STUDIES ON THE DISINFECTING ACTION OF HYPOCHLOROUS ACID GAS AND SPRAYED SOLUTION OF HYPOCHLORITE AGAINST BACTERIAL AEROSOLS

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(With Plate 1 and 3 Figures in the Text)

These studies, carried out during the period 1940–2, were undertaken to test the relative merits of hypochlorous acid gas and sprayed solutions of sodium hypochlorite, when these systems are used as aerial disinfectants, and to ascertain the factors determining their efficiencies in this capacity. The ulterior motive was the use of such systems as a counter measure against the danger of air-borne infection. The more practical aspects of their large-scale application, in shelters and other compartments, have been the province of others of our colleagues and will be reported upon separately.

The potential value of sprayed hypochlorite as a means of disinfecting the atmosphere of a room was demonstrated in 1928 by Douglas, Hill & Smith. They atomized saline suspensions of *B. coli communis* in a room of 5208 cu.ft. capacity, and then studied the effect of spraying a solution of electrolyzed sea water into the atmosphere. Analyses made by exposing nutrient agar plates at intervals yielded evidence of a very definite bactericidal effect. The concentration of hypochlorite used, however, was stated to be such as would probably prove objectionable to some people, but nevertheless the authors were convinced of the possibilities of the method as a means of reducing the content of viable bacteria in the air of occupied rooms, and at the same time having a 'freshening' effect. Trillat (1938) reported having found aerosols of sodium hypochlorite effective in killing air-borne organisms, but he, too, considered that the odour might be disagreeable.

Masterman (1938) described investigations on the bactericidal power of sprayed solutions of sodium hypochlorite containing saline. The numbers of bacteria in the air of occupied rooms of various types were found to be reduced by the order of 90 % through atomizing dilute hypochlorite solution (1 % available chlorine) at regular intervals. Masterman explained the action of the hypochlorite aerosols as essentially due to the hypochlorous acid gas liberated by the interaction of the carbon dioxide of the air with the sodium hypochlorite.

The effectiveness of hypochlorite sprays was further confirmed in 1939 by Pulvertaft & Walker. They favoured the use of 2 % solution of sodium hypochlorite with 20 % glycerine [Masterman (1941) points out that glycerine decomposes hypochlorite] and found that, against atomized saline and broth suspensions of *B. prodigiosus* and *Streptococcus haemolyticus*, hypochlorite aerosols having a concentration 1/6,000,000 that of the solution sprayed would effect a 99·8 % kill in 30 min. The advantages possessed by hypochlorites were pointed out: 'they are very cheap and produce a pleasantly fresh atmosphere as well as being completely innocuous to mucous membranes.'

Baker, Finn & Twort (1940) and Twort (1941) reported on tests of the aerial disinfecting power of hypochlorite sprayed under various conditions and compared its effectiveness with that of hypochlorous acid gas and chlorine. Their hypochlorite solution contained 1 % sodium hypochlorite and 16·5 % saline, and as test organism, *B. coli* was generally employed, although others were occasionally used. The results obtained with hypochlorous acid gas and chlorine, while showing that these gases were bactericidal for air-borne organisms, indicated that they were less effective than the equivalent hypochlorite mists. The findings were apparently at variance with the view that sodium hypochlorite sprayed into the air exerts bactericidal action by virtue of the hypochlorous acid gas liberated by carbon dioxide. The authors stressed some of the disadvantages of hypochlorite sprays, viz. their lack of persistence, probable objectionable odour and irritant effect in the concentrations necessary, corrosion of metallic surfaces due to combined action of hypochlorous acid and saline, and finally possible bleaching of fabrics.

Masterman (1941) contended anew that hypochlorites exert their bactericidal action through liberated hypochlorous acid gas, and restated the chemical argument in favour of this view. He further considered that much of the data given by Baker *et al.* (1940) did, in fact, support the 'gas' as opposed to the 'aerosol' theory.

TOLERANCE FOR HYPOCHLORITE AEROSOLS, HYPOCHLOROUS ACID GAS AND CHLORINE

A search of the literature failed to reveal any record of direct experimental evidence as to the tolerance shown by man for hypochlorous acid gas when inhaled for long periods. Masterman (1938), in the experiments already mentioned, observed that 'in no case was the slightest discomfort or annoyance caused to any of the occupants of the rooms, offices or factories.... A very faint, but not unpleasing smell, was experienced.... Respiration was in no instance adversely affected.' The concentration of hypochlorous acid gas in the air was in the region of 1–0·1 parts per million (p.p.m.) by volume. Pulvertaft & Walker (1939) stated that aerosols of dilute sodium hypochlorite solutions are 'completely innocuous to mucous membranes'. Masterman (1941) quotes Pulvertaft as having found that 'the maximum concentration (1 % NaOCl) devoid of all inconvenience is 1 part in 10,000,000 parts of air'. Baker *et al.* (1940) expressed the view that sodium hypochlorite (1 %) sprayed as a mist of concentration approx. 1 p.p.m., such as would be effective against air-borne pathogens, would generally be considered irritating and its odour objectionable.

Observations of our own have indicated that some people raise objection to atmospheres containing hypochlorous acid gas in excess of 0·3 p.p.m., but will breathe 0·1 p.p.m. without comment. Concerning the cumulative effect, if any, of breathing such dilute hypochlorous acid gas over long periods, nothing definite is known. The dangerous effects to health of inhaling chlorine gas, on the other hand, are well appreciated. The maximum concentration permissible for prolonged exposure is 1·0 p.p.m. (see Henderson & Haggard, 1927).

PRESENT INVESTIGATIONS

Organisms used

(1) *Streptococcus salivarius.* A serum broth (10 % horse serum) culture, 20–24 hr. at 37° C., was used in all tests with this organism.

(2) *Streptococcus haemolyticus* Group C (Lancefield)—the strain isolated by Glover (1941) from the nasal discharge of a ferret suffering with a mixed streptococcal and influenzal infection. A serum broth culture, 20–24 hr. at 37° C., was always used, appropriately diluted.

(3) *Staphylococcus epidermidis albus.* This organism was grown on nutrient agar slopes, containing 10 % horse serum and casein digest broth, 20–24 hr. at 37° C. Suspensions were then prepared in water or serum broth as required.

Sprays used

(1) *For bacterial suspensions.* (*a*) Spray 'P', a modified form of the spray incorporated in the Collision Inhaler, furnished an exceedingly fine uniform aerosol. (*b*) An all-glass spray, of the scent-spray type, yielded a very heterogeneous aerosol, resembling more closely that produced by a natural sneeze.

(2) *For hypochlorites.* (*a*) Spray 'P' with the nozzle made of Perspex. (*b*) Ebonite box spray, as used by our colleagues, Drs Bourdillon, Lidwell & Lovelock, in shelters (work, so far, unpublished). This spray, in which a horizontal jet of air traverses at high velocity the opening of a vertical capillary through which the fluid is automatically drawn from the reservoir, yielded a relatively coarse heterogeneous mist. Its high output made it very suitable for tests in the experimental room.

