1950

Action of certain viruscidal agents on lactic streptococcus bacteriophage

Frederick William Bennett
Iowa State College

NOTE TO USERS

ACTION OF CERTAIN VIRUSCIDAL AGENTS ON LACTIC STREPTOCOCCUS BACTERIOPHAGE

by

Frederick William Bennett

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Dairy Bacteriology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State College
1950

TABLE OF CONTENTS

INTRODUCTION

Failure of lactic acid cultures to develop acid at a normal rate often has resulted in seriously upsetting plant routine, lowering the quality of the product and in many cases causing a complete loss of the material.

Since the announcement in 1935 of the discovery of bacteriophages as an important cause of culture failure, much research has been devoted to possible means of avoiding or controlling this source of trouble. Some of the most effective methods which have been developed are carrying cultures in special sealed cans in a separate building some distance from the main plant, rotation of a series of cultures of different bacteriophage sensitivities, use of special combinations containing organisms of different bacteriophage sensitivities and changing to a new culture after a culture has failed. Each of these methods is at best inconvenient, expensive or not always entirely satisfactory otherwise.

There is a limited amount of experimental data to indicate that chemical agents which have been used so effectively in the control of bacteria in the dairy industry also may be helpful in the control of bacteriophages. However, such available information is so meager that more research on the subject seemed desirable.

The study herein reported was undertaken for the following purposes:

(1) To measure the persistence of active lactic streptococcus bacteriophage in air and on surfaces contaminated with atomized infected whey which has been mentioned so often as an important mode of transmission.

(2) To investigate the possibility of inactivating lactic streptococcus bacteriophage in the air by means of certain viruscidal* agents suitable for use in dairy plants.

(3) To determine the action of certain viruscidal agents upon lactic streptococcus bacteriophage contained in certain dilutions of whey.

---

* Spelling according to Webster's New International Dictionary of the English language, 2d ed., unabridged, 1948, Springfield, Mass., G. and C. Merriam Co. "Viricidal" is used more commonly and has greater scientific acceptance.

REVIEW OF LITERATURE

Sources and Modes of Dissemination of Lactic Streptococcus Bacteriophage

The general characteristics of bacteriophage have been discussed in many places in the literature. It is beyond the scope of this presentation to review this subject exhaustively. However, citation of certain articles containing material on sources and modes of dissemination of lactic streptococcus bacteriophage seemed desirable.

Evidence of lactic streptococcus bacteriophage was first demonstrated in lactic acid cultures used in butter. Hammer (1933) at the Iowa Agricultural Experiment Station reported that addition to pasteurized or sterilized milk of cultures that had coagulated slowly resulted in delay of coagulation by normal cultures. Whitehead and Cox (1935) reported the first isolation of lactic streptococcus phage. The phage isolated by Whitehead and Cox (1936) proved active only against a particular strain of Streptococcus cremoris among several tested. Aeration of the milk in which the phage-infected cultures were grown was reported to stimulate phage development.

Nelson, Harriman, and Hammer (1939) reported that filtrates from slow cultures showed varying degrees of inhibitory effect upon the growth of a considerable number of strains of lactic streptococci. Under proper conditions the inhibitory principle caused lysis of a suspension of organisms

of a sensitive strain.

Sutton (1939) in New South Wales isolated bacteriophage from whey by an enrichment procedure in milk culture. Coagulation seldom was delayed beyond 24 hr. but a sample of the resultant whey caused lysed areas to develop on smeared agar plates and brought about disintegration of the streptococcus growing in milk culture.

Whitehead and Hunter (1941) demonstrated the presence of bacteriophage affecting lactic streptococci by recovering the phage from sterile water through which air was aspirated, from sterile skim milk exposed to the air in petri dishes for 10 min. and from exposed inoculated agar surfaces. The phage also was recovered from whey, dust and earth from under the whey tanks and dust from beams and ledges. The greatest concentration of air-borne phage was found near the whey separator. Failures in acid development in the manufacture of cheese seemed to occur almost entirely as a result of phage contamination of mother culture or bulk culture, rather than from other causes.

Mazé (1941) reported the isolation of five bacteriphages from the excreta of pigs being fed whey from a nearby cheese factory. The phages were specific for the cultures being used by the factories.

Failure of single strain starters as a result of bacteriophage infection was confirmed in England by Anderson and Meanwell (1942). A minute infection of bulk starter milk with bacteriophage was found to increase 1,000 fold after overnight incubation in the presence of the susceptible starter. Bacteriophage of mixed starter cultures retained their full strength on storage. Bulk starter was successfully protected from bacteriophage infection by the use of a special air-tight can with a small

opening plugged with cotton as suggested by Whitehead and Hunter (1941).

Nichols and Wolf (1942) demonstrated phage in cheese 3 wk. old and 3.5 mo. old made from a starter which had phaged. Phage was demonstrated in cheese made in seven different vats using three starters in two distant localities. In one of the older batches of cheese, phage was present in quantities of cheese as small as 0.00005 g.

Jensen (1943) presented direct evidence of the release of bacteriophage from starter bacteria by rennet. It was considered that some strains of lactic streptococci act as carriers of bacteriophage which they may release in the cheese vat.

Johns (1943) found evidence that the source of phage in a plant was the milk supply. Positive results in tests for bacteriophage also were obtained from the investigator's hands and from his saliva.

Nichols and Wolf (1944) found the concentration of phage in cheese to be fairly constant when tested periodically during 1 yr. The significance of such persistence on phage dispersal, its re-introduction into the dairy and carry-over from one season to the next was pointed out.

Hunter (1944) found the presence in the cheese milk of phage active against the organisms of the single strain culture in use as starter led to its multiplication during the cheese making process. The extent to which the presence of phage was made evident in the vat depended upon the amount of the initial infection. A heavy infection permitted sufficient multiplication of phage to cause lysis of the organism and consequent cessation of acid development before the manufacturing process was completed. A light infection might have no noticeable influence upon the performance of the starter in the vat. Intermediate amounts had an effect

on the rate of acid development in the later stages of the process but, within certain limits, there was little adverse influence on the quality of the cheese produced.

Whitehead and Hunter (1945) listed the following channels of infection of cheese milk: (a) Phage falls into cheese vat as a dust or droplet infection. (b) Whey separator or other equipment spreads droplet infection through the atmosphere. (c) Farmers' milk cans are contaminated by being used to carry home whey for feeding. Flash pasteurization does not destroy phage in contaminated milk. (d) Bulk starter culture may become infected. Dishes of sterile skim milk exposed for 10 min. as far as 25 yd. from a manufacturing building were found to contain phage. Phage also was demonstrated (a) 8 to 10 ft. from dripping whey, (b) in a room in which 1 gal. of phage preparation was splashed on concrete floor 24 hr. previously, and (c) in a cheese vat and the atmosphere the second day after resuming manufacture of cheese following 3 mo. suspension. Isolated starter rooms were recommended. The use of sealed vessels with small inlets plugged with cotton, the filtering of air into the building and keeping the pressure slightly greater on the inside also were advised.

Babel (1946) described the effect of bacteriophage on the manufacture of cheddar and cottage cheese at Iowa State College. Slow acid production in the manufacture of cheddar cheese usually was apparent at the time of draining the whey or shortly thereafter. The presence of bacteriophage resulted in almost complete cessation of acid production in the manufacture of cheddar cheese. Cheddaring for as long as 4 or 5 hr. did not result in an appreciable increase in acidity when acid production

was slow because of bacteriophage action. When a vat of skim milk intended for cottage cheese was contaminated slightly with bacteriophage active against a culture employed, acid formation was very slow for about 24 hr. after setting. During this same period the bacteria count showed a slight increase, then a large decrease and finally a large increase. The bacteriophage titer increased for 7 to 12 hr. following setting and then remained practically constant. Secondary growth usually occurred after 24 hr. and acid production proceeded normally.

Babel (1947) attempted to control difficulty in cheese manufacture from bacteriophage by the use of cultures that were not sensitive to the bacteriophage types responsible for the outbreak. Five cultures selected for study developed sensitivity to the bacteriophage after being used in the manufacture of cheese for 1-2 days. An additional culture developed sensitivity toward bacteriophage after approximately 3 mo. Still another culture was satisfactory after more than 6 mo.

Whitehead and Hunter (1947) found the phage titer of whey as drawn from the vat to range from 10^{-1} to 10^{-4}. The following morning it sometimes had a titer as high as 10^{-15}. Hunter and Whitehead (1949) reported a race of phage growing in symbiotic relationship with a culture of lactic streptococci but the phage was capable of attacking another culture of lactic streptococci. Hunter (1949) found that phage race and homologous organism react differently to a change in incubation temperature. It was therefore considered possible that a race of phage might be present and unobserved at one temperature but become very active with a change in temperature.

Nichols and Hoyle (1949) fed seven strains of lactic streptococci to pigs but were able to recover only one phage. They admitted that a phage may persist in a piggery or in the gut of a pig for some time, but that the claim of Mazé that the gut of the pig is a usual source of phage hardly could be sustained by their results. They were unable to obtain from cow dung any phages for 23 strains of Streptococcus cremoris. Although making numerous attempts, Nichols and Hoyle were unable to demonstrate any lysogenic strains of lactic streptococci. They admitted that a further search might yield a suitable indicator strain which might reveal a lysogenic strain.

Action of Glycols on Viruses and Bacteriophages

Reports in the literature on the effectiveness of glycol aerosols and vapors against certain viruses may lead one to expect that they would be very effective against air-borne bacteriophages also. No attempt has been made to present an exhaustive review of these reports but the following examples may be interesting.

Henle and Zellat (1941) stated that atomized propylene glycol aerosol reduced the chance of air-borne infection with a virus of influenza A and might be effective in preventing air-borne spread of the disease. They did not investigate the practicability of its use for this purpose.

All of 32 mice which Robertson et al. (1942) subjected to propylene glycol vapor in concentrations of 1 g. to 2,000,000 ml. of air and 0.39 ml. of a 10^{-2} dilution of influenza virus remained well. All of the 35 control mice subjected to the virus without the glycol vapor died.

Robertson et al. (1942) found that concentrations of 1 g. of propylene glycol vapor in 2,000,000 to 4,000,000 ml. of air produced immediate and complete sterilization of air into which pneumococci, streptococci, staphylococci, H. influenzae and other micro-organisms, as well as influenza virus, had been sprayed.

Stokes and Henle (1942), in experiments under controlled conditions, proved propylene glycol vapor more effective than ultraviolet irradiation in preventing air-borne infection of mice by influenza A virus. Propylene glycol was vaporized to a concentration in air of approximately 1:2,000,000 to 1:4,000,000 from an electric hot plate.

Propylene, triethylene, and dipropylene glycols were found suitable for practical use as aerosols by Robertson et al. (1943). A 2 to 3 ml. aluminum cup heated by a radio resistor carrying 15 to 20 volts was used to vaporize 0.1 to 0.2 ml. of triethylene glycol in 3 to 5 min. For 40 to 60 min. after the introduction of influenza virus in the air enough virus remained to kill 90 per cent of test mice. One g. of triethylene glycol to 200,000,000 ml. of air protected the mice.

Bigg, Jennings, and Olson (1945) observed that maintenance of concentrations of vaporized triethylene glycol between 0.0025 and 0.003 mg. per liter of air in test and control barracks of a military installation during the housing of approximately 1,000 men in each group produced a significant reduction in total bacteria count of the treated air, practical elimination of hemolytic streptococci in the treated air and a reduction of incidence of air-borne infections.

Rosebury et al. (1946) tested the effect of triethylene glycol vapor

on meningopneumonitis virus strain Cal 10 and psittacosis virus strain 6BC which had been sprayed in broth suspension into a closed cloud chamber. Triethylene glycol was nebulized or vaporized in a boiling water bath at relative humidities in the chamber between 55 and about 60 per cent. Reduction in virus concentration in the samples ranged from 0 to 93 per cent and averaged 62 per cent. Reduction of infective response in mice ranged from 55 to 98 per cent with an average of 73 per cent.

An in vitro test of glycol on vaccinia virus by Dunham and MacNeal (1943), for example, suggests the investigation of the action on bacteriophage in liquids also. The virus was inactivated by 70 per cent propylene glycol when exposed for 3 min. in watery suspension. On the other hand Tilley and Andersen (1947) found 100 per cent ethylene glycol ineffective against the virus of Newcastle disease.

Wolf, Nichols and Ineson (1946) reported inconclusive but apparently not very satisfactory results from propylene glycol mist in attempts to destroy air-borne lactic streptococcus bacteriophage.

Action of Hypochlorites and Chloramines on Bacteriophages

Wolf, Nichols and Ineson (1946) concluded from a series of tests that a fine mist produced by spraying 4 ml. of 9-12 per cent available chlorine solution for each 1,000 cu. ft. of air space gave protection from phage for 0.5 hr. when the humidity was well in excess of 50 per cent. Low humidities markedly reduced effectiveness.

Beckwith and Rose (1930) demonstrated that most strains of phage from sewage are resistant to chlorine *in vitro*. Diénert (1934) also found that bacteriophage from water contaminated with fecal matter resisted concentrations of 0.3 mg. of chlorine per liter.

Lactic streptococcus bacteriophage has been more readily affected by chlorine. Hunter and Whitehead (1940) found permanganate and active chlorine from hypochlorite were the most desirable agents for the destruction of bacteriophage of lactic streptococci in whey. Concentration of 0.05 per cent available chlorine was sufficient in a 1:2 dilution of whey to inactivate the bacteriophage in less than 1 min. when the protein content of the whey was standardized to 0.49 per cent. The phage preparations used varied in pH from 5.23 to 6.11.

Whitehead and Hunter (1947) found that 144 ppm. of available chlorine would prevent phage increase in whey free from curd but would not destroy the phage. One hundred and fifty ppm. caused reduction in phage titer in whey during 24 hr. storage. More than 200 ppm. were required to destroy low titer phages and over 500 ppm. of available chlorine for high titer phages. Over 600 ppm. of available chlorine were needed to eliminate phage from high titer whey. Destruction was judged by the more delicate lytic test.

Action of Quaternary Ammonium Compounds on Bacteriophages

Apparently no tests have been made of the effect of quaternary compounds in aerosols upon bacteriophages. A few tests in liquids have been reported.

Maier (1939) employed alkyldimethylbenzylammonium chloride solution

in the preservation of vaccine and venom solutions. A dilution of 1:50,000 did not interfere with the reproduction of staphylococcal bacteriophage. Reproduction of phage from such a mixture was obtained after 3 mo. of contact in the refrigerator.

In vitro tests with influenza A virus by Klein and Stevens (1945) revealed a definite viruscidal action with cetylpyridinium chloride, p-tertiaryoctylphenoxyethoxyethyldimethylbenzylammonium chloride and alkyldimethylbenzylammonium chloride. The compounds listed were viruscidal for the influenza A virus in 10 min. in dilutions ranging from 1:2,000 to 1:8,000, while dilutions of 1:500 to 1:2,000 gave a complete kill in 60 sec. Klein et al. (1945) found the same compounds to be viruscidal for vaccinia virus. Although the N(alkyl acid esters of colaminoformylmethyl)-pyridinium chloride showed but a suggestion of action against influenza virus, this compound was effective in concentrations of 1:4,000 against vaccinia virus. All three compounds proved lethal for Shigella dysenteriae and Staph. albus phages. None of them inactivated E. coli bacteriophage.

