STUDIES ON AIR-BORNE VIRUS INFECTIONS

III. THE KILLING OF AERIAL SUSPENSIONS OF INFLUENZA VIRUS BY HYPOCHLOROUS ACID

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(With 1 Figure in the Text)

Recently considerable interest has been taken in the possibility of combating air-borne infection by means of antiseptics dispersed in the air as fine particles. The effect of such bactericidal mists upon virus particles suspended in the air has therefore been studied. A brief reference has already been made to the results of these experiments which showed that aerosols of influenza virus could be rendered non-infective (Andrewes *et al.* 1940). The technical methods employed have recently been published (Edward, Elford & Laidlaw, 1943) and it is now possible to give details of the investigation. Preliminary tests suggested that influenza virus was susceptible to mists of hexyl resorcinol in propylene glycol, but only the action of hypochlorite was studied in detail as it appeared the more likely to be of practical value under wartime conditions (Andrewes *et al.*).

Technique

The apparatus and methods were a modification of those previously described (Edward *et al.* 1943). The PR8 and W.S. strains of influenza virus A were used. The lungs of mice were harvested 3 days after intranasal inoculation with the former strain and 2 days after inoculation with the latter. Crude 5% suspensions of virus were obtained by emulsifying the lungs in a mixture of equal parts of nutrient broth and physiological saline. To obtain for some experiments suspensions containing as little protein as possible the crude suspension was centrifuged at 3000 r.p.m. for 10 min. and the supernatant fluid clarified by filtration through asbestos pulp in a sintered glass filter (Jena, size G.1). About 20 c.c. of this fluid was then centrifuged at 10,000 r.p.m. for 3 hr. and the deposit resuspended in 1 c.c. of a mixture of equal parts of nutrient broth and physiological saline. Chemical tests showed that these purified suspensions contained only small amounts of protein.

An Aerograph Pencil Spray was used for atomization. The coarser droplets were removed by impingement on the bottom of the small flask into which the spray was fitted.

Effect of hypochlorite mists

For these experiments purified virus suspensions of the W.S. strain were used.

About 1 c.c. was atomized into a 9 l. space above a saturated solution of calcium chloride in an aspirator jar (*A*) (see Fig. 1) and allowed to stand for 15 min. to allow the larger particles to settle. One-half ($4\frac{1}{2}$ l.) of this aerosol was then displaced into a similar jar (*B*), where it was mixed with an equal volume of air to serve as a control. The jar (*A*) was now transferred into a room (3200 *cu. ft. capacity*) filled with a mist prepared by spraying, as a fairly fine baffled spray, 32 c.c. of a 1% slightly alkaline solution of sodium hypochlorite. Immediately $4\frac{1}{2}$ l. of this hypochlorite mist were aspirated into the jar through a tube which had an internal diameter of $\frac{1}{2}$ in. and was as short as possible to minimize any loss of hypochlorite. The contents of the jar were now well mixed. The amount of hypochlorite sprayed in the room was calculated to give a concentration equivalent to 1 vol. of hypochlorous acid gas in 2×10^6 vol. of air when diluted with the virus aerosol in the jar.

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After standing for 15 min., during which the contents of both jars were kept well mixed by repeated squeezing of the mixing bulbs (the balloons served to minimize sudden changes of pressure), the contents of each jar were displaced into a desiccator containing six mice, which were allowed to inhale the mixtures for 30 min. The mice were kept under observation for 10 days when the survivors were killed and they, and any mice which had died, were examined for influenzal lesions.

The result of such an experiment was as follows. All the control mice were dead by the seventh day. Among the six mice exposed to the treated aerosol there was one non-specific death and one influenzal death on the eighth day. The rest of the mice when killed on the tenth day showed influenzal lesions which were large in one mouse and of moderate size in the others. From experience gained in the titration of influenza aerosols this difference in the severity of the infection suggests a 90% kill of the virus.