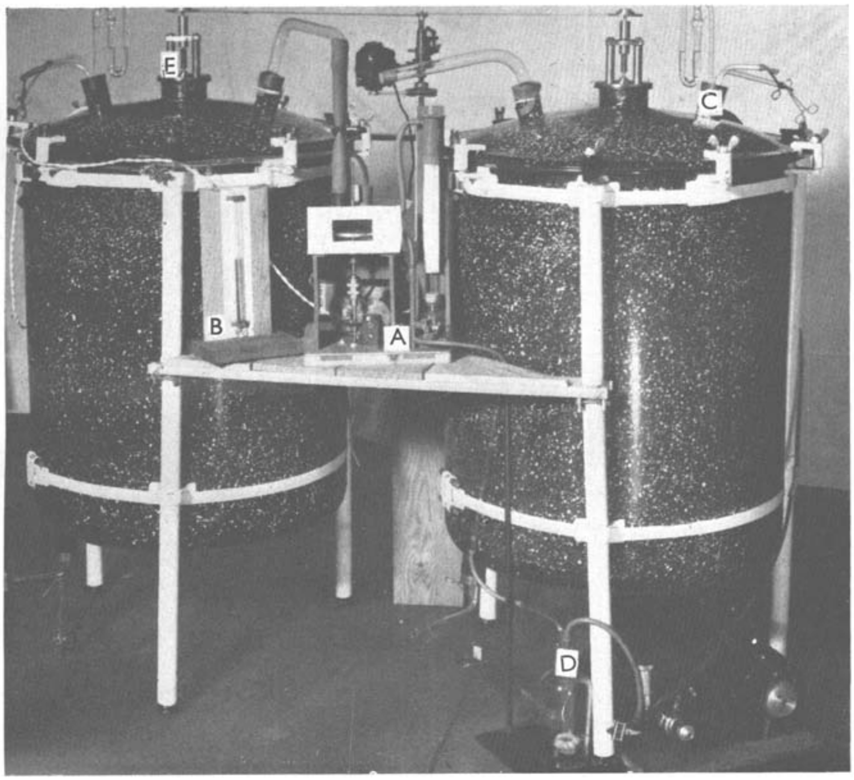
Experimental chambers

(*a*) *Glass-lined tanks.* Experiments under controlled conditions of humidity were conducted in two cylindrical glass-lined tanks (Pfaudler), each of 380 l. capacity (see Pl. 1). A fan, the spindle of which passed an air-tight oil seal in the lid, enabled the atmosphere in the tank to be well mixed, and a water manometer served to indicate any pressure differences that might develop during the experiment. Facilities were available for aerating the tanks by means of a steam extractor and the tanks could be sterilized by steaming.

(*b*) *Experimental room.* This room was of 21,000 l. capacity and was equipped as described in an earlier paper (Elford & van den Ende, 1942).

Methods of sampling and analysis

Bacteria. (*a*) *Plate exposure.* This method was used in the experimental room with the special facilities described earlier (Elford & van den Ende, 1942). (*b*) *Slit sampler.* The slit sampler, described by Bourdillon, Lidwell & Thomas (1941), was used both in the experimental room and in the tank experiments. The arrangement in the former instance was as shown by Elford & van den Ende (1942), while that for the tanks can be seen in Pl. 1. (*c*) *Syringe sampling.* This method, which has been used in a number of the tank experiments, consists in taking a known volume of the atmosphere into a glass syringe, together with a measured volume of broth. The syringe, after being thoroughly shaken, is placed in the dark for a given period to allow the organisms to settle. Then, after reshaking, the contents are expelled into the tube which originally contained the measured volume of broth, and the bacteria are estimated by plating the broth on agar. The technique of this simple method (cf. Meyer, 1921), which has proved very effective in studies



GLASS-LINED TANKS AS ASSEMBLED FOR THESE STUDIES

A, slit sampler, readily connected to either tank; B, flow-meter used when sampling tank atmospheres for [HOCl] estimations; C, water manometer, and also tubes for making additions to, or taking samples from, the air in the tanks; D,suction pump and aerosol trap used with slit sampler. E, drive for rotating fan inside tank.

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with experimental aerosols, was worked out in conjunction with our colleague, Dr C. Todd, in the course of earlier aerosol studies, reports on which have not so far been published.

Hypochlorous acid gas

(1) *o*-Tolidine method. The procedure outlined for the estimation of chlorine (D.S.I.R. 1939) was followed with minor modifications; in particular, when sampling, the hand pump was replaced by a flow-meter and suction from a water pump controlled by a variable leak.

(2) Starch-iodide method. A known volume of the air was drawn through neutral 1 % KI, followed by titration of the liberated iodine using *M*/1000 sodium thiosulphate and starch as indicator (cf. Elford & van den Ende, 1942).

The two methods compared in tests on chlorine water gave identical results, but in analysing atmospheres containing low concentrations of HOCl, the figures given by the starch-iodide method were, in general, about twice that by the *o*-tolidine method. Results with the two methods were concordant for higher gas concentrations. The *o*-tolidine method yielded better absorption at 20° C. than at 0° C., the rate of reaction, presumably, being the governing factor; whereas, in the case of absorption in KI, which is better at 0° C., the volatility of iodine is all important.

Hypochlorites

(1) *o*-Tolidine method, as for hypochlorous acid gas. It is essential to make use of the impingement principle to trap effectively the aerosol particles.

(2) The amount of fluid atomized is determined by weighing the spray before and after. The concentration can then be expressed in terms of weight of NaOCl sprayed, or the equivalent HOCl. Direct determinations of [HOCl] give figures lower than those anticipated from the amounts of NaOCl sprayed. This lack of agreement is attributable to a combination of at least three factors, according to the conditions: (1) The differential distillation of solvent with spray of type P, an effect already described by Baker *et al.* (1940) for other systems. The calculated amount of NaOCl dispersed in the air, based on the weight of fluid sprayed, will therefore be too high. (2) The complex nature of the reaction between carbonic acid and sodium hypochlorite, as suggested by Masterman (1941), may result in only a portion of the HOCl being made available. (3) The rapid decay of hypochlorous acid gas must inevitably result in direct estimations of the amount of the gas in the air being less than would be expected from the quantity of solution sprayed.

Humidity

The conditions of humidity were ascertained at the commencement of each experiment by means of a wet- and dry-bulb thermometer. In the experimental room, where no provision was made for stabilizing the humidity, after it has been adjusted to the desired level by spraying a fine mist of distilled water, the variation during an experiment was indicated by a hair hygrometer. The atmospheres of the two glass-lined tanks were maintained at 34–36 % R.H. (relative humidity) by saturated aqueous calcium chloride and at 82–84 % R.H. with saturated potassium chloride solution respectively, but were always directly checked.

