THE COMPARATIVE ACTION OF SODIUM HYPOCHLORITE, CHLORAMINE-T, AND AZOCHLORAMID ON ORGANIC SUBSTRATES

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Langheld (1) found that sodium hypochlorite reacts with α-amino acids to form chloramino acids, which more or less readily break down to aldehydes or ketones, ammonia, carbonic acid, and sodium chloride. Milroy (2) pointed out that in the case of aromatic or heterocyclic amino acids, such as tyrosine or tryptophane, partial chlorination of the ring takes place in addition to oxidation. Hypochlorites have long been used as germicidal agents, but their efficiency is greatly reduced in the presence of albuminous material due to the above reactions. In order to have continuous germicidal activity over a long period of time, it is essential that the chlorine remain as positive or "available" chlorine. In other words, the positive chlorine which is reduced in oxidizing or chlorinating a ring is of no more value as a germicide. On the other hand, any chlorine which has substituted a hydrogen atom of an amino group and which is present as $—N{=}Cl_2$ or $=N—Cl$, is still active as a germicide, as Milroy has suggested.

Schmelkes and Marks (3) have described a new germicide —N—N′-dichloroazodicarbonamidine (azochloramid), $H_2N(ClN{:})C—N{=}N—C({:}NCl)NH_2$. This compound is of special interest as it possesses high germicidal power, despite displaying but slight reactivity with organic matter, such as tissue and body fluids. This paper presents a comparison of its action upon various organic substrates with that of sodium hypochlorite and chloramine-T (sodium *p*-toluenesulfonchloramide). The procedure used was as follows: A solution or suspension of the various substrates (in stoichiometrically equivalent amounts when a substrate of definite chemical composition was used) was incubated at 37° in a solution containing 200 parts per million of available chlorine.[1] 25 cc. portions were withdrawn from time to time over a period of 24 hours and titrated for residual available chlorine with 0.01 N sodium thiosulfate after being acidified with acetic acid. It will be noticed that in our titration there is no distinguishing between OCl', NCl, or Cl_2, and hence the loss in available chlorine represents only that which has been converted to Cl' or to a ring substitution, and does not distinguish between the chlorine present in its original form and that used to form N-chloro compounds.

The substrates investigated can be divided roughly into two classes, depending upon whether they contain nitrogen or not. There was practically no consumption of chlorine at all by the non-nitrogenous substrates, as was to be expected. Seven sugars were tested—three monosaccharides, glucose, levulose, and mannitol (if we may call this a sugar); three disaccharides, maltose, lactose, and sucrose; and one trisaccharide, raffinose. Other non-nitrogenous substrates were cholesterol, sodium taurocholate, and sodium oleate. In all cases the drop in available chlorine after 24 hours was negligible, except in the case of levulose (which is one of the more reactive sugars (4)), when 69 parts per million of available chlorine were consumed from a sodium hypochlorite solution having an original concentration of 200 parts per million of chlorine.

The nitrogenous substrates were obviously more reactive. In all cases studied, the amount of chlorine consumed from azochloramid was very much less than from chloramine-T, which in turn was considerably less than that consumed from sodium hypochlorite. Thus, by plotting parts per million of available chlorine consumed (after subtraction of a blank) against the time, we get in all cases curves which are in general like those shown in Fig. 1. The results are in general agreement with those of Milroy. The behavior of the substrates investigated is shown in Table I. The *Escherichia coli* and *Lactobacillus acidophilus* were killed within

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[1] In the case of the azochloramid, the available chlorine is two-thirds of the titration figure, since only 4 of the 6 atoms of iodine released by it from KI are due to chlorine.

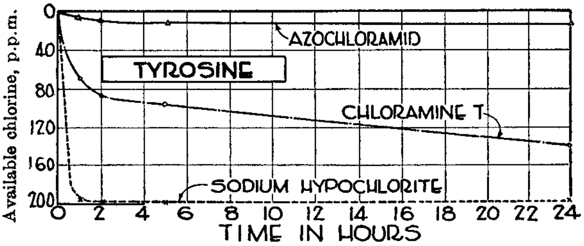

FIG. 1. Decrease of available chlorine in a solution containing 0.00025 M tyrosine and chlorinating agents in a concentration of 200 parts per million of available chlorine. Temperature 37°.

TABLE I

Parts per Million of Available Chlorine Consumed by Various Organic Substrates from Chlorinating Agents Having an Original Concentration of 200 Parts per Million of Available Chlorine after Incubation at 37° for 1 and 24 Hours

The concentrations of the substrate are final concentrations after addition of chlorinating solution.