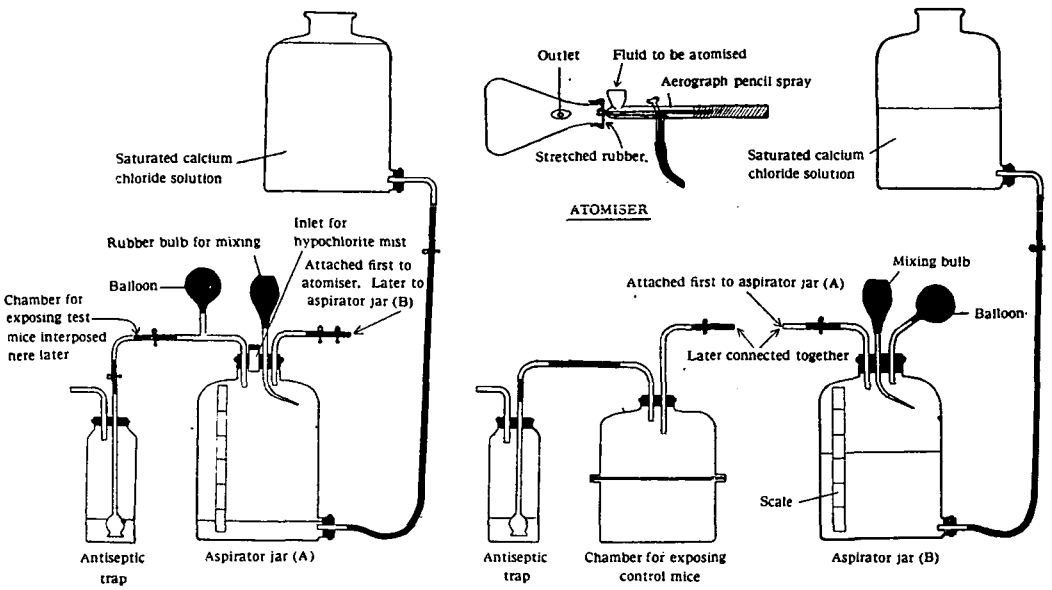

Fig. 1.

In an attempt to follow and evaluate the antiseptic action more closely, samples of the virus-hypochlorite and virus-air mixtures were taken at intervals after the mixtures had been made by displacing them into pots of 225 c.c. capacity, adding 2·25 c.c. of broth and centrifuging to deposit the virus. The broth samples were then titrated by inoculating mice intranasally. The results of such an experiment are shown in Table 1 and confirm a 90% kill of the virus, this action being effective within 7½ min.

Table 1. *Action of hypochlorite mist on an aerosol of influenza virus. Result of titration of broth samples in mice*

Time mixture was in contact min.	Control mixture of equal parts influenza aerosol and air			Test mixture of equal parts influenza and hypochlorite aerosols		
	1/1	1/10	1/100	1/1	1/10	1/100
7½	5, 5, 3	2, 0, 0	—	1, 1, 0	0, 0, 0	—
15	5, 4, 3	2, 1, 0	1, 0, 0	3, 0, 0	0, 0	0, 0
30	3, 2, 1	3, 0, 0	—	0, 0	0, 0	—

Resulting infection of mice is expressed in terms of a numerical index. Thus 5 represents death with influenzal consolidation of the lungs. Among survivors killed on the tenth day almost complete consolidation is represented by 4, consolidation affecting three-quarters of the lungs by 3, half by 2 and a quarter or less by 1.

The experimental technique necessary for the handling of viruses is likely to be less favourable for demonstrating the antiseptic action of hypochlorite than the methods usually employed for bacteria, viz. atomizing both the bacteria and the antiseptic directly into rooms. This was confirmed by an experiment

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carried out similarly to those described above but using a suspension of *Streptococcus salivarius* in equal parts of nutrient broth and physiological saline in place of the virus suspension. At the end of 15 min. contact, the bacteria-hypochlorite and the bacteria-air mixtures were sampled. No killing of the streptococcus could be demonstrated. Under normal conditions of temperature and humidity a concentration equivalent to 1 vol. of hypochlorous acid gas in 4×10^6 vol. of air will effect a better than 95% kill of an aerosol of *Str. salivarius* within 5 min. (Bourdillon, Lidwell & Lovelock, 1942). The complete absence of any detectable kill in the above experiment using twice this concentration indicates either very considerable losses of hypochlorite in the aspirator jars, probably by absorption on their relatively large wet internal surfaces, or that the relative humidity over the saturated calcium chloride solution was so low as to inhibit the bactericidal action of the hypochlorite.

These experiments therefore suggest that a hypochlorite mist is at least as effective as an aerial antiseptic against influenza virus as it is against *Str. salivarius*.