I. EXPERIMENTS WITH HYPOCHLOROUS ACID GAS

The hypochlorous acid gas has been generated from 'stabilized' bleaching powder (Imperial Chemical Industries Ltd.). Our colleague, Dr Lidwell, had exposed trays of bleaching powder in front of a fan in a fairly large room and found that a concentration of hypochlorous acid gas of the order 1 p.p.m. could be readily attained in this way. This was sufficient to effect the rapid killing of *Str. salivarius* sprayed into the air from serum-broth suspension. In extending these observations, we exposed the bleaching powder in shallow trays (depth of layer of powder approx. ½ in.) on the floor of the experimental room. The air was well circulated by the fan, and in about 2 hr. the concentration of hypochlorous acid gas would reach about 1 p.p.m. At relative humidities ranging from 30 to 50 % and temperatures 15–18° C., hypochlorous acid gas, so produced in concentrations up to 1·25 p.p.m., was found to exert a slow, and, at best, only a partial killing action on aerosols of *Streptococcus* 'C', *Str. salivarius* and *Staphylococcus albus* sprayed from serum-broth suspensions in spray P. An aqueous suspension of *Staph. albus*, similarly sprayed, was inactivated to the extent of 90 % in 15 min., when the concentration of HOCl was 0·5–1 p.p.m. and R.H. = 55 %, but at lower humidities, the killing effect was much less.

The lack of control over the level of hypochlorous acid gas concentration given by this otherwise attractively simple procedure led us to seek an alternative means of generating the gas, so that a desired concentration could be secured quickly and with some degree of certainty. An excellent output of HOCl resulted when a stream of carbon dioxide was bubbled through an aqueous suspension of bleaching powder. Consequent upon experiments in which the strength of the suspension in water and in buffer solutions was varied and different mixtures of air and carbon dioxide used for aeration, the following procedure was adopted:

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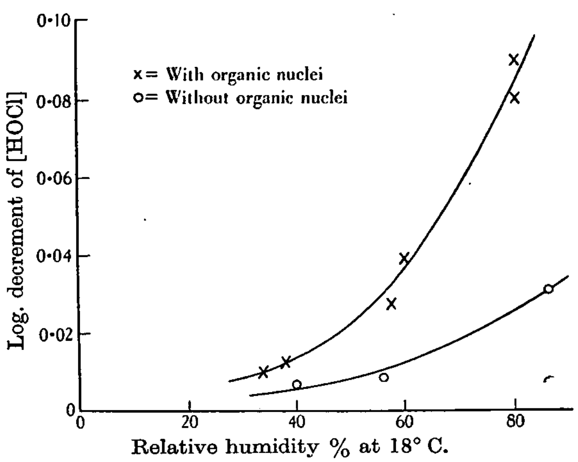
Carbon dioxide from a cylinder was bubbled at a measured rate—10–20 l. per min.—through a 2 % suspension of bleaching powder, in tap water. Under given atmospheric conditions, the passage of a known volume of CO_2 enabled a desired concentration of hypochlorous acid gas to be attained in the experimental room. In experiments with the glass-lined tanks, the carbon dioxide was bubbled at 0·5 l. per min. and was generated from a Kipp's apparatus. No measurable amount of chlorine could be detected in the gas thus generated, although analysis showed it to contain 0·1 % HOCl by volume.



Text-fig. 1. Decay of [HOCl] in experimental room at different humidities, and the influence of organic matter.

Persistence of hypochlorous acid gas

It is essential to know the degree of persistence of an aerial disinfectant, particularly in relation to different atmospheric conditions. The decay curves of hypochlorous acid gas concentrations in the experimental room were logarithmic in character, and those given in Text-fig. 1 show how the mean logarithmic decrement varied with the prevailing humidity at 18° C. The persistence depended very much on the content of organic matter in the air, especially at high R.H. values. The upper curve shows how the decay varies in relation to humidity in the presence of an aerosol formed by spraying a suspension of bacteria in serum broth, while the lower shows the relationship in normal air. Clearly in the upper curve, at high humidities, the gas is rapidly used up through interaction with the organic matter, but at low humidities the persistence tends to be normal.

Bactericidal power

The bactericidal action of hypochlorous acid gas on aerosols of the organisms already cited has been determined under known conditions of temperature and humidity and for varied concentrations of the gas. The experimental results are summarized in Tables 1–3, while Text-figs. 2 and 3 give the graphical representation. The importance of the prevailing humidity in determining whether or not hypochlorous acid gas can interact to kill the dispersed bacteria is strikingly illustrated by data for each of the three organisms. Concentrations of the gas, even in excess of 1 p.p.m., were ineffective at 36 % humidity, while at 80–85 % R.H., excellent kills were registered with concentrations below 0·1 p.p.m. The least concentration of the gas that would effect a rapid and complete kill in a humid atmosphere was determined by several factors, viz. (1) the medium in which the organism was contained when sprayed; (2) the type of spray used and the quality of the mist it generated; (3) the concentration of organisms and attendant organic matter dispersed in the air; (4) the particular organism being attacked.

When an aqueous suspension is sprayed, the nuclei in the resulting aerosol consist of naked organisms whether the mist, as generated, is fine and uniform or coarse and heterodisperse. Hypochlorous acid gas in concentrations 0·1 p.p.m. and less can kill such unprotected organisms rapidly at R.H. 82 %. Likewise a serum-broth suspension with spray P furnishes an exceedingly fine, uniform aerosol, in which the organisms must be covered only with a minimal protective layer of serum protein. It appears that this coating is readily penetrated by the hypochlorous acid gas, which still kills *Str. salivarius* effectively at concentration 0·1 p.p.m. and less at 82 % R.H. The majority of observations have been made with heterodisperse mists of serum-broth suspensions of the organisms, since these more closely resemble the product of a natural sneeze. The simple all-glass spray used yielded mists of varying quality, depending on the precise air pressure employed, and on the spraying time, particularly when this was generally only a matter of seconds in the glass-lined tanks. In the experimental room, agar plates were exposed and the slit sampler used in analysing the content of viable organisms in the air. It is interesting to note the more rapid initial rate of decay apparent in terms of the plate-exposure analysis than with the slit sampler. This, of course, is a reflexion of the heterogeneous character of the aerosol and the selective nature of the agar-plate test, which, being dependent on settling rate, is weighted in favour of the coarser particles, whereas the slit sampler records at least 90 % of the viable organisms in a given volume of the air. The apparent killing effect of the hypochlorous acid gas for a given set of conditions was generally greater when indicated by the slit sampler than by the plate exposure, a result to be anticipated, since the smallest nuclei, i.e. organisms associated with least amount of serum

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Table 1. *Action of hypochlorous acid gas on* Streptococcus salivarius *aerosols*

