

LXXIV. THE OXIDATION OF AMINO-ACIDS BY HYPOCHLORITE.

I. GLYCINE.

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From the work of Langheld [1909] with hypochlorite and of Dakin [1916] with chloramine-T it is known that active chlorine may produce from an amino-acid either the aldehyde or cyanide with one less carbon atom together with carbon dioxide and (in the first instance) ammonia. Chlorination of the amino-group has been supposed to constitute the first stage of the reaction. Wright [1926] considered the nature of the reaction to depend on the relative proportions of active chlorine and amino-acid present; with excess of chlorine it was regarded as oxidation and with excess of amino-acid as chlorination.

In the work to be presented, the optimum conditions for the complete oxidation of glycine by hypochlorite have been determined and the course of the reaction studied quantitatively. The investigation arose out of an attempt to use a "chlorine demand" figure for the evaluation of sewage and certain trade wastes.

EXPERIMENTAL.

The reaction between glycine and chlorine was followed iodimetrically. The source of chlorine was the commercial hypochlorite solution of Messrs Laporte Ltd. (Luton), a typical analysis of which showed:

Available chlorine	>15.0
Sodium chloride	13.4
Sodium hydroxide	0.77
Sodium carbonate	0.74

In all experiments, controls consisting of the hypochlorite solutions without glycine were run concurrently. All experiments were carried out at room temperature unless otherwise stated.

(a) *Amount of chlorine used.*

Six series of bottles, each series containing 14 mg. of available chlorine and different amounts of glycine ranging from 1 to 30 mg. in 100 ml. of water, were allowed to stand $\frac{1}{2}$, 1, 2, 3, 4 and 5 hours and the residual Cl titrated. The results for the 2, 3, 4 and 5 hour series were practically identical, indicating that the reaction under these conditions is complete within 2 hours. In Fig. 1 the percentage of available Cl remaining is plotted against mg. of glycine present for the $\frac{1}{2}$, 1 and 2 hour series. The curves are generally similar to those given by Wright [1926], who interpreted the downward portion of the curve as oxidation and the upward portion as mainly chlorination. As an alternative view it seemed possible that glycine in the upward part of the curve was reacting only very slowly.

Several series of oxidations were then run at various levels of glycine. The time allowed was, in every case, 2 hours. The mg. Cl added were plotted against mg. Cl used and a curve obtained for each level of glycine as given in Fig. 2. For 2 mg. of glycine it is seen that when 10 mg. of Cl are added, the curve

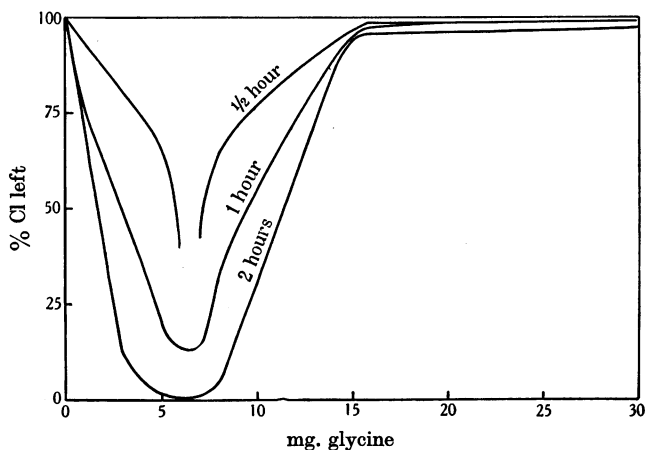


Fig. 1. Percentage of Cl used by glycine in various amounts. 14 mg. chlorine; glycine varied.

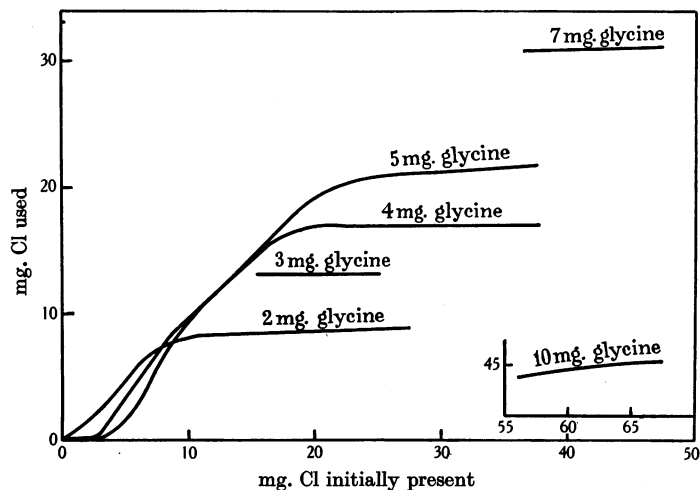


Fig. 2. Quantitative uptake of Cl by glycine.

levels off, indicating that 2 mg. of glycine react quantitatively with 8.55 mg. of Cl. In other words, the presence initially of at least 5 times as much Cl as glycine is necessary before the glycine will use a constant amount of chlorine. Examination of the other curves confirms this, and from them it was ascertained that 1 mg. of glycine uses 4.26 mg. of Cl.