Action of hypochlorous acid gas

There has been controversy concerning the mode of action of hypochlorite bactericidal mists.

Masterman (1941) claimed that it depended on the liberation of hypochlorous acid gas. Therefore, at the suggestion of Dr R. B. Bourdillon, the gas itself was tried as an aerial antiseptic. It was confirmed by one of us (O.M.L.) that hypochlorous acid gas delivered into the atmosphere as such (or more probably as chlorine monoxide, Cl_2O) is an effective aerial antiseptic for sprayed bacteria. It was thus considered desirable to study the effect of the gas on aerosols of influenza virus.

Table 2. *Summarized results of action of hypochlorous acid on influenza virus aerosols*

No. of exp.	Virus suspension atomized	Concentrations of hypochlorous acid gas by volume: Calculated; based on preliminary estimation	Estimated after 2 min.	Estimated after 15 min.	Test mice exposed to mixture of virus aerosol and HOCl	Control mice exposed to virus aerosol only
1	Purified W.S.	1 : 800,000	1 : 1,600,000	1 : 3,200,000	0, 0, 0, 0, 0, 0	5, 5, 5, 5, 4, 3
2	Purified W.S.	1 : 640,000	1 : 1,800,000	1 : 2,000,000	0, 0, 0, 0, 0, 0	5, 5, 5, 5, 5, 5
3	Crude W.S.	1 : 800,000*	—	1 : 2,000,000	0, 0, 0, 0, 0, 0	5, 5, 5, 5, 5, 4
4	Crude PR 8	1 : 640,000	1 : 900,000	1 : 1,200,000	? 1, 0, 0, 0	5, 5, 5, 3
5	Crude PR 8	1 : 1,300,000	1 : 1,200,000	1 : 1,400,000	2, 2, 2, 1, 1	5, 5, 5, 3, 3, 3

Resulting infection of mice expressed as a numerical index as in Table 1.

* First a volume of hypochlorous acid gas was admitted that had given a concentration of 1 : 1,200,000 in preliminary trial. The actual estimated concentration in the bottle was 1 : 2,800,000. As this was very low a further addition was made of half the previous volume.

Hypochlorous acid gas was generated by passing moist carbon dioxide in measured amounts through loosely packed 'stabilized' bleaching powder into an aspirator jar. After mixing, the concentration of hypochlorous acid could be estimated colorimetrically by aspirating with a hand pump through an acid solution of *o*-toluidine. The concentration produced was proportional to the volume of carbon dioxide used and it was easy to determine by preliminary experiment the volume necessary to give any desired concentration.

The experimental procedure was as follows. The clean dry jar was filled with virus mist prepared either from crude 5% suspensions of mouse lung, merely clarified by centrifuging at 3000 r.p.m. for 5 min., or from purified suspensions. Hypochlorous acid gas was then admitted in a known amount, and after well mixing its concentration was determined. Air was allowed to enter the jar to replace the mixture removed by sampling. After 15 min. the mixture was again well mixed and the concentration of hypochlorous acid estimated. Immediately the mixture was displaced into a desiccator in which six mice were exposed for 20 min.

A control experiment was then carried out in an identical manner, but without addition of hypochlorous acid.

The results of a series of these experiments are shown in Table 2. In four out of the five the destruction

of virus by the hypochlorous acid appears to have been at least 99%. The fifth experiment is puzzling. The destruction of virus is much less and in contrast to the other experiments the absorption of hypochlorous acid is negligible both initially and during the 15 min. period of standing. It is possible that in this experiment, which was the first to be done, either the estimator or the generator was at fault.

A mist produced by atomizing the mixture of equal parts of nutrient broth and physiological saline used as a suspending medium did not destroy more than an insignificant proportion of the hypochlorous acid gas. There was no significant difference in the behaviour of the crude and the purified suspensions so that the reduction in the concentration of this gas regularly observed in the experiments (except no. 5) must have been due to reaction with the virus itself or accompanying protein.