Suspension sprayed	Aerosol	R.H. %	T °C.	[HOCl] p.p.m.	% [organisms] at intervals after spraying: 2 min.	5 min.	10 min.	15 min.	HOCl added ↓	% [organisms] after periods of contact: 2 min.	5 min.	10 min.	15 min.	Method of sampling	Remarks
Str. salivarius in serum broth	Fine, uniform	36	20	3→2	100	86	—	70	Interval 3 min.	61	42	—	23	Syringe	Experiments in glass-lined tanks
,,	,,	36	19	1·25→1·0	100	98	—	87		62	41	—	10		
,,	,,	36	19	0·28→0·17	100	94	—	88		76	73	—	38		
,,	,,	36	20	Nil	100	90	—	81		—	—	—	68		
,,	,,	82	19	0·35–0·28	100	88	—	70		0	0	—	0		
,,	,,	82	21	0·17–0·15	100	90	—	76		0	0	—	0		
,,	,,	82	19	< 0·1	100	86	—	89		0	0	—	0		
,,	,,	82	20	Nil	100	89	—	87		—	—	—	70		
,,	Coarse, heterogeneous	36	19	0·4 –0·125	100	59	—	23		17	13	—	7	Slit sampler	
,,	,,	36	18	0·36–0·15	100	—	43	—		27	22	11	—		
,,	,,	82	18	0·36–0·15	100	73	44	—		0	0	0	—		
,,	,,	82	18	0·26–0·15	100	70	47	—		0	0	0	—		
,,	,,	82	19	< 0·1	100	64	50	—		0	0	0	—		
,,	,,	82	19	Nil	100	72	—	42		—	—	—	16		
,,	,,	38	17	0·3 –0·25	100	57	32	—		17	16	8	—		
,,	,,	38	18	0·1 –0·05	100	57	—	—		—	24	16	—	Plate	Experiments in experimental room
					100	83	—	—		—	43	35	—	Slit	
,,	,,	36	17	0·24–0·13	100	60	—	—		—	32	22	—	Plate	
					100	89	—	—		—	45	—	36	Slit	
,,	,,	36	17	0·15–0·09	100	55	—	—		—	33	—	11	Plate	
					100	91	—	—		—	43	—	18	Slit	
,,	,,	58	18	1·4 –0·55	100	48	28	—		—	1	< 0·1	—	Plate	
					100	90	41	—		—	—	< 0·1	—	Slit	
,,	,,	65	18	0·14–0·11	100	55	29	—		13	10	4	—	Plate	
,,	,,	65	17	0·19–0·08	100	54	—	—		—	27	11	—	Plate	
					100	75	—	—		—	—	3	—	Slit	
,,	,,	78	18	0·17–0·035	100	48	—	—		—	8	4	—	Plate	
					100	84	—	—		—	16	0·5	—	Slit	
,,	,,	84	17	0·64–0·17	100	49	36	—		2	0	0	—	Plate	
					100	79	—	—		—	< 1	0	0	Slit	
,,	,,	80	17	0·11–0·02	100	34	24	—		12	6	3	—	Plate	

Table 2. *Action of hypochlorous acid gas on* Streptococcus *'C' aerosols*

Suspension sprayed	Aerosol	R.H. %	T °C.	[HOCl] p.p.m.	% [organisms] at intervals after spraying: 2 min.	5 min.	10 min.	HOCl added	% [organisms] after periods of contact: 2 min.	5 min.	10 min.	15 min.	Method of sampling	Remarks
Streptococcus 'C' in serum broth	Coarse, heterogeneous	34	21	1·7 –1·25	100	60	39	↓ 3 min. interval	21	15	11	—	Slit sampler	Experiments in glass-lined tanks
,,	,,	80	22	0·6→	100	48	37		16	4	0	—		
,,	,,	84	20	4 –1·7	100	50	43		< 0·1	0	0	—		
,,	,,	84	22	1·25–0·5	100	89	70		3	0	0	—		
,,	,,	43	20	1·5 –0·75	100	53	33		—	16	13	10	Plate	Experiments in experimental room
					100	86	84		—	—	41	30	Slit	
,,	,,	60	18	1 –0·66	100	56	33		8	4	—	—	Plate	
					100	—	84		21	12	6	—	Slit	
,,	,,	70	18	0·54–0·24	100	54	20		4	1·5	—	—	Plate	
					100	91	87		3	0	0	—	Slit	
,,	,,	80	20	1·77–1·16	100	59	17		0	0	0	—	Plate	
					100	84	60		0	0	0	—	Slit	
,,	,,	80	20	0·62–0·4	100	64	30		0	0	0	—	Slit	
,,	,,	84	20	0·18–0·08	100	45	32		5	0·5	—	—	Plate	
					100	—	46		0	0	0	—	Slit	
,,	,,	78	18	0·8 –0·5	100	58	24		2	0	0	—	Plate	
					100	76	67		2	0	0	—	Slit	
,,	,,	75	17	0·12–0·07	100	48	24		11	7	3	—	Plate	
					100	80	61		30	17	12	—	Slit	
,,	,,	61	17	0·6 –0·3	—	—	—		100	58	39	—	Plate	HOCl added first
					—	—	—		100	98	88	—	Slit	
,,	,,	70	18	0·39–0·15	—	—	—		100	47	22	—	Plate	
					—	—	—		100	82	55	—	Slit	
,,	,,	80	20	2·3 –1·1	—	—	—		0	0	0	—	Plate	
					—	—	—		0	0	0	—	Slit	
,,	,,	82	17	1·25–0·73	—	—	—		0	0	0	—	Slit	
,,	,,	84	20	0·13–0·03	—	—	—		100	44	16	—	Plate	
,	,,	—	—	—	—	—	—		100	35	7	—	Slit	

Table 3. *Action of hypochlorous acid gas on* Staphylococcus albus *aerosols*

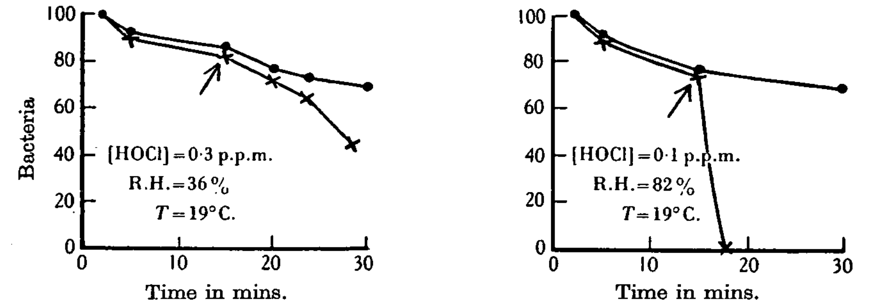
Suspension sprayed	Aerosol	R.H. %	*T* °C.	[HOCl] p.p.m.	% [organisms] at intervals after spraying: 2 min.	5 min.	10 min.	HOCl added	% [organisms] after periods of contact: 2 min.	5 min.	10 min.	15 min.	Method of sampling	Remarks
Staph. albus in water	Coarse, heterogeneous	84	20	0·33→	100	88	81	↓ 3 min. interval	20	1	0·1	—	Slit sampler	Experiments in glass-lined tanks
		86	24	0·1→	100	83	74		< 0·2	< 0·04	0	0		
Staph. albus in serum broth	Coarse, heterogeneous	36	20	0·8 –0·5	100	65	41		30	16	13	11		
		82	20	0·5 –0·25	100	79	50		0	0	0	—		
,,	,,	82	19	0·2 – < 0·05	100	88	72		28	3	—	—		
,,	,,	34	18	1·7 –0·5	100	70	39		—	22	15	—	Plate	Experiments in experimental room
					100	84	61		—	47	42	29	Slit	
,,	,,	36	18	0·24–0·21	100	67	—		40	—	16	—	Plate	
					100	89	—		—	80	67	—	Slit	
,,	,,	59	20	0·9 –0·4	100	63	—		—	20	10	—	Plate	
					100	78	66		—	—	11	1	Slit	
,,	,,	60	20	0·36–0·25	100	69	62		—	26	22	—	Plate	
					100	—	79		—	—	53	44	Slit	
,,	,,	83	19	0·33–0·17	100	51	34		6	2	—	—	Plate	
					100	93	83		2	0	0	—	Slit	
,,	,,	82	18	0·07→	100	50	—		35	23	10	—	Plate	
					100	75	—		35	—	20	—	Slit	
					2 min.	5 min.	10 min.	15 min.	30 min.	45 min.	60 min.			
Staph. albus in serum broth	Coarse, heterogeneous	80	18	Nil	100	71	50	39	21	10	8·6			Control