Substrate	Sodium hypochlorite		Chloramine-T		Azochloramid	
	1 hr.	24 hrs.	1 hr.	24 hrs.	1 hr.	24 hrs.
Glycine, 0.00025 M	90	97	65	68	6	10
Alanine, 0.00025 M	40	75	34	46	4	10
Tyrosine, 0.00025 M	197	200*	72	144	6	14
Tryptophane, 0.00025 M	185	195	75	112	5	16
Cystine, 0.000125 M	180	190	161	190	12	40
Gelatin, 0.01%	139	181	7	115	0	2
Casein, 0.01%	63	177	53	69	2	3
Egg albumin, 0.01%	158	195	57	77	6	11
Peptone, 0.01%	119	190	106	200*	2	9
Cow's milk, 2.5%	149	182	115	182	22	38
Sheep blood serum, 25%	160	200*	160	200*	40	146
Human cerebrospinal fluid, 50%	182	200*	35	75	1	8
“ pleural exudate, 50%	200*	200*	200*	200*	20	200*
“ “ “ 5%	155	200*	82	175	8	32
“ “ “ 0.5%	180	200*	15	70	5	5
Yeast, 0.05% (dry weight)	152	184	45	132	8	24
Escherichia coli, 0.05% (dry weight)	158	172	25	54	4	6
Lactobacillus acidophilus, 0.05% (dry weight)	135	158	16	42	0	4

* No available chlorine left.

1 hour[2] in spite of the fact that the available chlorine in the case of azochloramid remained practically unchanged.

In the above work no attempt was made to control the pH. We did, however, run a separate experiment to investigate the consumption of chlorine from sodium hypochlorite, chloramine-T, and azochloramid at different hydrogen ion concentrations. Price (5) investigated the consumption of chlorine from hypochlorites by gelatin at varying pH values from 5.5 to 10. His reaction time was 2 minutes, and he found that with increasing pH, more available chlorine was consumed. We took as our substrates glycine, tyrosine, and tryptophane. Solutions containing 200 parts per million of available chlorine in 0.00025 M solutions of amino acids, buffered at pH 3 to 11 inclusive, were incubated at 37° in brown glass-stoppered bottles and titrated after 21 hours. The buffers used were as follows (6):

pH	Buffer	Reference
2.9	0.1 N acetic acid	
4, 5	Acetic acid-sodium acetate	Cohn, Heyroth, and Menkin (6) pp. 219, 220
6, 7	Na_2HPO_4-KH_2PO_4	Sørensen (6) p. 210
7.7	$Na_2B_4O_7$-H_3BO_3	Palitzsch (6) " 213
9	H_3BO_3-NaOH-HCl	Sørensen (6) " 209
	Na_2CO_3-$NaHCO_3$	Remington and Trimble (7)
10, 11	Na_2CO_3-$Na_2B_4O_7$	Kolthoff and Vleschhouwer (6) p. 215

The pH values of the reaction mixtures at 2.9 to 7.7 inclusive were checked with the glass electrode at the beginning and end of the experiment. The maximum variation was ±0.07.

The curves are shown in Fig. 2. It will be seen that at all pH values azochloramid is far less reactive with amino acids than either sodium hypochlorite or chloramine-T. Although the results obtained are in apparent contradiction to those of Price, it will be

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[2] This is shown by absence of growth after 48 hours incubation in Dominick-Lauter broth (*Escherichia coli*) and plain nutrient broth with Andrade's indicator (*Bacillus acidophilus*) after neutralization of the chlorinating agent with an equivalent amount of sodium sulfite.

remembered that he was working with gelatin and not a pure amino acid, and that he took as his reaction time 2 minutes; whereas our time was 21 hours.

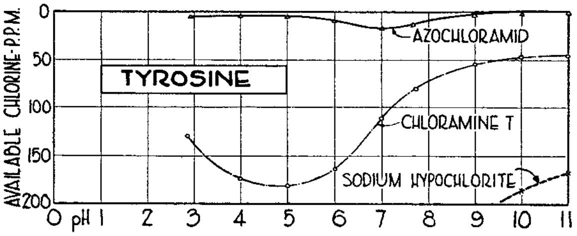

Fig. 2. Effect of hydrogen ion concentration on the decrease of available chlorine in a solution containing 0.00025 m tyrosine and chlorinating agents in a concentration of 200 parts per million of available chlorine. Reaction time = 21 hours. Temperature 37°.

SUMMARY

The action of sodium hypochlorite, chloramine-T, and azochloramid on organic substrates has been compared. Azochloramid is by far the least reactive of the three, being very inactive over a wide range from pH 3 to 11 inclusive. Chloramine-T is more reactive than azochloramid, but not nearly as reactive as sodium hypochlorite. This property is important in connection with the germicidal action of the three compounds.

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