These results show that it is possible to obtain a 90% and probably a 99% or more, destruction of suspended virus in an atmosphere to which sufficient hypochlorous acid gas is added to produce an initial concentration of 1 vol. in 800,000 volumes of air. This amount was needed to give a final concentration after reaction of about 1 in 2×10^6 vol. A virus aerosol has to be extremely dense in order to produce satisfactory infections in mice and therefore uses up appreciable amounts of hypochlorous acid. It is probable that the lower concentration obtaining at the end of the reaction is an adequate killing concentration when relatively small amounts of virus aerosol are present.

A saturated solution of calcium chloride absorbs significant amounts of hypochlorous acid from the atmosphere above it. It was therefore not desirable to allow the aerosol to dry over calcium chloride solution in these experiments as was done in the investigation of hypochlorite mists. The inability to find a substitute fluid which did not absorb hypochlorous acid gas, for displacing the contents of the aspirator jar, made it impossible to design experiments in which the same virus aerosol was used for the test experiment and the control.

Toxicity and irritation

Exposure of mice and cats to high concentrations of hypochlorous acid gas for a short period and to moderate concentrations for much longer periods (up to 1 : 120,000 vol. for short spells and up to 16 days at about $1 : 2 \times 10^6$) produced no observable general toxic effects but, in the higher concentrations only, acute irritation of the conjunctiva and mucous membrane of the upper respiratory tract, which did not lead to any apparent permanent damage. These experiments, though few in number and of a preliminary character only, do not suggest that there is any danger in allowing human beings to breathe the lower bactericidal concentrations which have been recommended for public use.

In order to investigate the possibility that the irritation of mucous membranes produced by hypochlorous acid gas might predispose to subsequent infection the following experiment was carried out. A batch of twenty-four mice was exposed for 3 hr. to a hypochlorite mist. The average concentration was equivalent to 1 vol. of hypochlorous acid gas in 1×10^6 vol. of air and the highest concentration reached was 1 in 400,000 vol. of air. Half an hour after being removed from this these mice, together with twenty-four controls, were exposed to an influenza virus aerosol. After 24 hr. and again after 3 days random samples of three mice were taken from the test and control batches; these were killed and the virus content of their lungs titrated. The rest of the mice were kept under observation for 10 days, when the survivors were killed. Nine of the test group and eleven of the controls died. All the mice showed large or moderate lung lesions. There was no significant difference in the death rate, the severity of the infection, or the virus content of the lungs between the two groups. The experiment, therefore, failed to reveal any increased susceptibility to infection following exposure to hypochlorous acid.

Discussion

The experimental basis for the recent use of hypochlorite mists in the field, including air-raid shelters, has been obtained only with sprayed bacteria (Pulvertaft & Walker, 1939; Andrewes *et al.* 1940; Masterman, 1941; Bourdillon *et al.* 1942, etc.). Evidence presented here suggests that hypochlorite mists are at least equally effective for killing air-borne

particles of influenza, a typical respiratory virus. In confirmation of the theory that their antiseptic action depends on the liberation of hypochlorous acid gas or its anhydride it has been shown that effective killing of influenza aerosols can be obtained by liberating hypochlorous acid gas itself into the atmosphere.

Since this investigation was completed there have been two reports of the successful killing of influenza virus aerosols by mists of propylene glycol (Henle & Zellat, 1941; Robertson, Loosli, Puck, Bigg & Millar, 1941) using a concentration equivalent to about 1 volume of glycol vapour in 10,000 vol. of air.

Summary

Experiments are described for determining the effect of hypochlorite mists and hypochlorous acid gas on aerosols of influenza A virus.

The virus aerosol is shown to be at least as easily killed by hypochlorite mists as *Streptococcus salivarius*. It is also killed by liberating hypochlorous acid gas itself into the atmosphere. A concentration of 1 vol. of gas in 2 million vol. of air is probably effective in destroying 99% or more of virus particles when the proportion of these in the air is small.

Preliminary experiments on mice and cats are recorded which failed to reveal any toxic effects produced by inhaling the gas in relatively high concentrations or for prolonged periods. Acute irritation of mucous membranes only was found. This did not appear to lead to any increased susceptibility of mice to subsequent infection with influenza.

We wish to acknowledge our indebtedness to the late Sir Patrick Laidlaw and to Dr R. J. V. Pulvertaft in co-operation with whom the earlier experiments were made. We also wish to thank Dr W. J. Elford, Dr F. C. MacIntosh, Dr R. K. Callow and Dr T. S. Work for their assistance and advice in parts of the investigation.

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