If, for all the values obtained thus far in both experiments (exclusive of the $\frac{1}{2}$ and 1 hour series in Fig. 1 which cannot be considered to have come to com-

pletion), the ratio of Cl initially added to glycine be plotted against the percentage of Cl used, a general curve for the reaction is obtained and shown as the continuous line in Fig. 3.

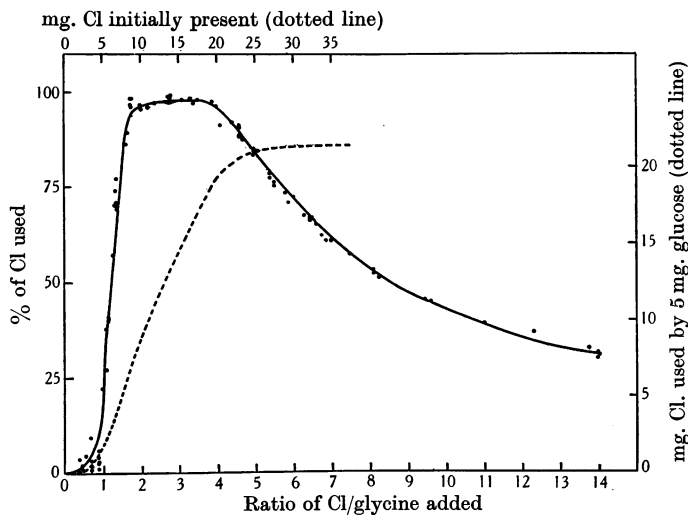


Fig. 3. Effect of Cl concentration.

Table I. *Determination of chlorides.*

A. Variation of Cl with 5 mg. of glycine.

Initial available Cl mg.	Ratio of initial Cl to glycine	Chloride-Cl			One half available Cl used mg.
		At start mg.	After 2 hours mg.	Increase mg.	
14.2 Contr.	—	9.25	9.25	—	—
14.2 Contr.	—	9.25	9.25	—	—
14.2	2.84	9.25	15.9	6.6	6.65
19.9	3.98	12.95	21.9	8.95	9.4
22.7	4.54	14.8	24.7	9.9	10.1
25.5	5.1	16.65	26.9	10.25	10.3
28.4	5.68	18.5	28.9	10.4	10.55
34.1	6.82	22.2	32.8	10.6	10.6
39.7	7.94	25.9	36.6	10.7	10.75

B. Variation of glycine with 14 mg. Cl.

Glycine mg.	Ratio of initial Cl to glycine	Chloride-Cl		One half available Cl used mg.
		After 2 hours mg.	Increase mg.	
Contr.	—	9.0	—	—
Contr.	—	9.0	—	—
1	14.0	11.2	2.2	2.15
3	4.66	15.0	6.0	6.15
4	3.5	15.4	6.4	6.75
5	2.8	15.6	6.6	6.75
7	2.0	15.3	6.3	6.65
8	1.75	14.2	5.2	6.15
9	1.55	13.6	4.6	5.5

C=Control containing no glycine.

(b) Determination of chlorides.

The hypochlorite solution employed contained alkali in the form of sodium hydroxide and sodium carbonate, together with a considerable amount of chloride. When the available Cl is determined by titration in the usual way, not only the Cl actually present in the NaOCl is titrated, but also an equivalent amount of Cl liberated from the chloride, the available Cl being twice the amount of Cl actually present as hypochlorite. If the reaction of Cl with glycine is considered ultimately to be one of oxidation, then for every two hypochlorite Cl atoms or for every four atoms of available Cl two mols. of chloride are formed. In other words, the increase of chloride should be equal to one-half the amount of available Cl used. To test this point, two series of experiments were made in duplicate—one keeping the glycine constant and varying the Cl, the other keeping the Cl constant and varying the glycine. After 2 hours one bottle was titrated for available Cl and the other for chloride. In all cases in which, by reason of the relative proportions of Cl and glycine present, the oxidation was complete (figures in bold type), the increase in chloride agreed well with one half the available Cl used (Table I). When oxidation was incomplete, chloride production lagged behind Cl utilisation.

(c) Factors affecting the rate of reaction.