Table 4. *The action of* HOCl *on bacterial aerosol particles settled on surfaces*

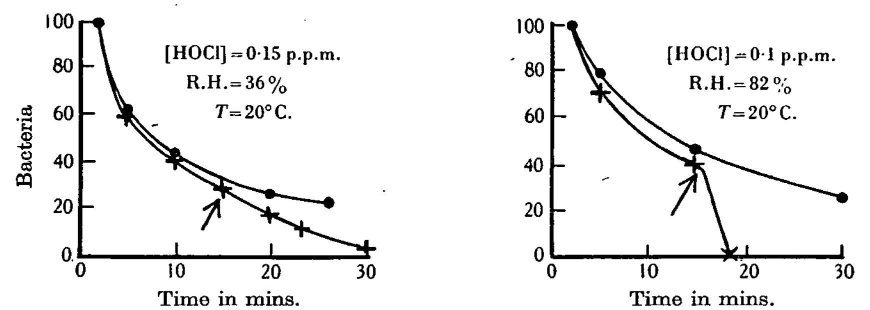
Suspension sprayed	Aerosol	R.H. %	*T* °C.	[HOCl] p.p.m.	Time of exposure min.	Observed percentage kills on various types of surfaces: Blood agar	Whatman no. 1 filter paper	Glass	Woollen serge
Staph. albus in serum broth	Fine, uniform	78→70	20	1·46→0·88	10	Nil	Nil	> 90	20
		,,	,,	1·46→0·68	20	Nil	20	> 90	58
Staph. albus in serum broth	Coarse, heterogeneous	76	20	2·85	15	Nil	80	> 99	97
		79	20	2·80	15	10	> 99	> 99	> 97
		72	20	1·47	15	Nil	65	> 99	70
Streptococcus 'C' in serum broth	Coarse, heterogeneous	78	20	1·4	15	Nil	20	> 95	55
		75	20	0·52	15	Nil	Nil	> 95	20
		72	20	0·25	15	Nil	Nil	> 95	Nil
Str. salivarius in serum broth	Coarse, heterogeneous	85	17	1·28	15	Nil	80	> 99	86
		79	17	0·74	15	Nil	50	> 99	30
		74	17	0·47	15	Nil	20	> 99	Nil

Nil = < 10%

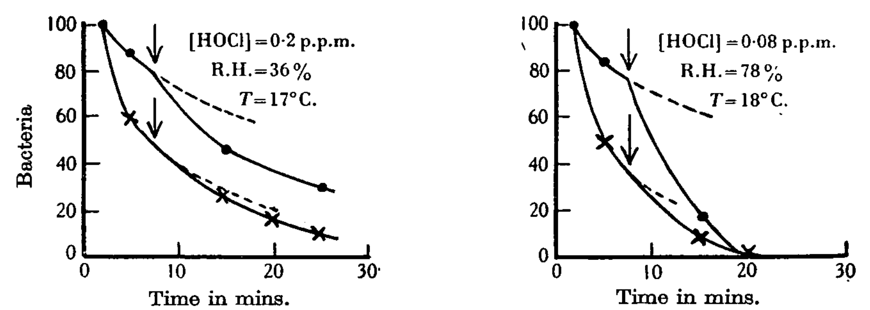
protein, will succumb most readily. The amount of hypochlorous acid gas required to disinfect quickly an atmosphere containing such coarse hetero-disperse bacterial aerosols was distinctly greater than with the fine aerosols even at high humidities. The data for *Str. salivarius* suggest at least 0·1 p.p.m. at 82 % R.H.; with *Streptococcus* 'C' and *Staph. albus* a somewhat higher value, c. 0·2 p.p.m. was necessary.



(*a*) Experiments in glass-lined tanks; syringe sampling; fine uniform bacterial aerosol.



(*b*) Experiments in glass-lined tanks; slit sampling; heterogeneous bacterial aerosol.



(*c*) Experiments in experimental room; slit and plate sampling; heterogeneous bacterial aerosol.

Text-fig. 2. Action of hypochlorous acid gas on aerosols of *Str. salivarius* sprayed from serum-broth suspension. In (*a*) and (*b*) ● = control curve without HOCl; × = experiment with HOCl. In (*c*) ● slit sample analysis; × = plate exposure analysis. The ↓ indicates point at which HOCl was added.

In most experiments the order of concentration of bacteria in the air was 1–10 organisms per 100 c.c. There were instances where considerably higher concentrations were studied, and here, when working with hypochlorous acid concentrations near the limiting value for effective killing, the apparent effectiveness of the gas was noticeably less. The explanation was obvious; with the increase in the amount of organic matter, the demand for HOCl increased, and to maintain a disinfecting concentration, a higher level is required initially. It should be borne in mind that the bacterial concentrations studied in these experiments are much higher than would be likely to be encountered under natural conditions, except, perhaps, in close proximity to a person sneezing (see Bourdillon & Lidwell, 1941).