Kalter et al. (1946) described a method for the isolation of E. coli bacteriophage from sewage by means of cationic detergents. N-(alkyl acid ester of colaminoformylmethyl)-pyridinium chloride, ethyldimethylbenzylammonium chloride and cetylpyridinium chloride in final dilutions of 1:5,000 were recommended for the isolation of bacteriophage from sewage.

Prouty (1949) working at 20-22°C., added 0.1 ml. portions of bacteriophage filtrate to 100 ml. amounts of dilutions of quaternary ammonium

compound ranging from 200 to 5 ppm. At intervals of 2 min. continuing over a period of 20 min., 0.1 ml. portions of each test mixture were transferred to tubes containing 10 ml. of skim milk inoculated with 1 per cent inoculum of the homologous culture. The presence or absence of active bacteriophage was determined by incubating the 10 ml. tubes of the inoculated skim milk plus the treated filtrate at 30°C. The quaternary ammonium compounds tested were alkyldimethylbenzylammonium chloride, diisopropylphenoxyethyldimethylbenzylammonium chloride, diisobutylphenoxyethoxyethyldimethylbenzylammonium chloride, 9-octadecenyldimethylethylammonium bromide, N-(acylcolamineformylmethyl)-pyridinium chloride and lauryldimethylbenzylammonium chloride. One hundred ppm. of each of the compounds was considered sufficient to inactivate the bacteriophage in all trials at 2 min. exposure except alkyldimethylbenzylammonium chloride which did not inactivate in 2 min. in one out of 17 trials.

EXPERIMENTAL METHODS

Selection and Propagation of Cultures

The three strains of bacteriophage and the respective susceptible cultures of Streptococcus cremoris used in this study were selected from the collection maintained by the Dairy Industry Section of the Iowa Agricultural Experiment Station. Three bacteriophages, F10, F67, and F69, which were specific in their action as to strain of S. cremoris and also immunologically distinct were selected. They were classified in Groups II, IV and VI, respectively, by Wilkowske (1950). The respective susceptible cultures of S. cremoris were 122-1, 144F and ML1.

The litmus milk used for the estimation of numbers of phage particles was prepared from fresh skim milk fortified with 200 g. of nonfat dry milk solids and 1 liter of filtered V-8 vegetable juice to each 3 gal. of skim milk. The litmus milk was distributed in tubes in approximately 8 ml. quantities and autoclaved at 15 lb. pressure for 20 to 23 min. Unfortified skim milk for the preparation of whey filtrates was distributed in 6-oz. bottles with screw caps, 140 ml. per bottle, and autoclaved at 15 lb. pressure for 23 min.

Twelve- to 24-hr cultures of S. cremoris, prepared with one drop or one loopful of inoculum per tube, were used in all assays for bacteriophage. When the cultures had developed to the point of coagulating the

milk they were stored in a refrigerated room at approximately 4°C. The incubation temperature for this study was 32° C. in all cases.

Additional whey filtrates containing each strain of phage separately were prepared by inoculating 140 ml. of sterile skim milk with 1 ml. of the susceptible S. cremoris culture and 1 ml. of whey filtrate containing the phage, incubating for 6 to 12 hr.; acidifying with 5.5-6.0 ml. of sterile 10 per cent lactic acid solution and filtering. The milk was completely coagulated by the lactic acid and was allowed to stand for at least 30 min. before filtering. Filtration was first through sterile filter paper and then through a # 03 Selas microporous porcelain filter into a suction flask. All glassware, porcelain filters, rubber stoppers and rubber tubing used for the purpose were sterilized before use.

The pH of the filtrates was adjusted by the addition of sterile, approximately normal solution of sodium hydroxide and tested with a Leeds and Northrup hydrogen-ion potentiometer with calomel and quinhydrone electrodes, or a Beckman Model G or a Beckman Model M pH meter with glass electrodes. The filtrates were stored at approximately 4° C. New filtrates were prepared as needed or whenever the titer became unsatisfactorily low. In most of the trials tests were made on all three of the phages simultaneously. Equal volumes of the filtrates containing the respective phages were mixed and the pH of the mixture adjusted as desired.

Selection, Preparation and Testing of Solutions of Viruscidal Agents

Viruscidal agents which lacked objectionable odors and were reasonably economical were selected. Ethylene glycol, triethylene glycol, propylene glycol, a commercial product containing calcium hypochlorite, two chloramine-T products and 10 per cent industrial solutions of alkyldimethylbenzylammonium chloride, N-(acylcolaminoformylmethyl)-pyridinium chloride, 9-octadecenyldimethylethylammonium bromide, methyldodecylbenzyltrimethylammonium chloride and pure diisobutyl-(p-tertiaryoctyl)-phenoxyethoxyethyldimethylbenzylammonium chloride were included. Except when otherwise stated, solutions for actual use were prepared in distilled water. The water in all cases was sterilized for 30 min. in the autoclave at 15 lb. pressure before the addition of the viruscide.

Tap water of the city of Athens supply was used for diluting calcium hypochlorite, when tap water was indicated. The following approximate analysis of the supply was furnished by the city engineer:

Residual chlorine	0-0.35 ppm.
Hardness as $CaCO_3$	25 ppm.
Total dissolved solids	48 ppm.
Alkalinity to methyl orange	20-21 ppm.
pH	8.2

The strengths of solutions of hypochlorite and chloramine-T were tested immediately before use by titrating with 0.1 N sodium thiosulphate using a 2 per cent solution of soluble starch as indicator. One ml. of 0.1 N sodium thiosulphate was regarded as being equivalent to

0.003546 g. of available chlorine. Glycols of technical grade were used. Test solutions of them were prepared volumetrically.

The solutions of the quaternary ammonium compounds were prepared volumetrically on the basis of the manufacturers' stated strengths of industrial solutions (usually 10 per cent) or prepared gravimetrically from the solid compound. Subsequently, solutions calculated to contain 1,000 ppm. from each compound were prepared and tested by the method of Miller and Milliker (1950). Since diisobutyl (or p-tertiaryoctyl)-phenoxyethoxyethyldimethylbenzylammonium chloride was available in solid form it was taken as the reference material. The volumes of Fisher Laboratory Aerosol required in the titration of the solutions of alkyldimethylbenzylammonium chloride, N-(acylcolaminoformylmethyl)-pyridinium chloride, 9-octadecenyldimethylethylammonium bromide and methyldodecylbenzyltrimethylammonium chloride were 1.88, 1.02, 0.77 and 1.60, respectively, times the volume required in the titration of the solution of diisobutyl(or p-tertiaryoctyl)-phenoxyethoxyethyldimethyl-benzylammonium chloride. A late communication from the junior author stated that the titration values obtained with a standard anionic solution do not follow a molecular ratio with quaternary ammonium compounds and suggested that controls be set up for each compound to be tested. Since only one of these compounds was available in pure form the above results are only empirical evaluations of the strengths of these stock solutions and were not used as a basis of preparing solutions in this investigation.

Determination of Most Probable Numbers of Bacteriophage Particles

The most probable numbers of active bacteriophage particles in test liquids were determined by the three-tube limiting dilution technique described by Krueger (1930), Harriman (1934), Nelson et al. (1939) and Babel (1946). Since simultaneous tests were made on the three phages, triplicate sets of tubes were prepared from each test sample and its serial dilutions. One set was inoculated with culture 122-1, a second set with culture ML1 and a third set with culture 144 F. The inocula were one drop of 12- to 24-hr. culture per tube. Tubes failing to show normal coagulation and reduction of litmus after 12 to 16 hr. incubation, the same as occurred in the phage-free control tubes, were considered as containing active bacteriophage. The most probable numbers of particles per milliliter were obtained by referring to the table of Buchanan and Fulmer (1928) prepared for three samples of each dilution.

Infection of Air with Lactic Streptococcus Bacteriophages

Whey filtrates were atomized into a room approximately 7 ft. 10.5 in. x 12 ft. 6.5 in. x 13 ft. 10 in. high. It had a smooth concrete floor and the plastered walls were painted with enamel. The length of the room ran approximately east and west. A 3 x 7 ft. door was located in the east wall near a corner of the room. A window approximately 3 x 7 ft. was located in the north wall in the eastern half. A steam radiator was located just under the window. The room was disinfected when necessary

to clear out infection from previous trials by spraying a liberal amount of chlorine disinfectant of high concentration or flushing all horizontal surfaces with a high concentration of quaternary ammonium compound in water. The room was kept closed during each trial, except as it became necessary to go in or out of the room to conduct portions of the experiment.

A metal work table with stainless steel top approximately 30 x 72 x 30 in. high was placed with an end against the west wall about midway between the north and south walls of the room. A 12-in. Emerson oscillating electric fan was set about midway along the south side of the table facing northward. The fan was tilted backward on the stand as far as possible and still allow the operation of the oscillator mechanism. Unless otherwise stated the fan was operated continuously throughout each trial.

The atomized filtrates for infecting the room were discharged from one of three DeVilbiss No. 127 atomizers 18-24 in. above the revolving electric fan. Another of the atomizers was used for dispersing the viruscidal solutions. The atomizers were cleaned and heated in flowing steam for one hour or immersed in distilled water to minimize heat shock of the thick glass reservoirs and autoclaved at 15 lb. pressure for 30 min. previous to each test. The atomizers were used interchangeably for atomizing both the filtrates and the viruscidal solutions. The capacity of the reservoir of each atomizer was slightly more than 20 ml. When larger volumes of liquids were handled, it was necessary to

refill the reservoir. Air pressure of 20-25 lb. per in.^{2} was supplied to the atomizers by an air compressor pump. Dry bulb and wet bulb temperatures of the room were taken with a Mason hygrometer and the relative humidities were determined from these temperatures by use of the relative humidity tables of the Taylor Instrument Co., reprinted by Lange (1946).

Measurement of Persistence of Active Lactic Streptococcus Bacteriophage in the Air

A 35 x 200 mm. test tube was used for sampling air. It was fitted with a rubber stopper through which two 15 mm. diameter glass tubes for inlet and outlet were inserted. The inlet extended nearly to the bottom of the test tube and the outlet tube just through the rubber stopper. Both tubes extended straight outward for distances of 8 to 20 cm. A sufficient quantity of glass beads approximately 3-5 mm. in diameter was placed in the tube so that they came approximately to the surface of the water when 25 ml. of distilled or buffered water was placed in the tube. The test tube containing the beads, rubber stopper, and the two 15 mm. diameter glass tubes was sterilized in the autoclave at 15 lbs. pressure for 30 min. After sterilization and cooling, 25 ml. of distilled water or of sterile 1/300 M phosphate buffer solution at pH 7.2 were measured into the sample tube. The outlet tube was connected by a sterile rubber tube to a 50 gal. aspirator located just outside the door of the room. The test tube was held in

an upright position by a clamp on a stand located on the east end of the metal work table. The glass inlet and outlet tubes of the sampler were protected against contamination during handling by short pieces of rubber tubing plugged on the opposite ends with cork stoppers.

A 50 gal. (6.7 ft.^{3}) aspirator for drawing air through the sample tube was made from a steel drum lying in a horizontal position and elevated about 6 ft. from the floor level to the bottom of the drum. The rubber tubing from the air sample tube was connected to the drum by means of a glass tube inserted in a rubber stopper in the middle of the upper side of the drum. The lower side of the east end of the drum was fitted with a section of 0.75 in. diameter water pipe and a 0.75 in. globe valve. A piece of rubber creamery hose was placed on the end of the water pipe and served to carry the water to the floor drain in the room adjoining the test room. A 6.7 ft.^{3} volume of air was sampled with this device in approximately 7 min.

Open sterile petri dishes were used to collect the bacteriophage settling from the air. One set of dishes on one end of the table was left open from the beginning of the atomizing of the filtrate containing the phage and individual dishes were closed after varying periods of exposure. Another set of dishes on the opposite end of the table was opened one by one for 7-min. periods after certain intervals following the atomizing of the whey filtrate. Certain dishes were rinsed with 20 ml. of sterile distilled water and the rinse water assayed for bacteriophage content. The majority of the dishes were rinsed with

20 ml. of 1/300 M phosphate buffer solution at pH 7.2, sterile rubber policemen being used for scrubbing the inside surfaces of the dishes.

Polished stainless steel discs approximately 90 mm. in diameter were placed in sterile petri dishes for setting tests also. After exposure 20 ml. of 1/300 M phosphate buffer at pH 7.2 were added to each petri dish containing a stainless steel disc and the surface was scrubbed thoroughly with a rubber policeman. Rinse water was assayed for bacteriophage content.

In order to test the effect of atomizing upon the activity of the bacteriophage, 20 ml. of the F69 phage filtrate were atomized into a 2-liter Erlenmeyer flask. The filtrate was tested for phage content before being atomized and also after collection in the flask following atomization. Similar tests also were made on filtrates diluted 10^{-2} and 10^{-4}. The results shown in Table 1 indicate that recovery of the atomized phages was about 10 per cent in 10 out of 39 comparisons but was 100 per cent or more in 27 of the comparisons. The recoveries in excess of 100 per cent may be explained by the breaking up of clumps of phage particles in the atomizing or by normal variations inherent in the method of assay.

In order to measure the possible effect of bubbling air through phage filtrates, air was drawn for 10 min. through undiluted filtrate and through diluted filtrates. The numbers of phage particles were determined in the filtrates before and after the bubbling with air.

TABLE 1

EFFECT OF ATOMIZING 20-ML. SAMPLES OF WHEY FILTRATES CONTAINING LACTIC STREPTOCOCCUS BACTERIOPHAGES INTO 2-LITER ERLENMEYER FLASKS

Filtrate	Mpn./ml. of phage particles on undiluted basis		
	F10	F67	F69
Undiluted, unatomized			4,500,000
Undiluted, atomized			450,000
Diluted 10^{-2}, atomized			450,000
Diluted 10^{-4}, atomized			950,000
pH 4.7			
Undiluted, unatomized	250,000	450,000,000	110,000
Undiluted, atomized	25,000	45,000,000	110,000
Diluted 10^{-2}, atomized	25,000	25,000,000	250,000
Diluted 10^{-4}, atomized	45,000	11,000,000	250,000
Undiluted, unatomized	250,000	45,000,000	110,000
Undiluted, atomized	250,000	1,100,000,000	250,000
Diluted 10^{-2}, atomized	25,000	75,000,000	150,000
Diluted 10^{-4}, atomized	25,000	110,000,000	250,000
pH 5.9			
Undiluted, unatomized	2,500,000	25,000,000	250,000
Undiluted, atomized	2,500,000	25,000,000	250,000
Diluted 10^{-2}, atomized	2,500,000	110,000,000	250,000
Diluted 10^{-4}, atomized	450,000	450,000,000	250,000
pH 6.65			
Undiluted, unatomized	1,100,000	25,000,000	1,100,000
Undiluted, atomized	1,100,000	150,000,000	4,500,000
Diluted 10^{-2}, atomized	1,100,000	110,000,000	2,500,000
Diluted 10^{-4}, atomized	2,500,000	150,000,000	1,100,000

The results shown in Table 2 indicate complete recovery of bacteriophage after air had been bubbled through the undiluted filtrate. In only two out of 10 comparisons were there approximate ten-fold reductions in numbers in the dilutions of filtrates as a result of the treatment. Other changes in numbers which appear to result from the treatment may be explained by the breaking up of clumps of particles and normal variations which may occur in the assay procedure.