Physical. Three bottles containing 4 mg. of glycine per 100 ml. and approximately 20 mg. per 100 ml. of available Cl and three controls of hypochlorite alone were set up. One sample and one control were left at 15–17°, one set was placed in a dark cupboard at the same temperature and the other set in an incubator at 30°. 100 ml. were withdrawn from each bottle at various time intervals and titrated for available Cl. All three controls showed no loss of available Cl (Table II).

Table II. *Effect of heat and light on chlorine utilisation.*

Time hours	Incubator, 30° darkness mg. Cl used	Room temp. daylight mg. Cl used	Room temp. darkness mg. Cl used
0.25	0.6	—	0.7
0.5	2.4	1.9	2.2
0.75	5.2	—	4.4
1.0	11.6	6.65	7.15
1.25	16.6	—	13.6
1.5	16.95	15.6	14.8
1.75	17.2	—	15.7
2.0	17.2	16.5	16.4
4.0	—	17.0	17.0

Table III.* *Effect of 500 candle-power light on Cl utilisation.*

Time hours	500 c.p. light mg. Cl used	No light mg. Cl used
0.5	8.55	7.65
0.75	12.75	11.8
1.0	14.5	13.9
1.25	15.8	15.8
1.5	16.5	16.4
1.75	16.9	16.9
2.25	17.2	17.1

* The hypochlorite used in this experiment was from a fresh source, so that the figures are not directly comparable with those of Table II because of alkalinity conditions, which will be discussed later.

The effect of a 500 c.p. light at a distance of 1 ft. on Cl utilisation was tried. The bottle subjected to the light and its control were placed in a glass water-bath in order to eliminate the effect of heat. Another set for comparison was placed in a water-bath of the same temperature but not exposed to the light (Table III).

Chemical. Reaction with hypochlorous acid. The effect of employing free hypochlorous acid instead of sodium hypochlorite was tested. The acid was prepared by passing Cl₂ into a suspension of mercuric oxide and then distilling under reduced pressure. 2 mg. of glycine as the Na salt per 100 ml. water were used. It was found necessary to let the bottles stand longer than the usual 2 hours for completion (Table IV).

Table IV. *Amount of Cl as HOCl used by 2 mg. glycine.*

Cl added mg.	Chlorine used					
	2 hours mg.	5 hours mg.	1 day mg.	2 days mg.	3 days mg.	4 days mg.
3	2.1	2.3	2.35	2.35	2.5	2.45
6	3.6	4.75	5.45	5.6	5.7	5.6
9	4.0	5.0	6.65	7.4	7.6	7.6
15	3.9	5.0	7.05	8.25	9.1	9.05
21	3.65	4.9	7.14	8.4	9.05	9.15

The reaction proceeded very slowly so that 3 days were required. Slightly more Cl (9.05 mg.) was used up by 2 mg. of glycine than was found with sodium hypochlorite (8.55 mg.). The HOCl solution is, of course, acid and completely masks the effect of the small amount of alkali (0.0266 m.mol. per 100 ml.) used to convert glycine into its sodium salt.

Reaction with chlorine water. A similar experiment was carried out with chlorine water. The controls tended to lose Cl at rather varying rates, so at each time interval three control determinations were made and the loss of available chlorine computed from an average of the three (Table V).

Table V. *Amount of Cl used up from Cl water by 2 mg. glycine.*

Initial Cl mg.	Chlorine used				
	2 hours mg.	5 hours mg.	1 day mg.	2 days mg.	3 days mg.
5.5	2.3	3.9	—	—	—
10.95	3.05	4.4	—	—	—
16.4	2.9	4.4	7.1	8.2	8.6
21.9	3.2	4.6	7.3	8.6	8.5
27.4	3.1	5.2	8.1	8.8	8.6

The reaction with chlorine water proceeded slightly faster than with HOCl, the amount of Cl used (8.6 mg. per 2 mg. glycine) agreeing closely with that from sodium hypochlorite.

Effect of acid and alkali on the rate of reaction. The hypochlorite solution employed contained 0.88 % sodium hydroxide and 0.76 % carbonate, exactly 10 ml. of the strong solution being diluted to 500 ml. as the source of chlorine for the following experiments. Large bottles were set up containing 4 mg. of glycine and 10 ml. of the dilute NaOCl solution (containing 20 mg. available Cl) per 100 ml. The acidity of the glycine (4 mg. = 0.0532 m.mol. of acid) and the

alkalinity of the hypochlorite (10 ml. dilute solution = 0.0727 m.mol. of alkali) were taken into consideration and various amounts of H_2SO_4 or NaOH were added so that a range from 25 m.mol. of alkali to 1 m.mol. of acid per 100 ml. of reaction mixture was covered. A large number of controls containing similar additions of acid and alkali were run simultaneously, since in stronger concentrations of acid and alkali the available Cl decreases slowly on long standing. 100 ml. were withdrawn and titrated at various time intervals, the results being given in Figs. 4 and 5. In those cases in which oxidation was complete (Fig. 4) 17 mg. of available Cl were