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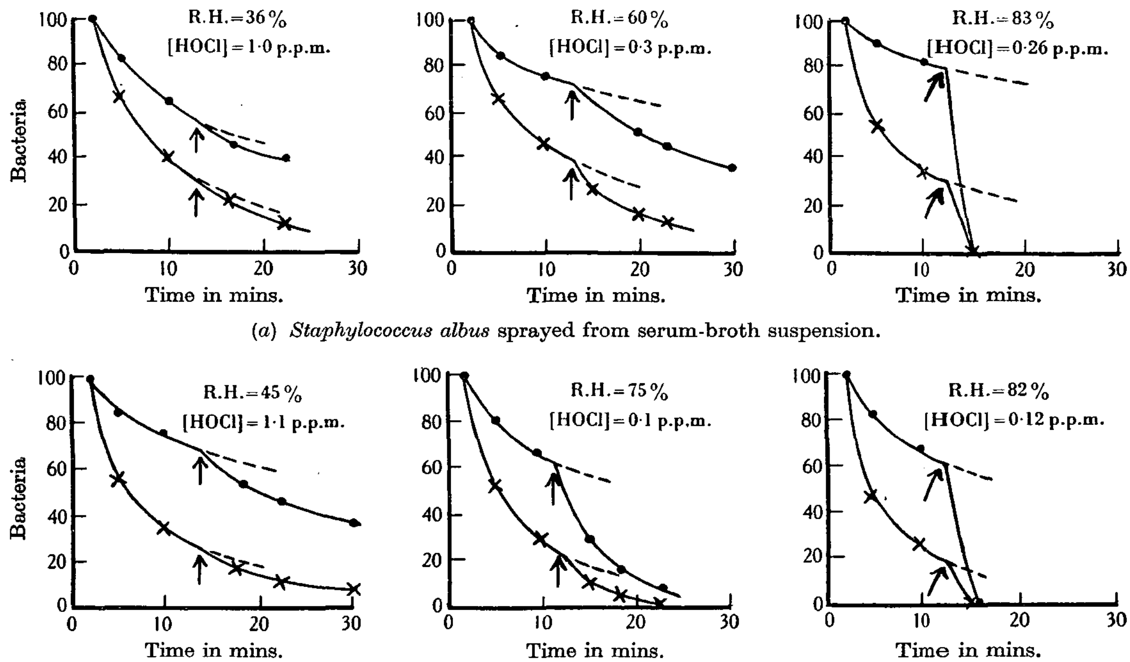
It appears, therefore, that in a relatively humid atmosphere, hypochlorous acid gas, present in concentrations 0·1–0·3 p.p.m., can exert a powerful disinfecting action on air-borne organisms, as typified by *Str. salivarius*, *Streptococcus* 'C' and *Staph. albus*. At concentrations even lower than 0·1 p.p.m., it can be effective against exceedingly fine, unprotected bacterial aerosol particles. When the atmosphere is relatively dry—R.H. less than 60 %—hypochlorous acid gas is correspondingly less effective in tolerable concentrations, and at 36 % R.H. concentrations even in excess of 1 p.p.m. achieve but little, if any, significant killing.



(*a*) *Staphylococcus albus* sprayed from serum-broth suspension.

(*b*) *Streptococcus* 'C' sprayed from serum-broth suspension.

Text-fig. 3. Action of hypochlorous acid gas on heterodisperse bacterial aerosols in experimental room at 18–20° C. ● = analysis by slit sampler; × = analysis by plate exposure; ↑ = indicates point at which HOCl was added; - - - - indicates course of normal decay.

The bactericidal action of hypochlorous acid gas on organisms that have settled out on various surfaces as dust

The particles from bacterial aerosols were allowed to settle on to several different types of surface: 2 % agar containing blood, glass, no. 1 Whatman filter-paper, and woollen serge. The contaminated surfaces were then exposed for given periods in atmospheres of known humidity, temperature and HOCl content. The numbers of organisms remaining viable were determined on the exposed and control non-exposed surfaces. The blood agar contained in 3·5 cm. Petri dishes was directly incubated; the glass surface was thoroughly washed with a measured volume of broth and the latter then plated; for the filter-paper and woollen serge surfaces, an impression technique was used (cf. similar tests with ozone, Elford & van den Ende, 1942). The results obtained are summarized in Table 4. Except in those instances where the limits are given, the concentrations of hypochlorous acid gas cited are the mean values for the period of exposure. The settled organisms are more resistant to hypochlorous acid gas than when dispersed in the air, but the measure of the increased resistance depends very much on the type of surface concerned. On glass, for example, the organisms studied were readily killed by HOCl present in less than 1 p.p.m. in atmospheres of 70–80 % R.H., while on filter-paper and woollen serge a concentration of 2 p.p.m. was indicated for rapid killing, and on blood agar a still higher concentration, at least 3 p.p.m., would be necessary. These facts are of practical importance since, although hypochlorous acid gas in tolerable concentration may be effective in killing infectious particles dispersed in the air, should any of these particles escape inactivation and settle on to clothes, they may survive there and remain a source of infection. Clearly, therefore, in order to exert its maximum effect as a counter to air-borne infection, an aerial disinfectant must be maintained at a level of concentration where its action can be rapid and complete. Then few organisms may survive even the short period required for them to settle on to cloth and other surfaces.

II. Experiments with aerosols of sodium hypochlorite

The parent solution in these studies has been 'Chloros' (Imperial Chemical Industries Ltd.). It contains approx. 12 % NaOCl, together with 11 % NaCl and a little sodium carbonate, the solution being coloured with a trace of permanganate. The *p*H of this solution, measured by the glass electrode, is close to 11·5. The stability is good, e.g. a batch tested soon after receipt showed 12·5 % NaOCl, and when retested 18 months later, having been stored meanwhile in a dark brown glass bottle, still showed 10·27 % NaOCl.

'Chloros', however, is too concentrated for use directly in sprays and in our experiments it has been diluted ten-fold in water, and as such it will be referred to as 'C/10'. It is immaterial whether tap water or distilled water is used as diluent, in so far as the resulting disinfecting power is concerned, but, again, for large-scale use tap water is desirable on grounds of economy.

The bactericidal efficiencies of hypochlorite aerosols have been studied both in the atmospheres of the glass-lined tanks and of the experimental room. Results obtained are summarized in Tables 5 and 6, where the amount of NaOCl sprayed, expressed in mg. per cu.m., and the equivalent concentration of hypochlorous acid gas, are cited for each test. Once again, as found in the experiments with hypochlorous acid gas already described, the importance of the prevailing humidity as a factor determining the bactericidal efficiency is strikingly demonstrated. Under relatively dry conditions, R.H. less than 50 %, hypochlorite aerosols have shown little value as aerial disinfectants, but at 70–80 % R.H. they become highly efficient. The bacteria in a heterogeneous aerosol, formed by atomizing a suspension of the organism in serum broth, can, at 80–85 % R.H., be rapidly and completely killed by spraying a fine aerosol containing as little as 1 mg. NaOCl per cu.m. of air. The equivalent concentration of hypochlorous acid gas is about 0·3 p.p.m. This is a somewhat higher figure than that found for the limiting effective concentration when HOCl gas was directly used.

Persistence of hypochlorite aerosols

The poor persistence of hypochlorite aerosols at 84 % R.H. was shown in a series of tests with aerosols ranging in concentration from 0·7 to 3·5 mg. NaOCl per cu.m. These concentrations of NaOCl sprayed into an existing aerosol of *Streptococcus* 'C' at 84 % R.H., had been found to effect a 99 % kill within 5 min. When the hypochlorite was sprayed into the tank first and allowed to age for known periods before introducing the organisms, the bactericidal power was found to have decayed so rapidly that in 2½ min. it had become negligible. Consequently hypochlorite aerosols must be sprayed continuously in order to maintain a high level of efficiency. This raises practical aspects of the problem outside the scope of this paper, namely the design of sprays, control of output, and their performance as regards quantity of mist and dependability over long working periods.

The use of more dilute hypochlorite solutions

Solutions containing less than 1·2 % NaOCl may be used satisfactorily for aerial disinfection, but proportionately larger volumes of fluid must be sprayed. The development of excessive humidity and the increase in consumption of power will set a practical limit for dilution.

