite and various amino-acids and proteins have been investigated, using buffered solutions as a means of controlling the *pH*.

2. The profound influence of acidity and alkalinity on the nature of the reactions has been demonstrated. In general it is found that acidity favours chlorination and alkalinity oxidation.

3. The bearing of the results on current theories of hypochlorite action is discussed.

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