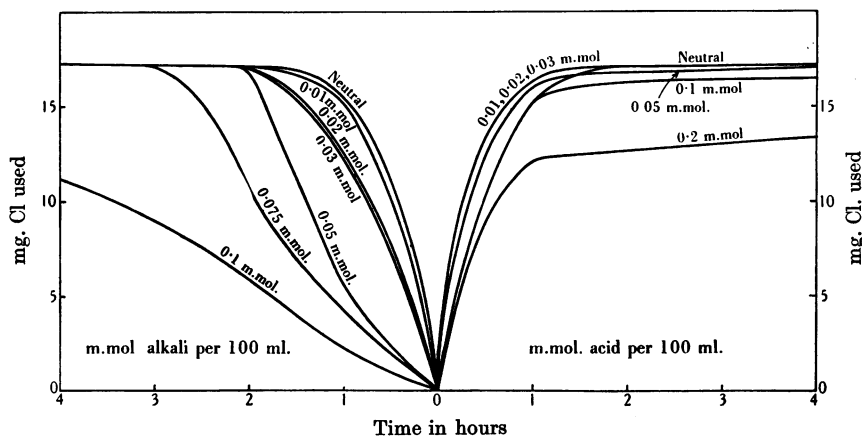


Fig. 4. Effect of acidity or alkalinity on rate of reaction. 4 mg. glycine present per 100 ml.

utilised by 4 mg. of glycine. As the alkali concentration increased, the rate of Cl utilisation decreased. On the acid side the 0.01–0.05 m.mol. reaction mixtures all used Cl at about the same rate, which was slightly faster than in the neutral¹ experiment. After the first hour the 0.1 m.mol. acid sample proceeded at a somewhat slower rate than the neutral one, and the 0.2 m.mol. sample was still slower, not having come to completion in 8 hours. A similar result was obtained with the 0.075 and 0.1 m.mol. alkaline reaction mixtures.

The curves in Fig. 5 represent concentrations of acid and alkali > 0.1 m.mol. per 100 ml. On the acid side, an increase in acid concentration again retarded the rate of reaction. The alkaline side is more complicated for, in contrast with the results in Fig. 4, the greater the amount of alkali the more Cl is used. It seems that small additions of alkali retard the reaction up to a certain point, after which further additions slowly increase the rate of reaction. A few more 2-hour determinations of the amount of available Cl used up were made around what seemed to be the critical point of 0.25 m.mol. of alkali per 100 ml. These, together with those already obtained, are given in Table VI.

This confirmed the fact that the point of reversal due to the effect of alkali was at 0.25 m.mol. of alkali. The slow reactions shown in Fig. 5 were followed for 20 days, but loss of Cl in the controls made interpretation difficult. In general it can be said that the rate of reaction was very slow after a few days in these comparatively strongly alkaline and acid mixtures.

¹ 10 ml. dilute NaOCl contain 0.0727 m.mol. alkali. 4 mg. glycine contains 0.0532 m.mol. acid. 0.0195 m.mol. acid was accordingly added per 100 ml. to neutralise the excess of alkali.

Table VI. *Additions of alkali to a mixture of chlorine and glycine.*

Cl concentration = 23 mg. per 100 ml. Glycine concentration = 4 mg. per 100 ml.

m.mol. alkali present per 100 ml.	mg. Cl used 2 hours
—	17.05
0.05	16.85
0.075	10.7
0.1	6.0
0.14	5.7
0.19	5.6
0.22	5.5
0.25	5.15
0.27	5.2
0.30	5.5
0.4	5.6
0.5	6.1
0.7	6.8
1.0	7.6

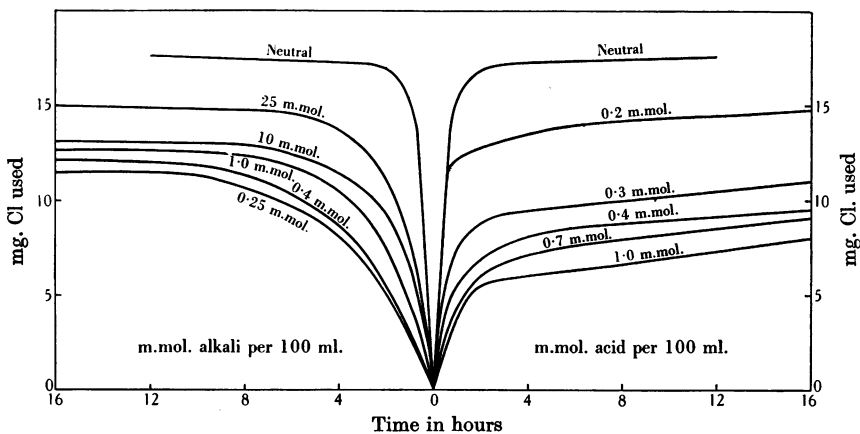


Fig. 5. Effect of acidity or alkalinity on rate of reaction. 4 mg. glycine present per 100 ml.