The role of carbon dioxide in determining the efficiency of hypochlorite aerosols

A limited number of tests were made to ascertain the extent to which the concentration of carbon dioxide in the atmosphere influences the efficiency of hypochlorite aerosols. Increasing the carbon dioxide content to 1 % by volume did not augment further the efficiency, either at low or high humidity, as shown by the data for *Staph. albus* in Table 5. The amount of CO_2 normally present in the air (0·03–0·04 %) is already more than adequate to liberate the hypochlorous acid from the amount of NaOCl involved. The reaction concerned is considered by Masterman (1941) to be

$$5NaOCl + H_2O + 2CO_2 = NaClO_3 + 2NaCl + 2Na_2CO_3 + 2HOCl.$$

Data from experiments in atmospheres of extremely low carbon dioxide content are summarized in Table 6. The glass-lined tanks were filled with air that had been passed through an absorption train comprising a soda-lime tower, 20 % potash, water, and thence via a tower of glass beads. Analysis of the air in the tank was made by drawing a measured volume through standard baryta water. The sprays were operated with nitrogen instead of air. The figures indicate that the efficiency of a hypochlorite aerosol is greatly reduced in atmospheres of extremely low carbon dioxide content, below 0·001 % CO_2. When the concentration of the aerosol was increased to 4 mg. NaOCl per cu.m., i.e. nearly ten times the limiting amount found adequate for a rapid kill under

Table 5. *Bactericidal action of sprayed hypochlorite on heterodisperse aerosols of* Staphylococcus albus *sprayed from serum-broth suspension*

[NaOCl] mg./cu.m.	Equiv. [HOCl] p.p.m.	R.H. %	*T* °C.	% [organisms] at intervals after spraying: 2 min.	5 min.	10 min.	Hypochlorite sprayed ↓	% [organisms] after given times of contact: 2 min.	5 min.	10 min.	20 min.	Spray for hypochlorite	Remarks
9·7	3·1	36	23	100	86	—	Interval 1 min.	—	62	51	45	Spray P	Experiments in glass-lined tanks
4·7	1·5	36	23	100	94	70	,,	—	—	30	17	,,	
5·0	1·6	82	22	100	61	48	,,	0·1	0·1	—	0	,,	
2·0	0·63	82	23	100	81	56	,,	1	0	—	0	,,	
1·2	0·37	84	22	100*	70	42	,,	6	3	1·5	0·5	,,	
0·58	0·19	84	22	100*	75	60	,,	22	20	16	0·2	,,	
0·48	0·15	84	23	100*	92	61	,,	25	21	10	—	,,	
0·96	0·30	82	19	100	81	73	Interval 5 min.	—	2	0	0	Spray P	Experiments in experimental room
0·48	0·15	80	19	100	80	68	,,	—	3	2	0	,,	
0·24	0·075	80	20	100	75	58	,,	35	24	20	14	,,	
4·8	1·5	90	19	100	72	—	Interval 2 min.	—	0	0	0	Ebonite box spray†	
6·2	2·0	90	19	—	—	—	Aged 15 min.	100	80	46	29		
6·0	1·9	82	19	—	—	—	Aged 10 min.	100	62	35	19		
4·8	1·5	80	20	—	—	—	Aged 5 min.	100	44	22	4	,,	
4·7	1·5	34	22	100	75	—	Interval 1 min.	—	40	27	22	Spray P	Experiments in glass-lined tanks with $[CO_2]=1$ %
0·46	0·15	84	22	100	67	—	,,	—	25	21	14	,,	

* Concentration of organisms in aerosol 2 to 3 times higher than usual.

† Hypochlorite sprayed first.

Table 6. *Bactericidal action of hypochlorite aerosols on* Streptococcus salivarius *and* Streptococcus *'C' aerosols in atmospheres with (a) normal, and (b) extremely low carbon dioxide contents*

Suspension sprayed	Spray	R.H. %	*T* °C.	[NaOCl] mg./m.³	Equiv. [HOCl] p.p.m.	% [organisms] at intervals after spraying: 2 min.	5 min.	10 min.	Hypochlorite sprayed ↓	% [organisms] after given times of contact: 2 min.	5 min.	10 min.	20 min.	$[CO_2]$ %	Remarks
Str. salivarius in serum broth	Coarse, heterogeneous	84	22	0·54	0·17	100	46	33	Interval 1 min.	5	3	2	1	0·04	All experiments conducted in glass-lined tanks
Streptococcus 'C' in serum broth	Coarse, heterogeneous	84	22	0·60	0·19	100	80	50	,,	2	0	0	0	0·04	
,,	Fine, uniform	90	22	1·2	0·38	100	61	54	,,	—	17	3	2	0·0001	Hypochlorite sprayed from spray P throughout
,,	Coarse, heterogeneous	92	23	2·3	0·74	100	63	50	,,	—	12	4	0	0·0002	
,,		92	22	0·54	0·17	100	52	44	,,	—	27	17	13	0·0004	
,,	,,	92	25	0·71	0·23	100	58	45	,,	—	19	10	4	0·0005	
,,	,,	92	24	1·1	0·35	100	69	62	,,	—	16	14	4	0·0008	
,,	,,	92	24	4·1	1·3	100	53	45	,,	—	1	0	0	0·001	

normal conditions, then effective bactericidal action was manifested in presence of 0·001 % CO_2. It would be instructive to determine the efficiencies of hypochlorite aerosols in atmospheres of pure nitrogen. The evidence furnished by the tests recorded here, however, clearly supports the view that hypochlorite aerosols owe their high bactericidal power in large measure to the presence of carbonic acid which reacts to liberate hypochlorous acid.

DISCUSSION

Humidity. The important role played by moisture is an outstanding factor in determining the bactericidal power both of hypochlorous acid gas, and of hypochlorite aerosols, against air-borne organisms. Our analysis of this effect has been based on determinations of relative humidities and hence the prevailing temperatures must also be emphasized (range 18–22° C.) since it is the absolute humidity with which we are really concerned. The degree of inactivation of streptococci or staphylococci, sprayed as aerosols from serum-broth suspensions, has been shown to be relatively slight or negligible as R.H. values fall below 50 %. In the region R.H. 50–70 %, however, a sharp rise in bactericidal efficiency is observed, until at 70–90 % R.H. the organisms are very rapidly killed by tolerable concentrations of the disinfecting systems. Clearly, observations made without regard to the prevailing conditions of humidity, as has been the case in most of the recorded earlier investigations, are bereft of value. The extensive work of Baker *et al.* (1940), planned to throw light on the mechanism of the action of hypochlorites in aerial disinfection, suffers in this respect, and in consequence it becomes quite impossible to interpret reliably the results obtained. Later, Baker & Twort (1941), in pointing out the importance of humidity, cited, amongst evidence on other types of aerial disinfectants, figures showing that an aerosol of the hypochlorite solution 'Milton' was found to have no apparent effect at 28 % R.H. on a certain '*coccus*' sprayed from broth suspension, whereas at 65–72 % R.H. it achieved a very good kill. This is in accord with the findings in the present study.