Areas of 4 $in.^2$ in various parts of the room were tested for the presence of active bacteriophage by dipping sterile cotton swabs in 4 ml. of sterile distilled water, sponging the areas, returning the swabs to the water and assaying the water for active bacteriophage. Attempts also were made to get active bacteriophage back into the air from the floor by sweeping vigorously with a dry broom. The air was then sampled and tested by the methods described above.

Determination of Action of Certain Aerosols on Lactic Streptococcus Bacteriophage

Air infected with bacteriophage in the manner described above was treated with aerosols of ethylene glycol, triethylene glycol, propylene glycol, and solutions of calcium hypochlorite, chloramine-T and alkyldimethylbenzylammonium chloride. The aerosols were made by atomizing the liquids with DeVilbiss No. 127 atomizers in the same manner as the filtrates were atomized. The air was treated at various intervals before, during and after infection with bacteriophage.

TABLE 2

EFFECT OF BUBBLING AIR FOR 10 MIN. THROUGH WHEY FILTRATES CONTAINING LACTIC STREPTOCOCCUS BACTERIOPHAGES

Filtrate	Mpn./ml. of phage particles-undiluted basis F10	F67	F69
Undiluted, untreated			4,500,000
Undiluted, treated			4,500,000
Diluted 10^{-4}, treated			9,500,000
pH 6.65			
Undiluted, untreated	200,000	110,000,000	1,100,000
Diluted 10^{-2}, treated	110,000	250,000,000	250,000
Diluted 10^{-3}, treated	250,000	11,000,000	250,000
Diluted 10^{-4}, treated	250,000	25,000,000	250,000
Undiluted, untreated	1,100,000	25,000,000	1,100,000
Diluted 10^{-2}, treated	1,100,000	110,000,000	2,500,000
Diluted 10^{-3}, treated	450,000	45,000,000	2,500,000
Diluted 10^{-4}, treated	115,000	30,000,000	2,500,000

Air samples were taken and tests of bacteriophage settling out of the air also were made by the methods described above. In addition, a series of petri dishes were open for successive 5-min. periods following the treatment of the air with calcium hypochlorite aerosol. In the trials of aerosols containing available chlorine, the air sampling tubes were charged with 25 ml. of a solution containing 3.2 mg. of sodium thiosulphate and 1/3200 M phosphate buffer. The reaction of the solution was adjusted to pH 7.2. The petri dishes each contained 20 ml. of the same solution. Buffered water without inhibitors was used in testing the action of the glycols and alkyldimethylbenzylammonium chloride.

Determination of Action of Certain Viruscidal Agents on Lactic Streptococcus Bacteriophages in Liquids

The action of viruscidal agents on bacteriophages in liquids was studied mostly in the dairy laboratories at the University of Georgia. A modification of the laboratory procedure proposed by Weber and Black (1948) for evaluating practical performance of quaternary ammonium compounds and other germicides proposed for sanitizing food utensils was followed.

Six to 10 ml. of the test solution of the viruscidal agent in double strength was placed in a 25 x 150 mm. test tube and covered with a 30 x 50 mm. glass or aluminum cap. Distilled water for preparing test solutions, the metal caps and all glassware used

in this group of trials were autoclaved at 15 lb. pressure for 30 min. Five ml. quantities of phage filtrate or diluted phage filtrate were carefully transferred to another 25 x 150 mm. tube, avoiding touching the sides of the tube in transfer. This tube also was covered with a 30 x 50 mm. glass or aluminum cap. Both capped tubes were placed in a 400 ml. beaker of water for obtaining and maintaining the test temperature.

The warmed tube containing the phage was set in a 2 oz. glass jar in a vertical position out of the water bath. With a 5 ml. tip-delivery bacteriological pipette with an aperture diameter of about 2 mm., 5 ml. of the viruscidal solution was transferred to the tube containing the phage. The contents of the pipette were discharged quickly by blowing them into the tube. As the transfer was begun a stop watch with a sweep second hand was started. The pipette was discarded quickly and the contents of the tube swirled vigorously. With an APHA 1.1 ml. bacteriological pipette, 1 ml. of the treated filtrate was removed from the tube and quickly discharged into a 9 ml. inhibitor blank prepared as described below at 15 sec. The inhibitor tube was swirled. Additional transfers of 1 ml. of treated filtrate each were made at 30 sec. and at 60 sec. The tube containing the treated filtrate then was returned to the water bath. A fourth transfer of 1 ml. of treated filtrate to a 9-ml. inhibitor blank was made at 120 sec. and a fifth transfer at 300 sec. Each tube containing the inhibitor blank was swirled after the addition of the 1 ml. portion.

Nine-ml. inhibitor blanks were prepared in 25 x 150 mm. Pyrex tubes which were plugged with cotton. The solution for preparing the 9-ml. inhibitor blanks to be used in testing 50 ppm. of available chlorine in hypochlorite or chloramine-T contained 80 mg. of sodium thiosulphate ($Na_2S_2O_3 \cdot 5 H_2O$) and 1.25 ml. of stock M/4 phosphate buffer made up to 1 liter and adjusted to pH 7.2. For higher concentrations of available chlorine, the concentration of sodium thiosulphate was increased proportionately.

The solution for prearing the 9-ml. inhibitor blanks as used for testing 200 ppm. quaternary ammonium solutions contained 2.222 g.. of asolectin, 15.6 ml. Tween 80 and 1.25 ml. stock M/4 phosphate buffer per liter and was adjusted to pH 7.2. For testing higher concentrations of quaternary ammonium compounds the asolectin and Tween 80 were increased proportionately. Both types of inhibitor solutions were sterilized in the autoclave for 20 min. at 15 lb. pressure.

In order to have sufficient volume of material for assay, 10 ml. of sterile distilled water were pipetted into each 9-ml. inhibitor tube to which 1 ml. of treated filtrate had been added. The contents of each tube then were assayed for each strain of bacteriophage by the method described above and the results doubled to compensate for the final water dilution. The most probable numbers of phage particles in controls were calculated from the titers of the stock phage preparations.

The pH of the original and treated filtrates was determined with a Beckman Model G pH meter with glass electrodes or a Leeds and Northrup hydrogen-ion potentiometer with calomel and quinhydrone electrodes.

RESULTS

Persistence of Lactic Streptococcus Bacteriophages in Infected Air

Results obtained in recovering the bacteriophages from the air by aspiration and by deposition on petri dishes when the room was infected with filtrate having a pH of 4.85 are presented in Table 3. The percentage of active particles recovered by aspiration during the second period, 104-111 min.after infection began, ranged from approximately 0.1 per cent in the case of F67 phage to 10 per cent in the case of F69 phage as compared with the numbers recovered during the period 10-17 min. after infection began. F10 and F67 phages were not recovered by aspiration 210 min. or longer after infection and F69, 670 min. or longer after infection. In the dishes exposed continuously the numbers of active particles tended to increase rapidly to a peak and then to decline at a more or less rapid rate. The peak number of F10 particles was obtained 17 min. after infection and the peak number of F67 and F69 particles, 111 min. after infection, disregarding the high count of F67 particles at 677 min. which is irregular and cannot be explained. The examination of the dishes exposed for 7-min. periods showed the largest numbers of active particles in the dish for the first period, 3- to 16-fold smaller numbers in the dish for the second period and from none to about 0.05 per cent of the initial numbers in the dishes

TABLE 3

BACTERIOPHAGE RECOVERY FROM ROOM INFECTED WITH ATOMIZED WHEY FILTRATE AT pH 4.85 CONTAINING LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period sample taken after infection began (min.)	Mpn./ft.^{3} of phage particles recovered from the air by aspiration through distilled water		
	F10	F67	F69
10- 17	940,000	940	94,000
104-111	3,600	1.1	9,400
*210-217	0	0	1,700
272-279	0	0	36
670-677	0	0	0
Period dish open after infection began (min.)	**Mpn./ft.^{2} of phage particles recovered by rinsing dishes with distilled water**		
	F10	F67	F69
0- 7	3,000,000	6,400	140,000
0- 17	64,000,000	1,100	110,000
0-111	3,000,000	11,000	300,000
*0-217	1,100	110	180,000
0-279	1,800	110	89,000
0-677	4.7	30,000	110
0- 7	3,000,000	6,400	140,000
10- 17	180,000	1,800	30,000
104- 111	640	0	24
*210- 217	0	4.7	30
272- 279	64	0	13
670- 677	0	0	0
677- 4 d.	0	2,400	30
677- 4 d.	0	4.7	0

60 ml. of whey filtrate containing 450,000, 15,000,000 and 25,000,000 particles/ml. of F10, F67 and F69 phages respectively were atomized into the room. Time required for atomizing was 7 min. Room temperature was 84-85°F. and relative humidity, 75-80%. Trial was made July 7-14, 1949.

*Oscillator mechanism of fan ceased operating between 111 and 210 min. after infection began and was not started again during trial.

for the later periods. The reason for the 2,400 particles of F67 phage collected during the interval from 677 min. to 4 days may be the long exposure, although the number is so large as to minimize this possibility.

Results of a similar test in which the pH of the filtrate was 4.45 and stainless steel discs were placed in the petri dishes as collection surfaces are shown in Table 4. The trends of the data are very much the same as those in Table 3, except that the peak in the numbers on the discs exposed continuously is not nearly as distinct as when dishes were used as in Table 3. Numbers varying from maximum to one-third of maximum persisted in the dish for the 0-497 min. period. During the 56-min. interval between the first and second periods the numbers of phage particles recoverable by aspiration decreased to approximately 0.3 to 1 per cent of the numbers recovered in the first period. No F10 particles were recovered after 131 min. and no F67 or F69 particles were recovered after 259 min. Approximately three- to nine-fold decreases in numbers of particles recovered occurred in the 8-min. interval between the first and second 7-min. exposures of the discs. After 495 min. no active phage particles were recovered on the discs during 7-min. exposures. Longer period of exposure may explain the recovery of the 300+ particles of F67 and F69 phages on the disc exposed all during the 497-1220 min. period.

The trial reported in Table 5 differed from that in Table 4 in that the pH of the filtrate was 5.6 and three petri dishes also were exposed during the 0-257, 0-1210 and 506-1210 min. periods. The trends

TABLE 4

BACTERIOPHAGE RECOVERY FROM ROOM INFECTED WITH ATOMIZED WHEY FILTRATE AT pH 4.45 CONTAINING LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period sample taken after infection began (min.)	Mpm./ft^3 of phage particles recovered from the air by aspiration through buffered distilled water		
	F10	F67	F69
10- 17	17,000	9,400	940,000
73- 80	110	110	2,800
131-138	0	1.1	36
259-266	0	0	0
490-497	0	0	0
Period disc exposed after infection began (min.)	**Mpm./ft.^{2} of phage particles recovered by rinsing stainless steel discs with buffered distilled water**		
	F10	F67	F69
0- 7	30,000	30,000	300,000
0- 17	11,000	30,000	53,000
0- 80	3,000	11,000	300,000
0-138	30,000	11,000	30,000
0-266	30,000	130,000	30,000
0-497	30,000	530,000	110,000
0-1220	5,300	480	11,000
0- 7	30,000	30,000	300,000
15- 22	350	11,000	110,000
78- 85	11	0	180
136-143	4.8	0	4.8
264-271	4.8	0	0
495-502	0	0	0
497-1220	0	300+	300+

60 ml. of whey filtrate containing 250,000,000, 25,000,000 and 110,000,000 particles/ml. of F10, F67 and F69 phages respectively were atomized into the room. Time required for atomizing was 5 min. Room temperature was 82°F. and relative humidity, 80-84%. Trial was made July 25, 1949.

TABLE 5

BACTERIOPHAGE RECOVERY FROM ROOM INFECTED WITH ATOMIZED WHEY FILTRATE AT pH 5.6 CONTAINING LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period sample taken after infection began (min.)	Mpm./ft.^{3} of phage particles recovered from the air by aspiration through buffered distilled water		
	F10	F67	F69
10- 17	1,700,000	-	-
72- 79	17,000	940	17,000
130- 137	9.4	940	3,600
250- 257	17	94	360
499- 506	0	0	0
Period dish open after infection began (min.)	**Mpm./ft.^{2} of phage particles recovered by rinsing stainless steel discs with buffered distilled water**		
	F10	F67	F69
0- 7	300,000	300,000	300,000
0- 17	300,000	110,000	1,100,000
0- 79	300,000	30,000	300,000
0- 137	180,000	11,000,000	530,000
0- 257	110,000	300,000	300,000
0- 257	*300,000	*300,000	* 53,000
0- 506	180,000	110,000	530,000
0- 1210	30,000	30,000	53,000
0- 1210	* 30,000	*11,000	* 89,000
0- 7	300,000	300,000	300,000
10- 17	3,000	3,000	30,000
72- 79	24	1,100	300
130- 137	4.8	30	300
250- 257	0	0	4.8
499- 506	0	0	0
506- 1210	0	0	0
506- 1210	* 0	* 0	* 0

60 ml. of whey filtrate containing 110,000,000, 25,000,000 and 250,000,000 particles/ml. of F10, F67 and F69 phages respectively were atomized into the room. Time required for atomizing was 5 min. Room temperature was 81-82°F. and relative humidity, 80-91%. Trial was made July 27, 1949.

*Petri dish alone without disc exposed for collection.

in the numbers obtained by aspiration and from continuously exposed disc samples are practically the same as in Table 4. No phage particles were recovered in samples from discs exposed for 7-min. periods after 499 min. or from the disc or dish exposed during the 506-1210 min. period. Five of the counts from the corresponding disc and dish samples were the same, the counts from the discs were greater twice, the counts from the dishes greater twice and the maximum difference in any instance was less than three-fold.

Petri dishes alone instead of discs were used in the trials reported in Tables 6 and 7. The pH of the filtrates was 5.5. The trends in the data seem to be essentially the same as those in Table 5. However, in Table 6 no reduction in F69 particle numbers occurred in the second aspiration sample and 28 F69 particles per $ft.^3$ were recovered during the 423-430 min. period. Also, in the same table, 4.8 F10 and 3.5 F69 particles per $ft.^2$ were recovered from the 1569-1576 min. dish.

The data from two similar trials using filtrates with a pH of 6.55 are presented in Tables 8 and 9. No consistent differences are apparent in the trends of these data and of those data from trials with filtrates having lower levels of pH. These data agree with those in Tables 3-7, inclusive, with the exception of parts of Tables 4 and 6, in indicating greater persistency of F69 particles than of F10 or F67 particles. The exceptions to this agreement with other data from Table 4 are the recovery of 4.8 particles of F10 phage per $ft.^2$ and no F69 particles on the disc for the 264-271 min. period and the recovery of possibly as many F67 particles as of F69 on the disc for the 497-1220

TABLE 6

BACTERIOPHAGE RECOVERY FROM ROOM INFECTED WITH ATOMIZED WHEY FILTRATE AT pH 5.5 CONTAINING LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period sample taken After infection began (min.)	Mpn./ft.^{3} of phage particles recovered from the air by aspiration through buffered distilled water		
	F10	F67	F69
10- 17	250,000	1,700,000	940,000
70- 77	450	36,000	940,000 +
423- 430	0	0	28
Period dish open after infection began (min.)	**Mpn./ft.^{2} of phage particles recovered by rinsing dishes with buffered distilled water**		
	F10	F67	F69
0- 7	5,300,000	3,000,000	3,000,000
0- 17	30,000,000	5,300,000	5,300,000
0- 77	3,000,000	3,000,000	11,000,000
0- 430	30,000 +	30,000 +	1,100,000
*0- 7230	300 +	25 +	300 +
0- 7	5,300,000	3,000,000	3,000,000
10- 17	110,000	110,000	1,100,000
70- 77	530	300	2,400
423- 430	53	24	110
441- 1576	30,000	30,000	30,000
1569- 1576	4.8	0	3.5

60 ml. of whey filtrate containing 200,000,000, 150,000,000 and 450,000,000 particles/ml. of F10, F67 and F69 phages were atomized into the room. Time required for atomizing was 7 min. Room temperature was 79-80°F. and relative humidity, 59-68%. Trial was made July 12, 1949.