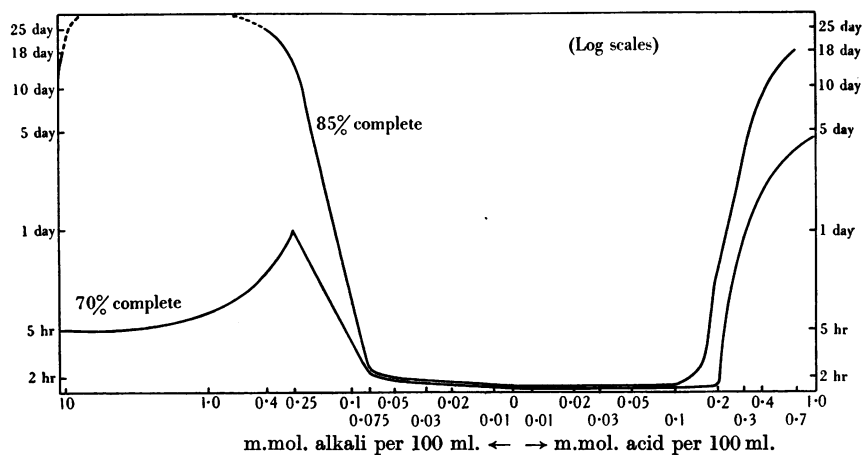


Fig. 6. General variation in rate of reaction.

The general variation in the rate due to the acid or alkali content is, perhaps, best shown in Fig. 6, where the time and the acid or alkali content are plotted on a logarithmic scale. The curves represent 70 and 85 % completion of the reaction, figures which have been arbitrarily chosen. Within the limits of 0.1 m.mol. acid and 0.1 m.mol. alkali per 100 ml. the reaction is rapid, outside these limits, slow, with a reversal on the alkaline side. A curve of a somewhat similar nature was given by Clibbens and Ridge [1927] for the hypochlorite oxidation of cotton.

As a result of these experiments which have shown that very small additions of acid or alkali may greatly affect the rate at which Cl is used by glycine, some former results may be better interpreted. The 2-hour curve in Fig. 1 is very similar to that given by Wright [1926] for 5 hours. Wright stated that he used an alkaline solution of glycine which no doubt retarded the reaction. Similarly the oxidation of glycine by chlorine water and by HOCl was slow, owing to the acidity of these solutions.

Hydrogen ion concentration.

The changes in p_H during the course of the reaction were followed by means of a glass electrode. Three samples, each containing 4 mg. of glycine and 10 ml. of dilute sodium hypochlorite solution (23.0 mg. Cl) per 100 ml. were set up. One received alkali to make the final concentration 0.168 m.mol. alkali per 100 ml., one received no addition of acid or alkali but had present 0.02 m.mol. of alkali per 100 ml. due to the alkali present in the hypochlorite solution, and the third received acid to make the final concentration 0.1 m.mol. acid per 100 ml. The changes in p_H and the Cl used were determined at various time

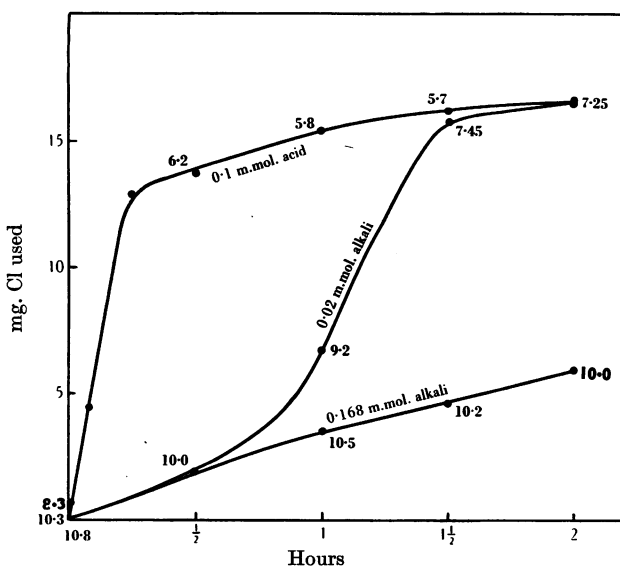


Fig. 7. Effect of p_H on Cl utilisation. Figures in bold type are p_H readings by glass electrode.

intervals. The data presented in Fig. 7 bring out the change in the rate of Cl utilisation due to differences in p_H . The process of oxidation of glycine produces a fall in p_H , the Cl uptake being most rapid between p_H 7.0 and 9.0. Reactions

which were proceeding slowly owing to a high initial p_H were greatly accelerated by suitable adjustment to neutrality (as calculated). Examples are given in Fig. 8, the dotted lines indicating the rate of the reaction if unadjusted.