Carbon dioxide. The experiments conducted in atmospheres of extremely low carbon dioxide content, 0·001 % and less, have clearly shown that the bactericidal efficiency of hypochlorite aerosols is greatly reduced compared with their behaviour in ordinary air, where the CO_2 concentration is 0·03–0·04 %. While the bactericidal power of hypochlorite aerosols is thus dependent upon carbon dioxide being available, the presence of this gas, even in concentration as high as 1 %, does not in itself satisfy all the requirements. Moisture also is necessary, as is demonstrated by the relative inefficiency of hypochlorite aerosols in dry atmospheres. These findings lend support to the view of Masterman, referred to earlier, namely, that hypochlorite aerosols owe their efficiency as aerial disinfectants to the hypochlorous acid gas liberated through the interaction with carbonic acid from the atmosphere. This presupposes the presence of adequate moisture, but even Masterman appeared not to appreciate the dominant role of humidity in the subsequent disinfecting process.

Persistence. The time during which tolerable concentration of an aerial disinfectant is able to exercise bactericidal action becomes a matter of much importance when considering practical application. Unfortunately, the persistence of bactericidal power shown by sodium hypochlorite aerosol and hypochlorous acid gas under conditions best suited to their efficiency, namely 70–90 % R.H., is very poor. This is not surprising in the case of hypochlorous acid gas which is known to react aggressively with any form of organic matter. When solutions of hypochlorite are sprayed, on the other hand, were the aerosol nuclei containing sodium hypochlorite responsible for the inactivation of air-borne organisms solely through a collision process, the decay of efficiency would hardly be expected to be as rapid as that observed. The fact that the persistence of bactericidal power shown by NaOCl aerosol decays rapidly, like that of HOCl gas, is not surprising if the gas is the disinfecting agent in each instance. This might be used as another argument in support of gaseous absorption as opposed to a collision process being the mechanism of the action of hypochlorite aerosol.

Limiting concentrations for rapid and complete killing. The limiting effective concentration of disinfectant under optimum conditions of temperature and humidity is determined very largely by the nature of the medium in which the organism is contained, either when experimentally sprayed or naturally sneezed. The naked unprotected organisms forming the nuclei in aerosols sprayed from aqueous suspensions are readily inactivated by concentrations of hypochlorous acid gas, as low as 1 part in 20 million parts of air. When the bacterium is coated with a layer of organic matter, as in a natural sneeze, or, as experimentally produced by spraying serum broth suspensions of the organism, then a definitely higher concentration of HOCl is required to assure rapid killing, viz. 0·1–0·3 p.p.m. according to the organism and its coating. *Staph. albus* was generally found rather more resistant than *Streptococcus* 'C' or *Str. salivarius*. Reference to Table 5 will reveal that the minimum effective concentration of NaOCl aerosol expressed in terms of equivalent HOCl (assuming all available) is found to be 2 or 3 times greater than that of the gas used directly. Bearing in mind the factors complicating

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the estimation of HOCl and NaOCl, the above relationship found for the limiting effective concentrations may be regarded as indicating good agreement between the two sets of experiments. This, together with the comparable persistences, implies that the decomposition of NaOCl in aerosol form by carbonic acid takes place almost instantaneously.

Practical considerations. It can be said without hesitation that hypochlorous acid gas, or sprayed aerosols of sodium hypochlorite, in concentrations of 0·1–0·3 p.p.m. available HOCl, may, in atmospheres of 70–90 % R.H., be applied with every prospect of success, for the rapid inactivation of air-borne streptococci and staphylococci. When the organisms have settled on to surfaces as dust, then, in general, considerably higher concentrations of disinfectant are required. The level of concentration indicated above for effective aerial disinfection may be considered tolerable, but any higher concentration would most certainly be complained against as being unduly irritating when breathed, even if not dangerous. Careful control of the concentration is a matter of first importance. Furthermore, the poor persistence of bactericidal power shown by hypochlorite aerosols will necessitate continuous spraying to ensure the maintenance of full efficiency. When sodium hypochlorite solutions are atomized, however, although the hypochlorous acid gas liberated decays quickly, the aerosol nuclei, containing NaCl, Na_2CO_3 and $NaClO_3$, will persist in the air and accumulate progressively. It is highly probable, therefore, that in a relatively short time the air laden with such particles will be found irritating when breathed. On this account the preferable course would appear to be to generate hypochlorous acid gas directly, rather than to spray hypochlorite solution when the continuous treatment of the atmospheres of crowded quarters is contemplated. The humidity would need to be measured, but, under such crowded conditions, in the absence of efficient ventilation, it will tend to reach a high level, 80 or maybe 90 %. The requirement for good disinfecting action will therefore prevail.

The adverse characteristics of hypochlorous acid gas, already mentioned, should on no account be overlooked in any plans for its application for aerial disinfection. The corrosive action on many metals, particularly rapid in humid atmospheres, may well be re-emphasized.

The studies here described have been supplemented by tests carried out by our colleagues, Drs Bourdillon, Lidwell & Lovelock (1941), on the efficacy of sprayed hypochlorite in reducing the number of viable organisms in a closed atmosphere following a sneeze. The findings have been concisely summarized: 'An average vigorous sneeze causes the emission of about 100,000 bacteria-carrying particles of a size small enough to remain in the air for more than 1 min.... All, or almost all, the bacteria thus emitted can be killed in 3–4 min. by a spray of 1 per cent sodium hypochlorite solution in a concentration of 2·1 c.c. per 1000 cu.ft. of air.' This latter quantity corresponds to 0·22 p.p.m. hypochlorous acid gas. The view was expressed that the practical lower limit of humidity for effective killing is 'certainly below 60 per cent at 70° F.'

Recently, Challinor (1943) has described the results of tests carried out in a poorly ventilated occupied (crowded) room, into which hypochlorite solution was sprayed to reduce the number of viable air-borne bacteria. Control bacterial counts were made for conditions obtaining before occupation of the room. It is important to note that the floor was not treated in any way, and there was no sneezing or coughing, and little conversation, amongst the occupants during tests. A concentration of hypochlorite, averaging 0·4 c.c. of 1 % sodium hypochlorite per million c.c. air (i.e. 1·2 p.p.m. HOCl by vol.), produced a perceptible characteristic odour and caused a slight tingling sensation in the nose and throat. This level of concentration was found to effect a 33 % reduction in the count of viable bacteria under favourable conditions of humidity. The hypochlorite was invariably ineffective at low humidities. The observed percentage kill is surprisingly low in comparison with the findings of Bourdillon *et al.* cited above, and with what one would expect to be achieved by the prevailing hypochlorite concentration in the light of our own experiments with sprayed bacterial aerosols. There can be little doubt, as Challinor himself has reasoned, that the organisms under consideration in his studies were predominantly 'dust organisms', known to be relatively resistant to antiseptics, while only a small proportion were freshly contributed to the atmosphere from the respiratory tracts of the persons in the room. It is these latter newly dispersed bacteria that succumb most readily to the disinfecting action of the hypochlorite.

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