*Fan was stopped 1576 min. after infection began.

TABLE 7

BACTERIOPHAGE RECOVERY FROM ROOM INFECTED WITH ATOMIZED WHEY FILTRATE AT pH 5.5 CONTAINING LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period sample taken after infection began (min.)	Mpn./ft.^{3} of phage particles recovered from the air by aspiration through buffered distilled water		
	F10	F67	F69
11- 18	560,000	56,000	1,500,000
71- 78	110	3,600	94,000
131- 138	9.4	9.4	940
254- 261	94	170	4,100
1212-1217	0	0	0
Period dish open after infection began (min.)	**Mpn./ft.^{2} of phage particles recovered by rinsing dishes with buffered distilled water**		
	F10	F67	F69
0- 7	890,000	35,000	480,000
0- 18	3,000,000	83,000	3,000,000
0- 78	530,000	530,000	530,000
0- 138	890,000	190,000	1,800,000
0- 261	1,400,000	35,000	1,100,000
0-1217	8,900	1,800	3,000,000 +
0-4081	530	480	3,000
0- 7	890,000	35,000	480,000
11- 18	53,000	1,300	110,000
71- 78	300	300	530
131- 138	300	300	13,000
254- 261	530	300	890

53 ml. of whey filtrate containing 15,000,000, 9,500,000 and 150,000,000 particles/ml. of F10, F67 and F69 phages respectively were atomized into the room. Time required for atomizing was 6 min. Room temperature was 79-80°F. and relative humidity, 57-64%. Trial was made July 22, 1949.

TABLE 8

BACTERIOPHAGE RECOVERY FROM ROOM INFECTED WITH ATOMIZED WHEY FILTRATE AT pH 6.55 CONTAINING LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period sample taken after infection began (min.)	Mpn./ft.^{3} of phage particles recovered from the air by aspiration through buffered distilled water		
	F10	F67	F69
10- 17	940,000	940,000	940,000
73- 80	94	9,400	9,400
130- 137	2,400	9,400	41,000
259- 266	0	2.2	94
490- 497	0	0	94 +

Period dish open after infection began (min.)	Mpn./ft.^{2} of phage particles recovered by rinsing dishes with buffered distilled water		
	F10	F67	F69
0- 7	1,100,000	5,300,000	3,000,000
0- 17	5,300,000	3,000,000	3,000,000
0- 80	1,100,000	890,000	3,000,000
0- 137	1,100,000	300,000	1,100,000
0- 266	1,100,000	3,000,000	3,000,000
0- 497	530,000	1,100,000	3,000,000
0-1217	180,000	89,000	1,300,000
0- 7	1,100,000	5,300,000	3,000,000
10- 17	110,000	300,000	180,000
73- 80	530	300	1,100
130- 137	18	110	890
259- 266	11	110	300 +
490- 497	30	53	300 +
497-1217	0	0	0

60 ml. of whey filtrate containing 25,000,000, 9,500,000 and 450,000,000 particles/ml. of F10, F67 and F69 phages respectively were atomized into the room. Time required for atomizing was 6 min. Room temperature was 80°F. and relative humidity, 79-87%. Trial was made July 21-22, 1949.

TABLE 9

BACTERIOPHAGE RECOVERY FROM ROOM INFECTED WITH ATOMIZED WHEY FILTRATE AT pH 6.55 CONTAINING LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period samples taken after infection began (min.)	Mpn./ft.^{3} of phage particles recovered from the air by aspiration through buffered distilled water		
	F10	F67	F69
10- 17	940,000 +	1,700	940,000 +
70- 77	150	1,700	410,000
250- 257	94	94	9,400 +
490- 497	1.1	11	36
1930-1937	0	0	1.5
Period dish open after infection began (min.)	**Mpn./ft.^{2} of phage particles recovered by rinsing dishes with buffered distilled water**		
	F10	F67	F69
0- 7	53,000,000	1,300,000	5,300,000
0- 17	5,300,000	1,100,000	5,300,000
0- 77	3,000,000	180,000	3,000,000
0- 257	3,000,000 +	130,000	3,000,000 +
0- 497	5,300,000	1,300,000	5,300,000
0-1937	300,000	30,000	300,000
0-4810	5,300	30,000	300,000
0- 7	53,000,000	1,300,000	5,300,000
10- 17	48,000	18,000	530,000
70- 77	530	110	2,400
250- 257	300	53	530
490- 497	300 +	300	530
1930-1937	4.8	4.8	18
4803-4810	0	0	0

60 ml. of whey filtrate containing 1,100,000,000, 11,000,000 and 450,000,000 particles/ml. of F10, F67 and F69 phages respectively were atomized into the room. Time required for atomizing was 4 min. Room temperature was 79-82°F. and relative humidity, 54-61%. Trial was made July 15-18, 1949.

period. In Table 6, as many or more of one or the other phages as of F69 were recovered from the dishes for the 0-7230 min., 441-1576 min., and 1569-1576 min. periods.

Tables 10 and 11 show data on trials differing from those of previous trials in the intervals of sampling. These results emphasize the fact that the largest numbers of active particles settled out of the air and/or became inactive during the first 30-40 min. period after the phages were introduced into the air. The counts of the air samples for the 30-37 and 40-47 min. periods ranged from 0.5 to 48 per cent and averaged 15 per cent of the counts for the 10-17 min. periods. The counts of the dish samples for the 30-60 min. and 40-70 min. periods ranged from 0.0006 to 1.0 per cent and averaged 0.6 per cent of the counts for the 0-30 and 0-40 min. periods. In the four cases on which definite comparisons can be made, the counts for the 60-67 min. and 70-77 min. periods ranged from 0.02 to 3.7 per cent of the counts of the particles recovered in the dishes for the 10-17 min. periods.

On Apr. 16, 20 ml. of filtrate containing 250,000 particles per milliliter of F69 phage were atomized into the test room. The temperature at the time of atomizing and of sampling was 72° F. The relative humidity at the time of atomizing was 33 per cent and at the time of sampling was 73 per cent. On Apr. 19, 2.6 phage particles per $ft.^3$ were recovered from the still room by aspiration. Sampling by aspiration was repeated immediately while the floor was dry mopped to stir up dust. The recovery was 940 phage particles per $ft.^3$

TABLE 10

BACTERIOPHAGE RECOVERY FROM ROOM INFECTED WITH WHEY FILTRATE AT pH 6.3 CONTAINING LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period sample taken after infection began (min.)	Mpn./ft.^{3} of phage particles recovered from the air by aspiration through buffered distilled water		
	F10	F67	F69
10- 17	3,600,000	75,000	940,000
40- 47	17,000	36,000	56,000
70- 77	36,000	15,000	56,000
Period dish open after infection began (min.)	**Mpn./ft.^{2} of phage particles recovered from rinsing dishes with buffered distilled water**		
	F10	F67	F69
0- 40	1,100,000	1,800,000	3,000,000
40- 70	11,000	8,900	30,000
10- 17	53,000	300,000	53,000
40- 47	530	11,000	5,300
70- 77	530	530	300

60 ml. of whey filtrate containing 75,000,000, 115,000,000 and 250,000,000 particles/ml. of F10, F67 and F69 phages respectively were atomized into the room. Time required for atomizing was 7 min. Room temperature was 82° F. and relative humidity, 61-65%. Trial was made Aug. 15, 1949.

TABLE 11

BACTERIOPHAGE RECOVERY FROM ROOM INFECTED WITH ATOMIZED WHEY FILTRATE AT pH 6.3 CONTAINING LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period sample taken after infection began (min.)	Mpn./ft.^{3} of phage particles recovered from the air by aspiration through buffered distilled water		
	F10	F67	F69
10- 17	940,000	1,500,000	3,600,000
30- 37	75,000	170,000	2,800
60- 67	1,700	9,400	75,000
Period dish open after infection began (min.)	**Mpn./ft.^{2} of phage particles recovered by rinsing dishes with buffered distilled water**		
	F10	F67	F69
0- 30	1,100,000	5,300,000	3,000,000
30- 60	30	30	30
10- 17	⌊48,000	⌊48,000	300,000
30- 37	11,000	11,000	30,000 +
60- 67	530	530	11,000

60 ml. of whey filtrate containing 75,000,000, 115,000,000 and 250,000,000 particles/ml. of F10, F67 and F69 phages respectively were atomized into the room. Time required for atomising was 8 min. Room temperature was 81-82°F. and relative humidity, 65-72%. Trial was made August 16, 1949.

On June 28, 60 ml. of filtrate containing 45,000, 95,000 and 250,000 particles per milliliter of F10, F67 and F69 phages, respectively, were atomized into the room. The room temperature was 79° F. at the time of atomizing and at the time of sampling and the relative humidity, 79 per cent. On the following day, no phage was recovered by aspiration although the floor was dry mopped and the fan operated during the sampling.

On June 30, 1949, 60 ml. of filtrate containing 250,000,000, 450,000 and 450,000,000 particles per milliliter of F10, F67 and F69 phages, respectively, were atomized into the room. The temperature at the time of atomizing and sampling was 80-81° F. and the relative humidity 72-74 per cent. On Jul. 2, the floor was dry mopped vigorously while an air sample was taken by aspiration. No phage was recovered. Immediately afterward, nine samples were taken by swabbing areas on the window sill, the door, a lacquered metal drum top, the pump base, the west and south walls at low levels and three widely separated sections of the floor. The recovery of F10 phage particles was 0 per ft.^{2} from three areas and 360 to 6,500 per ft.^{2} from six areas. From 3,600 to 36,000 + F67 particles per ft.^{2} were recovered. The recovery of F69 phage particles was 0 per ft.^{2} from two areas and 360 to 36,000 per ft.^{2} from seven areas. There was no apparent correlation between the kinds or positions of surfaces and the numbers of phage particles recovered.

Action of Aerosols of Certain Viruscidal Agents on Lactic Streptococcus Bacteriophages

Glycols

The effect of ethylene glycol aerosol on the bacteriophages in the infected room in two trials is indicated by the data presented in Tables 12 and 13. The ratios of volumes of glycol to the volume of the room were approximately 1:1,900,000 and 1:650,000 or 15 ml. and 44 ml. per 1,000 ft.^{3} respectively. The numbers of phage particles recovered apparently were normal for an untreated room.

Table 14 shows the results of a similarly ineffective treatment of the infected room with an aerosol of 1 ml. of propylene glycol in 1,900,000 ml. of air (15 ml. per 1,000 ft.^{3}). Treatment of the infected room with an aerosol of 1 ml. of triethylene glycol in 650,000 ml. of air (44 ml. per 1,000 ft.^{3}) likewise was ineffective as shown in Table 15.

Calcium hypochlorite and chloramine-T

The effects of calcium hypochlorite aerosols in the ratio of 1 g. of available chlorine to 155,000,000 ml. (0.18 g. per 1,000 ft.^{3}) of air are shown in the results of three trials in Tables 16, 17 and 18. From 0 to 30 active phage particles per square foot were recovered from the dishes exposed during the interval from 3-4 min. before infection to 22-27 min. after infection began. Numbers of particles averaging much lower than normal for the untreated room were recovered by the other samplings, except following the second infection in Table 16.

TABLE 12

EFFECT OF ETHYLENE GLYCOL AEROSOL ON NUMBERS OF PHAGE PARTICLES RECOVERED FROM ROOM INFECTED WITH LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period sample taken after infection began (min.)	Mpn./ft.^{3} of F69 phage particles recovered from the air by aspiration through distilled water
10- 17	17,000
40- 47	940

20 ml. of whey filtrate containing 250,000 particles/ml. of F69 phage were atomized into the room. 20 ml. of ethylene glycol were dispersed into the room 30 min. after infection of the room began. Room temperature was 72-75° F. and relative humidity, 38-58%. Trial was made April 9, 1949.

TABLE 13

EFFECT OF ETHYLENE GLYCOL AEROSOL ON NUMBERS OF PHAGE PARTICLES RECOVERED FROM ROOM INFECTED WITH LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period sample taken after infection began (min.)	Mpn./ft.^{3} of phage particles recovered from the air by aspiration through buffered distilled water		
	F10	F67	F69
13- 22	17,000	56,000	1,700,000
27- 34	9,400	9,400	9,400
43- 50	280	940	36,000
Period dish open after infection began (min.)	**Mpn./ft.^{2} of phage particles recovered from buffered distilled water standing in petri dishes**		
	F10	F67	F69
0- 13	3,000,000	180,000	3,000,000
0- 22	240,000	30,000	360,000
0- 34	1,100,000	3,000,000	3,000,000
0- 50	300,000	89,000	3,000,000
0- 13	3,000,000	180,000	3,000,000
13- 22	18,000	5,300	300,000
27- 34	1,800	890	5,300
43- 50	300	110	1,800

60 ml. of whey filtrate with a pH of 6.6, containing 75,000,000, 95,000,000 and 150,000,000 particles/ml. of F10, F67 and F69 phages respectively were atomized into the room. Time required for atomizing was 13 min. 60 ml. of ethylene glycol were dispersed in 20 ml. portions alternately with equal volumes of the filtrate, the filtrate first. Room temperature was 75°F. and relative humidity, 78-82%. Trial was made August 4, 1949.

TABLE 14

EFFECT OF PROPYLENE GLYCOL AEROSOL ON NUMBERS OF PHAGE PARTICLES RECOVERED FROM ROOM INFECTED WITH LACTIC STREPTOCOCCUS BACTERIOPHAGE

Period sample taken after infection began (min.)	Mpn./ft.^{3} of F69 phage particles recovered from the air by aspiration through distilled water
6- 14	56,000
60- 80	7,500
90- 98	940

20 ml. of whey filtrate containing F69 phage were atomized into the room. 20 ml. of propylene glycol were dispersed into the room beginning 25 min. after infection began. Room temperature was 70°F. and relative humidity, 72%. Trial was made April 27, 1949.

TABLE 15

EFFECT OF TRIETHYLENE GLYCOL AEROSOL ON NUMBERS OF PHAGE PARTICLES RECOVERED FROM ROOM INFECTED WITH LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period sample taken after infection began (min.)	Mpn./ft.^{3} of phage particles recovered from the air by aspiration through buffered distilled water		
	F10	F67	F69
10- 17	750,000	280,000	940,000
20- 27	940,000	170,000	940,000
40- 47	9,400	56,000	17,000
Period dish open after infection began (min.)	**Mpn/ft.^{2} of phage particles recovered from buffered distilled water standing in petri dishes**		
	F10	F67	F69
0- 7	1,100,000	530,000	3,000,000
0- 10	3,000,000	1,100,000	3,000,000
0- 17	5,300,000	3,000,000	3,000,000
0- 27	3,000,000	1,800,000	3,000,000
0- 47	530,000	1,100,000	3,000,000
0- 7	1,100,000	530,000	3,000,000
10- 17	300,000	30,000	530,000
20- 27	30,000	11,000	110,000
40- 47	8,900	5,300	3,000

55 ml. of whey filtrate with a pH of 6.6, containing 150,000,000, 45,000,000 and 150,000,000 particles/ml. of F10, F67 and F69 phages respectively were atomized into the room. 60 ml. of triethylene glycol were dispersed simultaneously with the filtrate. Room temperature was 77°F. and relative humidity, 52-56%. Trial was made August 2, 1949.