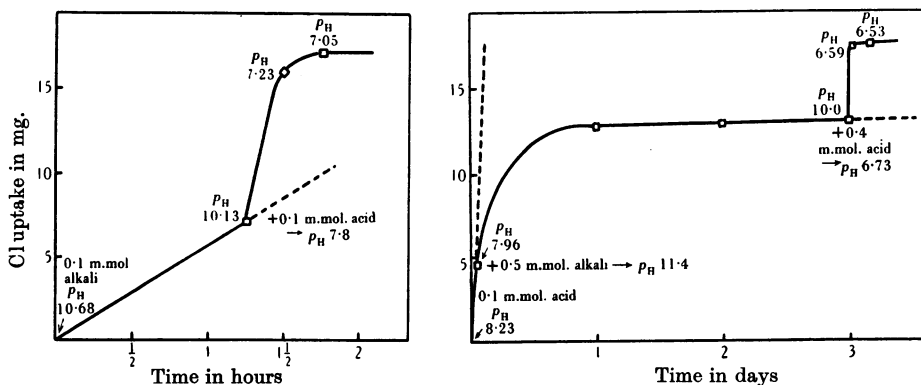


Fig. 8. Effect of adjustment of p_H on rate of oxidation (4 mg. glycine).

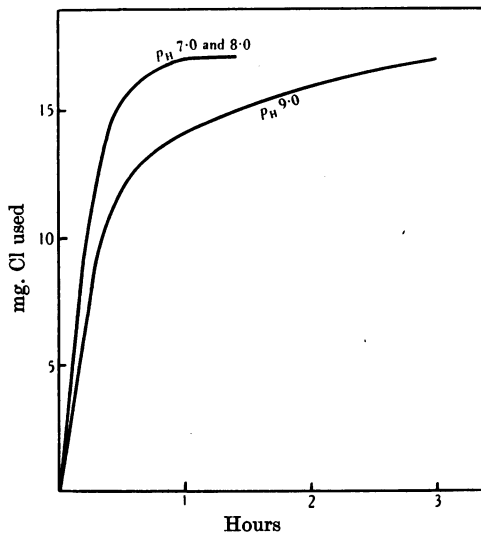


Fig. 9. Utilisation of Cl in buffered solutions.

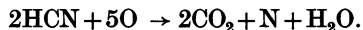
All observations show that, while the reaction may take place partially and slowly when the p_H is high, for complete oxidation approximate neutrality is required; retardation is also evident on the acid side. In order to determine the effect of stabilisation at a given p_H within the optimum range, solutions were buffered with Clark's [1928] phosphate and borate buffers to approximately p_H 7.0, 8.0 and 9.0, and titrated every 10 min. The solutions buffered at p_H 7.0 and 8.0 were very similar and are represented by the upper curve in Fig. 9. In the solution buffered at 9.0, while the initial rate of utilisation during the first half hour somewhat approximated to those in the other solutions, the rate subsequently decreased considerably so that 3 hours were required for complete oxidation of the glycine as compared with 1 hour for the other two.

The route of oxidation.

It has been experimentally determined above that 4.26 mg. of available Cl are utilised in completely oxidising 1 mg. of glycine (8.98 g.-atoms of available Cl per g.-mol. of glycine). Simple chlorination alone could account for but 4 atoms of available Cl. Dakin [1916] stated that the mono- and di-chloroamino-acids decompose spontaneously to give either the aldehyde and ammonia, or the nitrile respectively, CO₂ being liberated in both cases. In the case of glycine, HCHO or HCN would be formed by such decomposition, and using chloramine-T Dakin reported that he obtained traces of HCN from glycine. The uptake of 5 atoms of Cl by glycine remains unaccounted for, and it is evident that there must be a further utilisation of Cl by these initial fission products. Determinations of the amount of Cl used by ammonia, HCHO and HCN were therefore made.

(1) *Formaldehyde*. 2 mg. of formaldehyde were allowed to react with various amounts of sodium hypochlorite solution for 1 hour, 2 hours and 1 day, but in no case was there any loss of available Cl. This excludes the possibility of the formation of formaldehyde as an intermediate product.

(2) *Cyanide*. Solutions containing 1 and 2 mg. of KCN were allowed to react with various amounts of Cl as hypochlorite. The reaction was incomplete in 2 hours, but complete within 24 hours. The titration figures for nine samples varied from 2.8 to 3.15 mg. of Cl used per mg. of KCN present, with an average of 3.0 equiv. to 5.5 atoms of Cl per g.-mol. of cyanide. This is slightly in excess¹ of the theoretical figure of 5 atoms obtained from the following equation:



The reaction, however, is believed to be one initially of hydrolysis to formic acid and ammonia with the subsequent oxidation of these products.



(3) *Formic acid*. Preliminary experiments showed that H.COOH did utilise Cl at a slow rate. 5 mg. H.COOH and 11.2 mg. of available Cl (as sodium hypochlorite) per 100 ml. were allowed to react at room temperature. The amount of Cl used is given in Table VII.

Table VII. *Utilisation of chlorine by 5 mg. of formic acid.*

Time (days)	mg. Cl used
1	3.0
2	7.2
3	7.55
4	7.7
6	7.7

The uptake of 7.7 mg. Cl per 5 mg. of formic acid is equivalent to 2 atoms of Cl per mol. of acid. Evidently the formic acid is oxidised to carbon dioxide and water.

(4) *Ammonia*. 1 mg. of NH₃ was allowed to react with a large excess of Cl (30 mg.) for 2, 3 and 4 hours and 1 and 2 days. Oxidation is rapid and complete in 2 hours. The amount of Cl used is given in Table VIII.

The amount of Cl, 6.75 mg., used by 1 mg. of NH₃ is equivalent to 3.2 atoms of Cl for each mol. of ammonia, which agrees fairly well with the theoretical calculation for complete oxidation to nitrogen and water.

¹ The additional utilisation over and above the theoretical value is believed to be due to the formation of a small amount of nitrite, positive tests for which were obtained in certain cases. This point is being further examined.

Table VIII. *Utilisation of chlorine by 1 mg. of ammonia.*

Time	mg. Cl used
2 hours	6.85
3 "	6.75
3 "	6.75
4 "	6.75
1 day	6.6
2 days	6.65
2 "	6.85
Average	6.75

Thus of the possible intermediate products tested, cyanide, formic acid and ammonia have been shown to react with hypochlorite. The cyanide, most probably through the intermediate formation of formic acid and ammonia, accounts for the utilisation of 2+3 atoms of Cl. This, together with the 4 atoms of Cl required for the initial reaction with glycine, is in agreement with the uptake (by 1 g.-mol. of glycine) of 9 atoms of Cl as experimentally determined.

Carbon dioxide production.

During the reaction, there would be formed therefore, 2 mol. of CO₂ for each mol. of glycine oxidised, if the above theory of oxidation is true. Solutions containing 10 mg. of glycine, 25 ml. of phosphate buffer p_H 7.0 and 50 mg. of Cl, added as chlorine water, per 100 ml. of reaction mixture, were kept until the reaction was complete. Chlorine water was used instead of hypochlorite because the latter contained carbonate. After the reaction was complete the CO₂ was aerated off, absorbed in alkali and determined by back-titration. Three determinations gave 11.3, 11.6 and 11.5 mg. of CO₂. 10 mg. of glycine would give a theoretical figure of 11.7 mg. of CO₂.

The nature of the initial reaction.

There seems to be no clear evidence as to the nature of the initial reaction between an amino-acid and hypochlorite in excess. Two possibilities exist; either the amino-acid may be chlorinated to give, in the case of glycine, dichloroaminoacetic acid, or the acid may be directly oxidised to form the cyanide, with the liberation of CO₂ and water. Ammonia when treated with hypochlorite does yield chloroamine if the ammonia be present in excess [Chapin, 1929; 1931], an observation which indicates that even under such alkaline conditions hypochlorite may act as a chlorinating agent. Langheld [1909] and Wright [1926] stated that chlorination of the amino-group was the first stage in the action of hypochlorite on an amino-acid or protein in any proportion, and claimed that under certain conditions the dichloro-derivative of a monoamino-acid could be formed by simple mixture of the appropriate quantities of hypochlorite and acid. Since such chloroamino-derivatives are capable of liberating iodine from KI, no apparent loss of chlorine should be observed.

Repetition of Wright's experiment on this point, together with a similar experiment adjusted to neutrality, failed however to confirm his view (Table IX). A slow utilisation of chlorine was observed in the unaltered sample¹ and a more rapid one in the neutralised sample. Controls made at the same time showed a loss of only 0.2 mg. Cl in 3 days and 0.4 mg. in 10 days. 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 neutralised experiment than in the untreated experiment.