TABLE 16

EFFECT OF CALCIUM HYPOCHLORITE AEROSOL ON NUMBERS OF PHAGE PARTICLES RECOVERED FROM ROOM INFECTED WITH LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period sample taken after infection began (min.)	Mpn./ft.^{3} of phage particles recovered from the air by aspiration through buffered thiosulphate solution		
	F10	F67	F69
12- 19	9,400	9,400	94,000
28- 35	17,000	15,000	36,000
58- 65	750	750	3,600
Period dish open after infection began (min.)	**Mpn./ft.^{2} of phage particles recovered from buffered thiosulphate solution standing in petri dishes**		
	F10	F67	F69
4 before- 22 after	0	0	0
22- 58	54,000	54,000	54,000
12- 19	11,000	30,000	30,000
28- 35	11,000	1,400	30,000 +
58- 65	1,100	480	3,000

60 ml. of whey filtrate with a pH of 6.27, containing 45,000,000, 25,000,000 and 150,000,000 particles/ml. of F10, F67 and F69 phages respectively were atomized into the room. Time required for atomizing was 9 min. 10 ml. of calcium hypochlorite solution containing 2.5% av. Cl were dispersed into the room 4-3 min. before infection began. A second infection with 10 ml. of filtrate was made 22-24 min. after the first. Room temperature was 82°F. and relative humidity, approx. 76%. Trial was made August 16, 1949.

TABLE 17

EFFECT OF CALCIUM HYPOCHLORITE AEROSOL ON THE NUMBERS OF PHAGE PARTICLES RECOVERED FROM ROOM INFECTED WITH LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period sample taken after infection began (min.)	Mpn./ft.^{3} of phage particles recovered from the air by aspiration through buffered thiosulphage solution		
	F10	F67	F69
8- 15	<150	260	1,700
27- 34	0	1.1	17
57- 64	0	0	0
Period dish open after infection began (min.)	**Mpn./ft.^{2} of phage particles recovered from buffered thiosulphate solution standing in petri dishes**		
3 before- 27 after	0	0	30
27- 57	0	11	0
8- 15	11,000	11,000	300,000
27- 34	0	0	0
57- 64	0	0	0

60 ml. of whey filtrate with a pH of 6.27, containing 45,000,000, 25,000,000 and 150,000,000 particles/ml. of F10, F67 and F69 phages respectively were atomized into the room. Time required for atomizing was 7 min. 10 ml. of calcium hypochlorite solution containing 2.5% av. Cl were dispersed into the room 3-2 min. before infection began. Room temperature was 79°F. and relative humidity, approx. 75%. Trial was made August 18, 1949.

TABLE 18

EFFECT OF A CALCIUM HYPOCHLORITE AEROSOL ON THE NUMBERS OF PHAGE PARTICLES RECOVERED FROM ROOM INFECTED WITH LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period sample taken after infection began (min.)	Mpn./ft.^{3} of phage particles recovered from the air by aspiration through buffered thiosulphate solution		
	F10	F67	F69
13- 20	3,600	15,000	360,000
27- 34	94	1,700	3,600
57- 64	5.6	340	3,600
Period dish open after infection began (min.)	**Mpn./ft.^{2} of phage particles recovered from buffered thiosulphate solution**		
	F10	F67	F69
3 before-27 after	0	8.3	4.8
27- 57	110	300	300
13- 20	11,000	30,000	53,000
27- 34	300	1,800	3,000
57- 64	0	300	300

60 ml. of whey filtrate with a pH of 6.3, containing 15,000,000, 25,000,000 and 450,000,000 particles/ml. of F10, F67 and F69 phages respectively were atomized into the room. Time required for atomizing was 12 min. 10 ml. of calcium hypochlorite solution containing 2.5% av. Cl were dispersed into the room 3-2 min. before infection began. Room temperature was 77° F. and relative humidity, 67-74%. Trial was made August 19, 1949.

The second infection in Table 16 increased the numbers of F10 and F67 particles recovered by aspiration but the recovery of F69 particles was slightly more than one-third of that in the first sampling. Active phage was not recovered from the dish exposed during the period from 4 min. before to 22 min. after infection but was recovered from the dish exposed during the 12-19 min. period. An explanation of this difference was not apparent. It may have been the result of concentration of chlorine in a mist just above the dish and/or in the surface layer of the liquid in the former dish which was open during the dispersal of the aerosol. The second infection resulted in recovery of active phage in the dish exposed during the 22-50 min. period and in recovery of approximately the same numbers of F10 and F69 particles in the dish for the 28-35 min. period as in the dish for the 12-19 min. period. There was a 20-fold less recovery of F67 particles in the dish exposed during the 28-35 min. period than in the dish for the 12-19 min. period.

The results of treatments of the infected room with calcium hypochlorite aerosols producing 1 g. of available chlorine to 38,000,000-47,000,000 ml. of air (0.61-0.75 g. per 1,000 ft.^{3}) are presented in Tables 19, 20 and 21. In the first two trials (Tables 19-20) active phage particles were not recovered until the second infection was made (Table 20). From zero to near normal numbers of active particles were recovered after the second and third infections. Noticeably larger numbers of F69 particles than of F10 or F67 particles were recovered. In the third trial (Table 21) the aerosol was dispersed 3-2 min. before infection and small numbers of active phage particles were recovered in the

TABLE 19

EFFECT OF CALCIUM HYPOCHLORITE AEROSOL ON NUMBERS OF PHAGE PARTICLES RECOVERED FROM ROOM INFECTED WITH LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period sample taken after infection began (min.)	Mpn./ft.^{3} of phage particles recovered from the air by aspiration through buffered thiosulphate solution		
	F10	F67	F69
10-20	0	0	0
25-32	0	0	0
40-47	0	0	0
Period dish open after infection began (min.)	**Mpn./ft.^{2} of phage particles recovered from buffered thiosulphate solution standing in petri dishes**		
	F10	F67	F69
0-10	0	0	0
0-20	0	0	0
0-32	0	0	0
0-47	0	0	0
0-10	0	0	0
10-20	0	0	0
25-32	0	0	0
40-47	0	0	0

60 ml. of whey filtrate with a pH of 6.6, containing 75,000,000, 95,000,000 and 150,000,000 particles/ml. of F10, F67 and F69 phages respectively were atomized into the room. Time required for atomizing was 8 min. 10 ml. of calcium hypochlorite solution containing 8.3% av. Cl were dispersed into the room simultaneously with the filtrate. Room temperature was 77-78°F. and relative humidity, 53-63%. Trial was made August 5, 1949.

TABLE 20

EFFECT OF CALCIUM HYPOCHLORITE AEROSOL ON NUMBERS OF PHAGE PARTICLES RECOVERED FROM ROOM INFECTED WITH LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period sample taken after infection began (min.)	Mpn./ft.^{3} of phage particles recovered from the air by aspiration through buffered thiosulphate solution		
	F10	F67	F69
10- 17	0	0	0
46- 53	750	17,000	360,000
77- 84	170	17,000	75,000
Period dish open after infection began (min.)	**Mpn./ft.^{2} of phage particles recovered from buffered thiosulphate solution standing in petri dishes**		
	F10	F67	F69
0- 37	0	0	0
37- 47	30	30,000	110,000
10- 17	0	0	0
46- 53	0	24	1,100
77- 84	180	890	53,000

60 ml. of whey filtrate with a pH of 6.55, containing 1,500,000, 150,000,000 and 150,000,000 particles/ml. of F10, F67 and F69 phages respectively were atomized into the room. Time required for atomizing was 6 min. 10 ml. of calcium hypochlorite solution containing 8.3% av. Cl were dispersed into the room 7-9 min. after infection began. A second infection of 60 ml. of the filtrate was made 37-45 min. after the first began and a third of 20 ml. was made 71-73 min. after the first. Room temperature was 82-83°F. and relative humidity, 65-69%. Trial was made August 8, 1949.

TABLE 21

EFFECT OF CALCIUM HYPOCHLORITE AEROSOL ON NUMBERS OF PHAGE PARTICLES RECOVERED FROM ROOM INFECTED WITH LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period sample taken after infection began (min.)	Mpn./ft.^{3} of phage particles recovered from the air by aspiration through buffered thiosulphate solution		
	F10	F67	F69
7- 14	1.1	1.1	3.4
30- 37	360	17,000	75,000
54- 61	940	56,000	170,000
Period dish open after infection began (min.)	**Mpn./ft.^{2} of phage particles recovered from buffered thiosulphate solution standing in petri dishes**		
	F10	F67	F69
0- 22	0	30	24
22- 50	540	30,000	180,000
7- 14	0	8.0	0
30- 37	0	2,400	3,500
54- 61	300	11,000	30,000 +

60 ml. of whey filtrate with a pH of 6.5, containing 4,500,000, 4,500,000 and 75,000,000 particles/ml. of F10, F67 and F69 phages respectively were atomized into the room. Time required for atomizing was 7 min. 12 ml. of calcium hypochlorite solution containing 8.5% av. Cl were dispersed into the room 5-2 min. before infection began. A second infection of 60 ml. of filtrate was made 22-29 min. after the first and a third of 20 ml. was made 50-54 min. after the first. Room temperature was 83-84° F. and relative humidity, 69-80%. Trial was made August 10, 1949.

first samplings. Considerable numbers of active particles were recovered after the second infection and greater numbers were recovered after the third infection. Greater numbers of F69 than of F10 or F67 particles survived but not in proportion to the numbers added to the room.

The results of an additional trial in which the infected room was treated with 1 g. of available chlorine to 155,000,000 ml. of air (0.18 g. per 1,000 ft.^{3}) are presented in Table 22. These data agree with those in Tables 16, 17 and 18. In addition, the numbers of phage particles recovered in petri dishes during successive 5-min. periods are given. No active particles were recovered in the dish exposed during the period 3 min. before to 2 min. after the infection was begun. The largest numbers were recovered during the following 5-min. period. The numbers of F10 particles recovered remained constant for the third and fourth periods and then declined three- and six-fold, respectively, for the fifth and sixth periods. The numbers of F67 particles recovered declined 36-fold for the third period, declined nearly three-fold for the fourth and remained about constant for the fifth and sixth periods. When compared with the respective preceding periods, nine-, six- and less than two-fold and no declines occurred in recoveries during the third to sixth periods. Whey filtrate added to a portion of the contents of the dish collected for the first 5-min. period and assayed for active phage particles indicated that the contents of the dish had no inhibitory effect on the phages.

The results of treating the infected room with a chloramine-T aerosol supplying 1 g. of available chlorine to 39,000,000 ml. of air

TABLE 22

EFFECT OF CALCIUM HYPOCHLORITE AEROSOL ON NUMBERS OF PHAGE PARTICLES RECOVERED FROM ROOM INFECTED WITH LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period sample taken after infection began (min.)	Mpn./ft.^{3} of phage particles recovered from the air by aspiration through buffered thiosulphate solution		
	F10	F67	F69
9 - 16	150	2,800	9,400
30 - 37	9,400	11,000	7,500
57 - 64	520	3,600	17,000
Period dish open after infection began (min.)	**Mpn./ft.^{2} of phage particles recovered from buffered thiosulphate solution standing in petri dishes**		
	F10	F67	F69
* 3 before - 27 after	0	13	110
27 - 57	53,000	53,000	110,000
9 - 16	360	11,000	30,000
30 - 37	30,000	30,000	48,000
57 - 64	53	295	530
* 3 before - 2 after	0	0	0
2 - 7	300	30,000	48,000
7 - 12	300	830	5,300
12 - 17	300	300	830
17 - 22	110	350	480
22 - 27	18	300	480

60 ml. of whey filtrate with a pH of 6.25, containing 250,000,000, 45,000,000 and 150,000,000 particles/ml. of F10, F67 and F69 phages respectively were atomized into the room. Time required for atomizing was 7 min. 10 ml. of calcium hypochlorite solution containing 2.5% av. Cl were dispersed into the room 3-2 min. before infection began. A second infection of 10 ml. of filtrate was made 27-28 min. after the first began. Room temperature was 76-77° F. and relative humidity, 66-67%. Trial was made Aug. 24, 1949.

* 1 ml. of filtrate diluted 10^{-4} was added to approximately 9 ml. of buffered thiosulphate solution in each dish and assayed with the following results:

TABLE 22 (con'd)

	F10	F67	F69
Dish 1			
Calculated mpn./ml. of mixture, assuming no viruscidal action	2,700	490	1,600
Actual mpn./ml. of mixture	250	450	2,500
Dish 2			
Calculated mpn./ml. of mixture, assuming no viruscidal action	2,700	490	1,600
Actual mpn./ml. of mixture	2,500	2,500	2,500

(0.73 g. per 1,000 ft.^{3}) are presented in Table 23. The chlorine from this source apparently was slightly less effective than an equivalent amount of chlorine from calcium hypochlorite in the Aug. 10 trial (Table 21).

Alkyldimethylbenzylammonium chloride

Table 24 is a presentation of the results of treating the infected room with an alkyldimethylbenzylammonium chloride aerosol. A concentration of 1 g. of this compound to 39,000,000 ml. of air (0.73 g. per 1,000 ft.^{3}) reduced the numbers of active phage particles considerably below the numbers normal for an untreated room (cf. Tables 3-11) but failed to completely inactivate them. Large numbers of active particles were recovered after the second and third infections. This aerosol had a highly astringent effect upon the respiratory tract which alone would make its use impracticable in rooms where workers were present.

Action of Certain Viruscidal Agents on Lactic Streptococcus Bacteriophages in Liquids

Glycols

The results of treatment of the bacteriophages in 10^{-1} and 10^{-2} dilutions of whey filtrate using ethylene glycol, propylene glycol and triethylene glycol are presented in Tables 25, 26 and 27, respectively. Little, if any, destructive action of any of the three compounds in concentrations as high as 1,000 ppm. in a filtrate diluted 10^{-1} occurred in 300 sec. Some destruction by ethylene glycol in a 10^{-2} dilution may

TABLE 23

EFFECT OF CHLORAMINE-T AEROSOL ON NUMBERS OF PHAGE PARTICLES RECOVERED FROM ROOM INFECTED WITH LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period sample taken after infection began (min.)	Mpn./ft.^{3} of phage particles recovered from the air by aspiration through buffered thiosulphate solution		
	F10	F67	F69
10- 17	94	94	9,400
33- 40	940	3,600	94,000
63- 70	940	19,000	19,000
Period dish open after infection began (min.)	**Mpn./ft.^{2} of phage particles recovered from buffered thiosulphate solution standing in petri dishes**		
	F10	F67	F69
7 before - 23 after	300	5,400	2,400
23- 30	540	54,000	300,000
10- 17	0	300	0
33- 40	5,400	30,000	54,000
63- 70	5,400	5,400	30,000

60 ml. of whey filtrate with a pH of 6.25, containing 45,000,000, 95,000,000 and 25,000,000 particles/ml of F10, F67 and F69 phages respectively were atomized into the room. Time required for atomizing was 6 min. 42 ml. of chloramine-T solution containing 2.375% av. Cl were dispersed into the room 7-2 min. before infection began. A second infection of 60 ml. of filtrate was made 23-30 min. after the first began and a third infection of 20 ml., 60-62 min. after the first. Room temperature was 83° F. and relative humidity, 76-80%. Trial was made August 12, 1949.