¹ Unaltered sample contained 0.17 m.mol. acid.

Table IX. *Utilisation of 14.3 mg. Cl by 15 mg. glycine.*

Time	mg. of available Cl used	
	Unaltered	Neutralised
2 hours	1.0	1.8
5 "	1.2	3.5
17 "	1.8	5.35
1 day	2.1	6.1
3 days	3.7	8.3
6 "	6.15	10.45
10 "	9.0	11.8

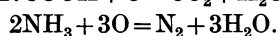
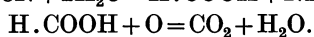
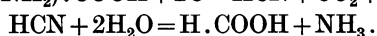
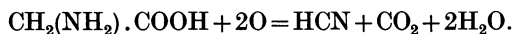
Some other evidence may be cited for the view that ordinary hypochlorite solution does not act as a chlorinating reagent. Cross *et al.* [1908] noted that gelatin treated with chlorine formed a chloro-derivative which may be washed, and subsequently titrated iodimetrically. However, gelatin immersed in hypochlorite retains very little titratable chlorine. Three amounts of 0.2 g. gelatin were allowed to stand with 60 mg. Cl as hypochlorite in 45 ml. water. To one experiment were added 5 ml. *N* acetic acid and to another 5 ml. *N* NaOH, the third receiving only 5 ml. water. After $\frac{1}{2}$ hour the liquid was drained off through fine cloth and the gelatin extensively washed till the washings gave no test for chlorine. This took several hours. The gelatin was then suspended in dilute acidified KI and the iodine liberated titrated in the usual way. The acid sample had retained 16.5 mg. Cl, the alkaline sample 1.4 mg. and the sample with hypochlorite alone 2.5 mg. Calculated on the nitrogen content of the gelatin (17%) these results correspond to the chlorination of one NH group out of every five in the acid sample, one out of every 66 in the alkaline sample and one out of 37 in the unaltered sample. Chlorination is thus shown to be relatively small, except under acid conditions in which free chlorine would be present.

Indirect evidence against chlorination by hypochlorite is provided by the behaviour of lignified plant tissues after exposure to this reagent. No colour is given on subsequent treatment with cold sodium sulphite solution [Norman and Jenkins, 1933] whereas if acidified hypochlorite or gaseous chlorine be used a characteristic rich purple colour is obtained.

In the light of this rather conflicting evidence the nature of the initial reaction between an amino-acid and chlorine as hypochlorite in excess must remain open. The theory of direct oxidation to cyanide seems at least as well founded as that of the formation of chloroamino-acids.

CONCLUSION.

Whatever be the first step of the reaction, oxidation or chlorination, the reaction between glycine and an excess of hypochlorite probably results in the intermediate formation of hydrocyanic acid, which is then hydrolysed to formic acid and ammonia. These products may then be supposed to be completely oxidised with the liberation of carbon dioxide and gaseous nitrogen. The whole reaction may be given in the equations below, the possible validity of each of which has been experimentally verified.



SUMMARY.

1. Glycine is rapidly oxidised by hypochlorite. At least five times as much chlorine as glycine must be present for completion of the reaction. Under such conditions 1 mg. of glycine uses 4.26 mg. of chlorine, equivalent to 9 atoms of chlorine or $4\frac{1}{2}$ atoms of oxygen per mol. of glycine.

2. When there is a sufficient excess of chlorine to complete the reaction, the increase of chloride-chlorine is equal to one half of the amount of available chlorine used.

3. The rate of oxidation is most rapid and is complete in 2 hours between the acid and alkali concentrations of 0.05 m.mol. per 100 ml. (on either side). In general, further additions of acid or alkali greatly retard the rate of reaction. With alkali however a point of maximum retardation is reached at a concentration of 0.25 m.mol. per 100 ml. In the presence of alkali concentrations greater than this, the rate is very slowly increased, though the reaction is still not complete after a period of 25 days.

4. The change in p_H during the reaction has been followed by means of a glass electrode. The mixture becomes more acid as oxidation proceeds, and the reaction is most rapid in the region of p_H 7-9. Solutions buffered at p_H 7 and 8 are more rapidly oxidised than one buffered at p_H 9.

5. The oxidation of possible intermediate products in the reaction, H. CHO, HCN, H. COOH and NH_3 , was tested. All except H. CHO are completely oxidised by hypochlorite. From this it is established that the oxidation of glycine results first in formation of HCN, CO_2 and water. HCN is then hydrolysed to give formic acid and ammonia, both of which are oxidised to CO_2 , water and gaseous N. Quantitative recovery of carbon dioxide was obtained. In this way, the uptake of $4\frac{1}{2}$ atoms of oxygen per mol. of glycine is accounted for.

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