TABLE 24

EFFECT OF ALKYLDIMETHYLBENZYLAMMONIUM CHLORIDE AEROSOL ON NUMBERS OF PHAGE PARTICLES RECOVERED IN ROOM INFECTED WITH LACTIC STREPTOCOCCUS BACTERIOPHAGES

Period sample taken after infection began (min.)	Mpn./ft.^{3} of phage particles recovered from the air by aspiration through buffered distilled water		
	F10	F67	F69
10 - 17	0	1.1	940
38- 45	56,000	17,000	17,000
70- 77	75,000	940,000	94,000
Period dish open after infection began (min.)	**Mpn./ft.^{2} of phage particles recovered from buffered distilled water standing in petri dishes**		
	F10	F67	F69
0- 28	5,400	11,000	30,000
28- 58	540,000	1,400,000	240,000
10- 17	3,000	11,000	890
38- 45	54,000	110,000	110,000
70- 77	5,400	30,000	11,000

60 ml. of whey filtrate with a pH of 6.5, containing 25,000,000, 200,000,000 and 25,000,000 particles/ml. of F10, F67 and F69 phages respectively were atomized into the room. Time required for atomizing was 6 min. 10 ml. of 10% alkyldimethylbenzylammonium chloride solution were dispersed into the room 5-2 min. before infection began. A second infection of 50 ml. of filtrate was made 30-35 min. after the first began and a third, 60-62 min. after the first. Room temperature was 80-82° F. and relative humidity, 76-84%. Trial was made August 11, 1949.

TABLE 25

EFFECT OF ETHYLENE GLYCOL ON LACTIC STREPTOCOCCUS BACTERIOPHAGES IN DILUTIONS OF WHEY FILTRATES AT 25° C.

Bacteriophage	pH of filtrate	Ppm. of glycol in treated filtrate	pH of treated filtrate	**Calc. mpn./ml. in control	*Mpn./ml. after exposure time (in sec.) of: 15	30	60	120	300
				Whey filtrate diluted 10^{-1}					
F10	6.5	500	7.05	1,100					500
F69	6.5	500	7.05	25,000					5,000
F10	6.5	1,000	7.1	25,000					50,000
F69	6.5	1,000	7.1	95,000					50,000
F10	6.65	1,000	7.15	11,000					9,000
F67	6.65	1,000	7.15	1,100,000					500,000
F69	6.65	1,000	7.15	2,500					15,000
				Whey filtrate diluted 10^{-2}					
F69	ca. 4.7	100	-	45,000	19,000	19,000	5,000	19,000	2,300

* Assay of treated filtrate diluted 10^{-1} in sterile milk.

** Calc. = calculated.

TABLE 26

EFFECT OF PROPYLENE GLYCOL ON LACTIC STREPTOCOCCUS BACTERIOPHAGES IN DILUTIONS OF WHEY FILTRATES AT 25° C.

Bacteriophage	pH of filtrate	Ppm. of glycol in treated filtrate	pH of treated filtrate	Calc. mpm./ml in control	*Mpm./ml. after exposure time (in sec.) of: 30	60	120	300
				Whey filtrate diluted 10^{-1}				
F10	6.5	500	7.0	1,100				500
F69	6.5	500	7.0	25,000				22,000
F10	6.5	1,000	7.1	25,000				50,000
F69	6.5	1,000	7.1	95,000				50,000
F10	6.65	1,000	7.2	11,000				5,000
F67	6.65	1,000	7.2	1,100,000				900,000
F69	6.65	1,000	7.2	2,500				5,000
				Whey filtrate diluted 10^{-2}				
F69	ca. 4.7	100	6.0	45,000	9,000	3,000	9,000	50,000

* Assay of treated filtrate diluted 10^{-1} in sterile milk.

TABLE 27

EFFECT OF TRIETHYLENE GLYCOL ON LACTIC STREPTOCOCCUS BACTERIOPHAGES IN 10^{-2} DILUTIONS OF WHEY FILTRATES AT 25° C.

Bacteriophage	pH of filtrate	Ppm. of glycol in treated filtrate	pH of treated filtrate	Calc. mpn./ml in control	*Mpn./ml after 300 sec. exposure
F10	6.5	500	7.0	1,100	500
F69	6.5	500	7.0	25,000	22,000
F10	6.5	1,000	7.1	25,000	50,000
F69	6.5	1,000	7.1	95,000	50,000
F10	6.65	1,000	7.1	11,000	2,200
F67	6.65	1,000	7.1	1,100,000	2,200,000
F69	6.65	1,000	7.1	2,500	5,000

* Assay of treated filtrate diluted 10^{-1} in sterile milk.

have occurred.

Calcium hypochlorite and chloramine-T

The results of tests to determine the action of calcium hypochlorite upon the phages in whey filtrates diluted 1:2 are shown in Table 28. A concentration of 100 ppm. of available chlorine reduced the numbers of active particles in filtrates with a pH of 5.0 from 25,000,000-55,000,000 per milliliter to 1.4-5,000 per milliliter in 300 sec. with most of the destructive action occurring within 15 sec. of exposure time. The action of the chlorine diluted in tap water was not significantly different from that of the distilled water dilution of the chlorine. The effect of the chlorine was greater on F10 and F67 phages at 45° C. than at 25° C., but such was not the case with F69.

A concentration of 100 ppm. of available chlorine inactivated the F10 and F67 phages in filtrates with a pH of 5.25 within 15 sec., but the original numbers were very small.

The F10 phage appeared to be somewhat more susceptible to the concentrations of 150-200 ppm. of available chlorine but the effects of these concentrations on the F67 and F69 phages in filtrates at pH 5.0 appeared to be very little greater than that of 100 ppm. A concentration of 300 ppm. was more effective but still did not inactivate all three phages within 300 sec.

A concentration of 400 ppm. of available chlorine inactivated all three phages in filtrates with a pH of 5.0 within 15 sec. but when the filtrate had 10 per cent of milk culture of the homologous organisms

TABLE 28

EFFECT OF CALCIUM HYPOCHLORITE ON LACTIC STREPTOCOCCUS BACTERIOPHAGES IN 1:2 DILUTIONS OF WHEY FILTRATES AT 25° C. AND AT 45° C.

Bacteriophage	pH of filtrate	Ppm. of av. Cl in treated filtrate	pH of treated filtrate	Calc. mpn./ml. in control	*Mpn./ml. after exposure time (in sec.) of: 15	30	60	120	300
F10	5.0	100		25,500,000	500	500	500	90	1.4
F67	5.0	100		55,000,000	5,000	5,000	5,000	5,000	5,000
F69	5.0	100		55,000,000	500	500	90	90	30
F10	5.0	100		22,500,000	500	500	500	90	1.4
F67	5.0	100		55,000,000	5,000	5,000	5,000	5,000	5,000
F69	5.0	100		55,000,000	500	500	90	90	30
F10	5.0	#100		22,500,000	19	50	19	50	0.8
F67	5.0	#100		55,000,000	5,000	5,000	1,900	5,000	190
F69	5.0	#100		55,000,000	190	400	500	900	1,900
F10	5.0	#100		22,500,000	190	90	90	8	0
F67	5.0	#100		55,000,000	300	50	500	500	90
F69	5.0	#100		55,000,000	5,000	50	5,000	5,000	1,900
## F10	5.0	100		22,500,000	50	90	0.8	0	0
## F67	5.0	100		55,000,000	5,000	900	130	990	50
## F69	5.0	100		55,000,000	5,000	5,000	900	50	0.8

TABLE 28 (con'd)

Bacteriophage	pH of filtrate	Ppm. of av. Cl in treated filtrate	pH of treated filtrate	Calc. mpn./ml. in control	*Mpn./ml. after exposure time (in sec.) of: 15	30	60	120	300
##F10	5.0	100		22,500,000	1,900	19	1.8	0	0
##F67	5.0	100		55,000,000	50,000	5,000	5,000	5,000	90
##F69	5.0	100		55,000,000	5,000	5,000	1,900	1,900	50
F10	5.25	100		250	0	0	0		0
F67	5.25	100		250	0	900	0		0
F69	5.25	100		2,500,000	900	0	400		1,900
F10	5.25	100		250	0	0	0		0
F67	5.25	100		250	0	0	0		0
F69	5.25	100		2,500,000	900	900	400		1,900
F10	5.0	150		22,500,000	1.8	0	0	0	0
F67	5.0	150		55,000,000	1,900	190	190	90	4
F69	5.0	150		55,000,000	90	230	90	0.8	0
F10	5.0	150		22,500,000	0	0	0	0	0
F67	5.0	150		55,000,000	90	9	19	9	1.4
F69	5.0	150		55,000,000	50	50	90	5	0.8
F10	5.0	200		225,000	0	5	0.6	0	0
F67	5.0	200		2,250,000	400	900	5,000	1,950	500
F69	5.0	200		10,000,000	190	5,000	400	90	1.2

TABLE 28 (con'd)

Bacteriophage	pH of filtrate	Ppm. of av. Cl in treated filtrate	pH of treated filtrate	Calc. mpm./ml. in control	*Mpm./ml. after exposure time (in sec.) of: 15	30	60	120	300
F10	5.0	200		225,000	0	0	0	0	0
F67	5.0	200		2,250,000	900	900	900	300	150
F69	5.0	200		10,000,000	900	5,000	900	90	90
F10	5.0	300		1,250,000	0	0	0	0	0
F67	5.0	300		2,250,000	0	0.4	0	0	0
F69	5.0	300		3,750,000	0	0	0	0	0
F10	5.0	300		1,250,000	3		0	0	0
F67	5.0	300		2,250,000	150		50	90	50
F69	5.0	300		3,750,000	50		50	50	50
F10	5.0	400		1,250,000	0		0	0	0
F67	5.0	400		2,250,000	0		0.6	0.8	0
F69	5.0	400		3,750,000	0		0	0	0
F10	5.0	400		1,250,000	0		0	0	0
F67	5.0	400		2,250,000	0		0	0	0
F69	5.0	400		3,750,000	0		0	0	0
**F10	4.6	500	4.8	1,250,000	500	90	50	0.6	0
**F67	4.6	500	4.8	2,250,000	400	5	4	0.6	0
**F69	4.6	500	4.8	1,250,000	5,000+	90	3	0.6	0.6

TABLE 28 (con'd)

Bacteriophage	pH of filtrate	Ppm. of av. Cl in treated filtrate	pH of treated filtrate	Calc. mpn./ml. in control	*Mpn./ml. after exposure time (in sec.) of: 15	30	60	120	300
**F10	4.6	500	4.8	1,250,000	500	90	50	0.6	0.
**F67	4.6	500	4.8	2,250,000	50	50	50	50	50
**F69	4.6	500	4.8	1,250,000	9	50	9	1.4	0
F10	6.5	400	6.5	10,000,000	300		500	50	90
F67	6.5	400	6.5	7,500,000	400		5,000	5,000	220
F69	6.5	400	6.5	7,500,000	5,000		5,000	1,900	90
F10	6.5	400	6.6	1,250,000	50		9	5	0
F67	6.5	400	6.6	1,250,000	50		50	50	50
F69	6.5	400	6.6	2,250,000	220		90	50	5
F10	6.5	500	6.4	10,000,000	5		0	0	0
F67	6.5	500	6.4	7,500,000	50		50	50	1.4
F69	6.5	500	6.4	7,500,000	50		50	50	19

TABLE 28 (con'd)

Bacteriophage	pH of filtrate	Ppm. of av. Cl in treated filtrate	pH of treated filtrate	Calc. mpn./ml. in control	*Mpn./ml. after exposure time (in sec.) of: 15	30	60	120	300
F10	6.5	500	6.6	1,250,000	0		0	0	0
F67	6.5	500	6.6	1,250,000	50		90	0	0
F69	6.5	500	6.6	2,250,000	9		0	0	0

* Assay of treated filtrate diluted 10^{-1} in thiosulphate inhibitor
** 10% susceptible culture of lactic streptococcus added just before treatment with viruscide solution
\# Viruscide solution made with tap water
Test made at 45° C. All others at 25° C.

added, 500 ppm. was hardly sufficient for complete inactivation within 300 sec.

In filtrates with a pH of 6.5, a concentration of 500 ppm. of available chlorine inactivated all three phages within 120 sec. in one trial but small numbers of F67 and F69 phages survived an exposure of 300 sec. in another trial. At 400 ppm., a number of particles survived.

The results of tests of the action of calcium hypochlorite upon the phages in 10^{-1} dilutions of whey filtrates are given in Table 29. A concentration of 50 ppm. of available chlorine reduced the titers of the test mixtures from 1,100,000-11,000,000 to 50-90 within 15 sec. using filtrate at pH 4.6. However, active phage remained after 300 sec. A concentration of 100 ppm. inactivated all three phages in filtrates at pH 4.6-6.5 within 300 sec. in two out of five trials but survival of the individual phages in the other three trials ranged from 0 to 220 per milliliter. No difference in action at the different pH levels is evident in the data for the 10^{-1} dilutions of filtrates. Available chlorine concentrations above 100 ppm. were necessary for consistent phage inactivation.

The effect of calcium hypochlorite upon the phages in 10^{-2} dilutions of filtrates is shown in Table 30. A concentration of 15 ppm. of available chlorine reduced the titers of test mixtures from 25,000-45,000 per milliliter to 1.8-50 per milliliter within 15 sec. but failed to inactivate completely any of the three phages within 300 sec. in both of two trials, at pH 5.4-6.0. A concentration of 25 ppm.

TABLE 29

EFFECT OF CALCIUM HYPOCHLORITE ON LACTIC STREPTOCOCCUS BACTERIOPHAGES IN 10^{-1} DILUTIONS OF WHEY FILTRATES AT 25° C.

Bacteriophage	pH of filtrate	Ppm. of av. Cl in treated filtrate	pH of treated filtrate	Calc. mpm./ml. in control	*Mpm./ml. after exposure time (in sec.) of: 15	30	60	120	300
F10	4.6	50		4,500,000	50	50	19	4	1.4
F67	4.6	50		11,000,000	90	220	90	90	6
F69	4.6	50		1,100,000	50	50	50	19	9
F10	4.6	50		4,500,000	80	80	50	50	0.6
F67	4.6	50		11,000,000	90	220	150	220	50
F69	4.6	50		1,100,000	90	50	50	50	6
**F10	4.6	100	4.75	450,000	400	50	90	90	4
**F67	4.6	100	4.75	9,500,000	50	50	50	50	220
**F69	4.6	100	4.75	400,000	90	25	220	220	50
**F10	4.6	100	4.8	450,000	0	9	19	5	1.8
**F67	4.6	100	4.8	9,500,000	300	150	80	90	19
**F69	4.6	100	4.8	400,000	50	50	90	150	19
F10	5.0	100		4,500,000	0	50	0	0	0
F67	5.0	100		11,000,000	0	50	0	0	0
F69	5.0	100		11,000,000	0	50	0	0	0

TABLE 29 (con'd)

Bacteriophage	pH of filtrate	Ppm. of av. Cl in treated filtrate	pH of treated filtrate	Calc. mpn./ml. in control	*Mpn./ml. after exposure time (in sec.) of: 15	30	60	120	300
F10	6.5	100	6.9	2,000,000	5	1.4	0	0	0
F67	6.5	100	6.9	1,500,000	1.4	3	5	0.8	0
F69	6.5	100	6.9	1,500,000	19	6	2.2	0.6	0
F10	6.5	100	6.95	2,000,000	0.8	0.8	1.4	0	0.8
F67	6.5	100	6.95	1,500,000	1.8	1.8	0.8	0	1.8
F69	6.5	100	6.95	1,500,000	5	1.8	0.8	1.4	0

* Assay of treated filtrate diluted 10^{-1} in thiosulphate inhibitor

** 10% culture of susceptible organism added to filtrate immediately before treatment with hypochlorite solution

TABLE 30

EFFECT OF CALCIUM HYPOCHLORITE ON LACTIC STREPTOCOCCUS BACTERIOPHAGES IN 10^{-2} DILUTIONS OF WHEY FILTRATES AT 25° C.

Bacteriophage	pH of filtrate	Ppm. of av. Cl in treated filtrate	pH of treated filtrate	Calc. mpn./ml. in control	*Mpn./ml. after exposure time (in sec.) of: 15	30	60	120	300
F10	4.6	15	5.4	25,000	9	0.6	0	1.8	1.2
F67	4.6	15	5.4	45,000	19	4	5	0.6	0
F69	4.6	15	5.4	25,000	9	50	5	1.4	1.8
F10	4.6	15	6.0	25,000	19	1.8	1.4	3.2	9
F67	4.6	15	6.0	45,000	50	3	3	3	1.4
F69	4.6	15	6.0	25,000	1.8	9	2.2	1.8	1.2
F10	4.6	25	6.0	25,000	0	0	0	0	0
F67	4.6	25	6.0	45,000	0.8	0	0	0	0
F69	4.6	25	6.0	25,000	0	0	0.6	0	0
F10	4.6	25	5.8	25,000	0	0	0	0	0
F67	4.6	25	5.8	45,000	0	0	0	0	0
F69	4.6	25	5.8	25,000	0	0	0	0	0
F10	4.6	50	6.8	25,000	0	0	0	0	0
F67	4.6	50	6.8	450,000	0	0	0	0	0
F69	4.6	50	6.8	150,000	0	0	0	0	0

TABLE 30 (con'd)

Bacteriophage	pH of filtrate	Ppm. of av. Cl in treated filtrate	pH of treated filtrate	Calc. mpn./ml. in control	*Mpn./ml. after exposure time (in sec.) of: 15	30	60	120	300
F10	4.6	50	6.4	2,500	0	0	0	0	0
F67	4.6	50	6.4	450,000	0	0	0	0	0
F69	4.6	50	6.4	150,000	0	0	0	0	0
F10	6.5	25	7.4	200,000	0	0	0	0.6	0
F67	6.5	25	7.4	150,000	0	0	0.8	0.6	0
F69	6.5	25	7.4	150,000	0	0.8	0	0	0
F10	6.5	25	7.2	200,000	3	50	0	1.8	0.8
F67	6.5	25	7.2	150,000	50	50	50	50	50
F69	6.5	25	7.2	150,000	50	50	50	50	500

*Assay of treated filtrate diluted 10^{-1} in thiosulphate inhibitor.

inactivated all the phages at pH 5.8-6.0 within 120 sec. but failed to inactivate in 300 sec. in one of two trials when the pH was 7.2-7.4. A concentration of 50 ppm. inactivated within 15 sec. all the phages at pH 6.4-6.8.

Data showing the effect of chloramine-T on the phages are presented in Table 31. Very noticeably larger numbers of phage particles survived in these tests than in the corresponding tests with calcium hypochlorite (Tables 28-30), especially as the treated preparations were less dilute. The pH levels of 7.6-8.1 of the viruscide-filtrate mixtures were distinctly higher when chloramine-T instead of calcium hypochlorite was used, undoubtedly explaining some of the difference observed between the two compounds.

Quaternary ammonium compounds

The results of treating the phages in 1:2 dilutions of whey filtrates with alkyldimethylbenzylammonium chloride are given in Table 32. A concentration of 400 ppm. of the compound lowered the titers of the test mixtures from 1,300,000-3,800,000 to 50,000-500,000 in a period of 300 sec. when the pH of the original filtrate was 5.0. From 0 to 50 active particles per milliliter survived 1,000 ppm. for 300 sec. in the case of a filtrate at pH 4.6. When the pH of the filtrate was 6.5, the phages were inactivated within 300 sec. by 800 ppm. and within 30 sec. by 1,000 ppm.

The results of treating the phages in 10^{-1} dilutions of whey filtrates with alkyldimethylbenzylammonium chloride are presented in Table 33. A concentration of 300 ppm. of the compound reduced the active particles

TABLE 31

EFFECT OF CHLORAMINE-T ON LACTIC STREPTOCOCCUS BACTERIOPHAGES IN DILUTIONS OF WHEY FILTRATES AT 25° C.

Bacteriophage	pH of filtrate	Ppm. of av. Cl in treated filtrate	pH of treated filtrate	Calc. mpn./ml. in control	*Mpn./ml. after exposure time (in sec.) of: 15	30	60	120	300
Whey filtrates diluted 1:2									
F10	6.5	500	7.7	45,000	50,000	50,000	5,000	500	8
F67	6.5	500	7.7	75,000	8,000	8,000	8,000	5,000	140
F69	6.5	500	7.7	125,000	190,000	190,000	30,000	9,000	5,000
F10	6.5	500	7.7	1,250,000	50,000	500	220	50	50
F67	6.5	500	7.7	2,000,000	500,000	22,000	9,000	5,000	90
F69	6.5	500	7.7	1,250,000	220,000	50,000	50,000	5,000	50
Whey filtrates diluted 10^{-1}									
F10	6.5	100	7.6	250,000	500	5,000	900	500	500
F67	6.5	100	7.6	400,000	50,000	50,000	90,000	50,000	2,200
F69	6.5	100	7.6	250,000	500,000	220,000	2,200	2,200	220,000
F10	6.5	100	8.1	9,000	9,000	9,000	5,000	5,000	5,000
F67	6.5	100	8.1	15,000	5,000	5,000	5,000	5,000	400
F69	6.5	100	8.1	25,000	8,000	8,000	5,000	9,000	5,000

TABLE 31 (con'd)

Bacteriophage	pH of filtrate	Ppm. of av. Cl in treated filtrate	pH of treated filtrate	Calc. mpn./ml. in control	*Mpn./ml. after exposure time (in sec.) of: 15	30	60	120	300
					Whey filtrates diluted 10^{-2}				
F10	6.5	25	7.7	25,000	500	500	500	500	500
F67	6.5	25	7.7	40,000	5,000	5,000	5,000	5,000	5,000
F69	6.5	25	7.7	25,000	2,200	2,200	220	900	500
F10	6.5	25	8.1	900	2,500	500	500	180	180
F67	6.5	25	8.1	1,500	50	500	500	500	80
F69	6.5	25	8.1	2,500	500	500	500	900	180

* Assay of treated filtrate diluted 10^{-1} in thiosulphate inhibitor

TABLE 32

EFFECT OF ALKYLDIMETHYLBENZYLAMMONIUM CHLORIDE ON LACTIC STREPTOCOCCUS BACTERIOPHAGES IN 1:2 DILUTIONS OF WHEY FILTRATES AT 25° C.

Bacteriophage	pH of filtrate	Ppm. of virucide in treated filtrate	pH of treated filtrate	Calc. apm./ml. in control	*Mpn./ml. after exposure time (in sec.) of: 15	30	60	120	300
F10	5.0	400		1,250,000	900,000	3,000,000	500,000	500,000	50,000+
F67	5.0	400		2,250,000	500,000	500,000	500,000	500,000	50,000+
F69	5.0	400		3,750,000	90,000	500,000	190,000	500,000	50,000
F10	5.0	400		1,250,000	5,000,000	3,000,000	5,000,000	1,900,000	190,000
F67	5.0	400		2,250,000	5,000,000	4,000,000	900,000	5,000,000	500,000
F69	5.0	400		3,750,000	90,000	150,000	190,000	40,000	500,000
F10	4.6	1000	4.6	1,250,000	90	50	9	0.6	0
F67	4.6	1000	4.6	7,500,000	22,000	22,000	90	50	0.8
F69	4.6	1000	4.6	2,250,000	1,300,000	500,000	22,000	1,900	50
F10	4.6	1000	4.6	1,250,000	220	50	3.0	5.0	0
F67	4.6	1000	4.6	7,500,000	220,000	2,200	2,200	19	0.8
F69	4.6	1000	4.6	2,250,000	500,000	500,000	40,000	2,200	0

TABLE 32 (con'd)

Bacteriophage	pH of filtrate	Ppm. of virucide in treated filtrate	pH of treated filtrate	Calc. mpn./ml. in control	*Mpn./ml. after exposure time (in sec.) of: 15	30	60	120	300
F10	6.5	600	6.7	5,500,000	5,000	2,200	220	50	9
F67	6.5	600	6.7	2,250,000	500,000	9,000	5,000	50	19
F69	6.5	600	6.7	1,250,000	1,500	500	220	50	19
F10	6.5	600	6.6	5,500,000	90,000	50,000	5,000	50	0.6
F67	6.5	600	6.6	2,250,000	500,000	220,000	5,000	90	0.6
F69	6.5	600	6.6	1,250,000	5,000	5,000	900	90	1.4
F10	6.5	800	6.6	5,500,000	19	0	0	0	0
F67	6.5	800	6.6	2,250,000	900	5	0	0	0
F69	6.5	800	6.6	1,250,000	2,200	50	0	0	0
F10	6.5	800	6.6	12,500,000	5	0.6	0	0.6	0
F67	6.5	800	6.6	1,000,000	80	1.4	0	0	0
F69	6.5	800	6.6	2,250,000	5,000	90	5	0.6	0
F10	6.5	1,000	6.6	750,000	0	0	0	0	0
F67	6.5	1,000	6.6	1,250,000	0.6	0	0	0	0
F69	6.5	1,000	6.6	75,000	0	0	0	0	0
F10	6.5	1,000	6.65	750,000	0.6	0	0	0	0
F67	6.5	1,000	6.65	1,250,000	0	0	0	0	0
F69	6.5	1,000	6.65	75,000	9	0	0	0	0

* Assay of treated filtrate diluted 10^{-1} in asolectin-Tween 80 inhibitor

TABLE 33

EFFECT OF ALKYLDIMETHYLBENZYLAMMONIUM CHLORIDE ON LACTIC STREPTOCOCCUS BACTERIOPHAGES IN 10^{-1} DILUTIONS OF WHEY FILTRATES AT 25° C.

Bacteriophage	pH of filtrate	Ppm. of virucide in treated filtrate	pH of treated filtrate	Calc. mpm./ml. in control	*Mpm./ml. after exposure time (in sec.) of: 15	30	60	120	300
F10	4.6	300	5.0	4,500,000	150	50	50	19	0
F67	4.6	300	5.0	11,000,000	2,200	900	900	300	1.8
F69	4.6	300	5.0	1,100,000	900	90	50	80	0.8
F10	4.6	300	5.0	4,500,000	50	3	19	1.4	0
F67	4.6	300	5.0	11,000,000	90	500	220	50	1.8
F69	4.6	300	5.0	1,100,000	150	90	50	0.8	0
F10	4.6	600		11,000	1.8	0	0	0	0
F67	4.6	600		1,100,000	90	9	0	0	0
F69	4.6	600		1,100,000	50	0	0	0	0
F10	4.6	600	4.8	110,000	0	0.8	0.8	0	0
F67	4.6	600	4.8	450,000	19	1.8	0	0	0
F69	4.6	600	4.8	1,500,000	9	0.8	0.8	0	0
F10	4.6	700	4.9	250,000	1.8	0.8	0	0	0
F67	4.6	700	4.9	11,000,000	90	19	0.8	0	0
F69	4.6	700	4.9	95,000	50	0.8	0	0	0

TABLE 33 (con'd)

Bacteriophage	pH of filtrate	Ppm. Af virucide in treated filtrate	pH of treated filtrate	Calc. mpm./ml. in control	*Mpm./ml. after exposure time (in sec.) of: 15	30	60	120	300
F10	4.6	700	4.85	250,000	0.8	0.8	0	0	0
F67	4.6	700	4.85	11,000,000	9	0.8	0	0	0
F69	4.6	700	4.85	95,000	0.8	5	0	0	0
F10	6.5	100	7.1	2,500,000	500,000	5,000	9,000	2,200	900
F67	6.5	100	7.1	200,000	50,000	22,000	9,000	900	50
F69	6.5	100	7.1	450,000	23,000	9,000	2,200	5,000	500
F10	6.5	200	7.05	2,500,000	5,000	5	0	0	0
F67	6.5	200	7.05	200,000	9,000	300	1.8	0.6	0
F69	6.5	200	7.05	450,000	22,000	5,000	19	9	0.6
F10	6.5	200	7.05	1,100,000	0	0	0	0	0
F67	6.5	200	7.05	450,000	0	0	0	0	0
F69	6.5	200	7.05	250,000	0	0	0	0	0
F10	6.5	300	6.9	1,100,000	0	0	0	0	0
F67	6.5	300	6.9	450,000	0	0	0	0	0
F69	6.5	300	6.9	250,000	0	0	0	0	0
F10	6.5	300	6.9	1,100,000	0	0	0	0	0
F67	6.5	300	6.9	450,000	0	0	0	0	0
F69	6.5	300	6.9	250,000	0	0	0	0	0

* Assay of treated filtrate diluted 10^{-1} in asolectin-Tween 80 inhibitor

to relatively small numbers within 15 sec. but in two trials only F10 was inactivated by exposure for 300 sec. at pH 5.0. Seven hundred ppm. inactivated the phages in 120 sec. and 600 ppm. gave practically the same results. As little as 100 ppm. displayed some destructive effect at pH 7.1, while 200 ppm. inactivated F10 and F67 in 300 sec. and 300 ppm. inactivated all three diluted phages in 15 sec. at this pH range.

Table 34 contains the results using alkyldimethylbenzylammonium chloride to treat the phages in 10^{-2} dilutions of whey filtrates. Fifty ppm. inactivated the phages in 15 sec. at pH 5.1, but 25 ppm. was insufficient for inactivation in 300 sec. at pH 7.0-7.05.

Results of similar tests with diisobutyl (or p-tertiaryoctyl)-phenoxyethoxyethyldimethylbenzylammonium chloride, methyldodecylbenzyl-trimethylammonium chloride, N-(acylcolaminoformylmethyl)-pyridinium chloride and 9-octadecenyldimethylethylammonium bromide are presented in Tables 35-38, inclusive. No significant differences between the actions of these compounds and of alkyldimethylbenzylammonium chloride appear in these data. No consistent difference in the susceptibility of the three phages to the action of the quaternary compounds is evident in these data. In Table 35 there was a considerable difference between the action of the quaternary ammonium compound when the treated filtrate was at pH 6.8 or 6.9 and at pH 7.4, when 25 ppm. of viruscide was employed.

TABLE 34

EFFECT OF ALKYLDIMETHYLBENZYLAMMONIUM CHLORIDE ON LACTIC STREPTOCOCCUS BACTERIOPHAGES IN 10^{-2} DILUTIONS OF WHEY FILTRATES AT 25° C.

Bacteriophage	pH of filtrate	Ppm. of virucide in treated filtrate	pH of treated filtrate	Calc. mpn./ml. in control	*Mpn./ml. after exposure time (in sec.) of: 15	30	60	120	300
F10	4.6	50	5.1	4,500	0	0	0	0	0
F67	4.6	50	5.1	25,000	0	0	0	0	0
F69	4.6	50	5.1	45,000	0	0	0	0	0
F10	4.6	50	5.1	4,500	0	0	0		0
F67	4.6	50	5.1	25,000	0	0	0		0
F69	4.6	50	5.1	45,000	0	0	0		0
F10	4.6	100	5.25	2,500	0	0	0	0	0
F67	4.6	100	5.25	25,000	0	0	0	0	0
F69	4.6	100	5.25	3,500	0	0	0	0	0
F10	4.6	100	5.3	2,500	0	0	0	0	0
F67	4.6	100	5.3	25,000	0.6	0	0	0	0
F69	4.6	100	5.3	3,500	0	0	0	0	0
F10	4.6	200	5.3	25,000	0	0	0	0	0
F67	4.6	200	5.3	45,000	0	1.8	0	0	0
F69	4.6	200	5.3	25,000	0.6	0	0	0	0

TABLE 34 (con'd)

Bacteriophage	pH of filtrate	Ppm. of virucide in treated filtrate	pH of treated filtrate	Calc. mpm./ml. in control	*Mpm./ml. after exposure time (in sec.) of: 15	30	60	120	300
F10	4.6	200	5.1	25,000	0	0	0	0	0
F67	4.6	200	5.1	45,000	1.8	0	0	0	0
F69	4.6	200	5.1	25,000	0	0	0	0	0
F10	4.6	300	5.2	25,000	0	0	0	0	0
F67	4.6	300	5.2	1,100,000	4	0.6	0	0	0
F69	4.6	300	5.2	9,500	0	0.8	0	0	0
F10	4.6	300	5.3	25,000	0	0	0	0	0
F67	4.6	300	5.3	1,100,000	0	0	0	0	0
F69	4.6	300	5.3	9,500	0	0	0	0	0
F10	6.5	25	7.0	15,000	900	800	500	300	90
F67	6.5	25	7.0	25,000	5,000	5,000	500	50	5
F69	6.5	25	7.0	1,500	1,900	500	400	500	9
F10	6.5	25	7.05	15,000	5,000	1,900	5,000	900	500
F67	6.5	25	7.05	25,000	5,000	5,000	5,000	5,000	1,950
F69	6.5	25	7.05	1,500	5,000	5,000	5,000	5,000	5,000

*Assay of treated filtrate diluted 10^{-1} in asolectin-Tween 80 inhibitor

TABLE 35

EFFECT OF DIISOBUTYLPHENOXYETHOXYETHYLDIMETHYLBENZYLAMMONIUM CHLORIDE ON LACTIC STREPTOCOCCUS BACTERIOPHAGES IN DILUTIONS OF WHEY FILTRATES AT 25° C.

Bacteriophage	pH of filtrate	Ppm. of virucide in treated filtrate	pH of treated filtrate	Calc. mpm./ml. in control	*Mpm./ml. after exposure time (in sec.) of: 15	30	60	120	300
Whey filtrates diluted 1:2									
F10	6.5	800	6.5	45,000	0	1.8	0	0	0
F67	6.5	800	6.5	75,000	0.8	0.8	0	0	0
F69	6.5	800	6.5	550,000	9,000	50	90	1.8	5
Whey filtrates diluted 10^{-1}									
F10	6.5	200	7.1	9,000	0	0	0	0	0
F67	6.5	200	7.1	15,000	0	0	0	0	0
F69	6.5	200	7.1	110,000	5	0	0	0	0
Whey filtrates diluted 10^{-2}									
F10	6.5	25	7.4	900	0	0	0	0	0
F67	6.5	25	7.4	1,500	0	0	0	0	0
F69	6.5	25	7.4	11,000	0	0	0	0	0
F10	6.5	25	6.8	250	50	50	50	90	50
F69	6.5	25	6.8	7,500	5,000+	5,000+	5,000+	5,000+	5,000+

TABLE 35 (con'd)

Bacteriophage	pH of filtrate	Fpm. of virucide in treated filtrate	pH of treated filtrate	Calc. mpn./ml. in control	*Mpn./ml. after exposure time (in sec.) of: 15	30	60	120	300
F10	6.5	25	6.9	250	900	220	220	220	500
F69	6.5	25	6.9	7,500	50,000	90,000	50,000	50,000	50,000

*Assay of treated filtrate diluted 10^{-1} in asolectin -Tween 80 inhibitor

TABLE 36

EFFECT OF METHYLDODECYLBENZYLTRIMETHYLAMMONIUM CHLORIDE ON LACTIC STREPTOCOCCUS BACTERIOPHAGES IN DILUTIONS OF WHEY FILTRATES AT 25° C.

Bacteriophage	pH of filtrate	Ppm. of virucide in treated filtrate	pH of treated filtrate	Calc. mpn./ml. in control	*Mpn./ml. after exposure time (in sec.) of: 15	30	60	120	300
				Whey filtrate diluted 1:2					
F10	6.5	800	6.7	125,000	500	0	0	0	0
F67	6.5	800	6.7	475,000	5,000	300	9	0.8	0
F69	6.5	800	6.7	2,250,000	50,000	50,000	2,200	500	9
F10	6.5	800	6.6	12,500	1.8	5	0	0	0
F69	6.5	800	6.6	375,000	9,000	900	50	1.8	5
				Whey filtrate diluted 10^{-1}					
F10	6.5	200	7.3	25,000	1.8	0	0	0	0
F67	6.5	200	7.3	95,000	50	5	0	1.2	0
F69	6.5	200	7.3	450,000	2,200	220	5	1.8	0
F10	6.5	200	7.1	2,500	0	0	0	0	0
F69	6.5	200	7.1	75,000	0	0	0	0	0
				Whey filtrate diluted 10^{-2}					
F10	6.5	25	7.4	2,500	220	90	90	50	50
F67	6.5	25	7.4	9,500	5,000	2,200	300	220	50
F69	6.5	25	7.4	45,000	5,000	500	220	90	50

* Assay of treated filtrate diluted 10^{-1} in asolectin-Tween 80 inhibitor

TABLE 37

EFFECT OF N-(ACYLCOLAMINOFORMYLMETHYL)-PYRIDINIUM CHLORIDE ON LACTIC STREPTOCOCCUS BACTERIOPHAGES IN DILUTIONS OF WHEY FILTRATES AT 25° C.

Bacteriophage	pH of filtrate	Ppm. of virucide in treated filtrate	pH of treated filtrate	Calc. mpm./ml. in control	Mpn./ml. after exposure time (in sec.) of: 15	30	60	120	300
				Whey filtrate diluted 1:2					
F10	6.5	800	7.0	125,000	1.8	0.8	0	0	0
F67	6.5	800	7.0	1,250,000	220	220	19	0	0
F69	6.5	800	7.0	2,250,000	5,000	500	300	9	5
F10	6.5	800	6.6	5,500	0	0	0	0	0
F69	6.5	800	6.6	125,000	0	0	0	0	0
				Whey filtrate diluted 10^{-1}					
F10	6.5	200	7.4	25,000	0	0	0	0	0
F67	6.5	200	7.4	250,000	5	3	0	0	0
F69	6.5	200	7.4	450,000	90	50	1.4	1.8	0
F10	6.5	200	7.0	2,500	0	0	0	0	0
F69	6.5	200	7.0	75,000	19	5	0	0	0

TABLE 37 (con'd)

Bacteriophage	pH of filtrate	Ppm. of virucide in treated filtrate	pH of treated filtrate	Calc. mpn./ml. in control	*Mpn./ml. after exposure time (in sec.) of: 15	30	60	120	300
					Whey filtrate diluted 10^{-2}				
F10	6.5	25	7.4	2,500	0.8	9	50	50	9
F67	6.5	25	7.4	25,000	9	90	500	2,200	90
F69	6.5	25	7.4	45,000	19	5,000	950	5,000+	500
F10	6.5	25	6.7	250	220	220	50	50	50
F69	6.5	25	6.7	7,500	2,200	500	2,200	2,200	900

*Assay of treated filtrate diluted 10^{-1} in asolectin-Tween 80 inhibitor.

TABLE 38

EFFECT OF 9-OCTADECENYLDIMETHYLETHYLAMMONIUM BROMIDE ON LACTIC STREPTOCOCCUS BACTERIOPHAGES IN DILUTIONS OF WHEY FILTRATES AT 25° C.

Bacteriophage	pH of filtrate	Ppm. of virascide in treated filtrate	pH of treated filtrate	Calc. mpm./ml. in control	*Mpm./ml. after exposure time (in sec.) of: 15	30	60	120	300
				Whey filtrates diluted 1:2					
F10	6.5	800	6.9	125,000	0	0	0	0	0
F67	6.5	800	6.9	475,000	50	1.8	0	0.8	0
F69	6.5	800	6.9	2,250,000	5,000+	5,000+	19	5	0
				Whey filtrates diluted 10^{-1}					
F10	6.5	200	7.35	25,000	0	0	0	0	0
F67	6.5	200	7.35	95,000	1.8	0	0	0	0
F69	6.5	200	7.35	450,000	1.8	0.8	0	0	0
				Whey filtrates diluted 10^{-2}					
F10	6.5	25	7.25	2,500	50	50	50	50	50
F67	6.5	25	7.25	9,500	50	50	50	50	50
F69	6.5	25	7.25	45,000	500	220	50	50	50
F10	6.5	25	6.9	250	0	0	0	0	0
F69	6.5	25	6.9	7,500	0	0	0	0	0

*Assay of treated filtrate diluted 10^{-1} in asolectin-Tween 80 inhibitor.

DISCUSSION

The known modes of dissemination clearly indicate that the control of lactic streptococcus bacteriophage involves its destruction in the air, on surfaces and in liquids. When chemical viruscidal agents are to be used to disrupt dissemination channels such agents logically would be applied in the form of either aerosols or liquids. Both applications were included in this investigation, although contaminated surfaces were not treated.

Although the dissemination of this type of bacteriophage through the air has been emphasized more than have other modes, little data on the number of phage particles which might be retained in the air over a given period of time were found in the literature. The information obtained by the procedures followed in this investigation seemed essential as a basis of measuring the viruscidal action of aerosols to be tested. Attempts to obtain such information therefore constituted the beginning of the present research.

The experiences of Whitehead and Hunter (1941) and Wolf et. al. (1946) demonstrated the advantages of sampling the air by aspiration through liquid or by exposing petri dishes containing a liquid rather than solid medium. Therefore modifications of their procedures were adopted. Comparisons of counts of phage particles before and after bubbling air for 10 min. through 10^{-2}, 10^{-3} and 10^{-4} dilutions of whey filtrate did not reveal any large degree of phage destroying effect as a

result of this treatment (See Table 2). Distilled water or buffered distilled water rather than broth, therefore was used for aspiration samples to avoid trouble with foam. Counts of phage particles in samples of air obtained by aspiration after being held for 7 hr. or longer were as high as immediately after sampling.

Preliminary tests made on the persistence of active phage in still air indicated a very rapid decrease in numbers. A fan therefore was used for the purpose of reducing such rapid settling. The fan also caused a more rapid and thorough distribution of the aerosols. It is very probable that neither the atomized filtrates nor the aerosols were uniformly distributed throughout the room. The procedure did seem to simulate to a fair degree the conditions which likely would exist in a commercial dairy plant as whey or other liquids containing the phage might be incorporated in the air and then treated with aerosols. Operations were performed and samples were taken in similar positions so that the results of the different trials might be considered comparable.

The room was kept closed practically all the time during the tests. With the change in air which normally would occur with good ventilation in a commercial plant, the phage might be scattered more widely and the concentration in any one localized area correspondingly lowered.

The tests made on the effect of atomizing the whey filtrate into 2-liter flasks (Table 1) indicate that the mechanical action did not consistently inactivate any considerable numbers of particles. When the filtrates were atomized in the much larger space of the room there was opportunity for dehydration of the droplets which could not occur in

the flasks. The fact that the cumulative numbers of active phage particles recovered in petri dishes in the room (Tables 3-11) decreased so markedly soon after atomizing, indicates that the phages may have been inactivated to a greater degree than in the flasks. Simultaneous sampling of the air indicated that the number of active phage particles in the air also were decreasing. Nelson et al. (1939) demonstrated that desiccation under apparently less rigorous conditions caused from slight to almost complete inactivation of the principle inhibitory to acid development by cultures. The results of the trial presented in Table 3 when the filtrate atomized was at pH 4.85 showed much more drastic reductions than at higher pH levels but this was not true at pH 4.45 (Table 4).

Twort et al. (1940) presented data to show that droplets of about 5 microns in radius settled very rapidly. The finer droplets settled less rapidly and might be expected to dehydrate to still smaller sizes more readily. The sizes of the mist particles were not determined in the present investigation but probably were as small as ordinarily would be encountered in practical dairy plant operation.

The present data agree in general with those of Wolf et al. (1946) in demonstrating a rapid decrease in active phage particles recovered from the air. Although they were able to recover less than 5 per cent of the phage dispersed into the air, by means of aspiration through a slit sampler depositing particles upon gelatine plates, 4.8, 0.4 and 0.02 per cent recoveries in 3-min. periods were obtained 2, 12 and 30 min. respectively after spraying the phage. One hr. after spraying, no phage was recovered by deposition on agar plates. The present data show

recoveries of phage particles in greater numbers and over slightly longer periods of time in most trials (Tables 3-11).

The rapidity with which the phage settled out of the air or became inactivated seems to discredit to some extent the relative emphasis which has been placed upon the dissemination of lactic streptococcus bacteriophage through the air. It is true that dust which might be carried by the air was found to be a source of phage by Whitehead and Hunter (1941) and active phage particles were re-suspended in the air in some tests of the present investigation but they may not always be recovered in the air under these conditions and are in only small numbers when detected. However, entry of only a very small number of phage particles, possibly only one, into a susceptible culture might result in very high bacteriophage populations after incubation, because of the exceedingly rapid rate of increase characteristic of bacteriophages.

Humidity of the air, according to Baker and Twort (1941), is an important factor in the action of aerosols. Most of the present virucidal tests were conducted with relative humidities above 60 per cent which was considered the most effective range for glycol and hypochlorite mists. The humidity of dairy processing rooms would ordinarily be at least 60 per cent. More or less isolated culture laboratories might sometimes have lower humidities. Lester, Kaye, Robertson and Dunklin (1950) maintain an opposite view of the relation of humidity to the effectiveness of triethylene glycol vapors. They refer to unpublished data in press which is interpreted to indicate that glycol vapor is much less effective at humidities above 60 per cent than below. Pulvertaft

et al. (1945) concluded that if an aerosol was hygroscopic, atmospheric humidity will not be of much importance. Glycol is rather hygroscopic.

It is a well recognized fact that higher temperatures generally accelerate the action of disinfectants. All of the tests of aerosols in this investigation were conducted at relatively warm temperatures such as might be encountered in the summer in many dairy plants and in the winter in well-heated processing rooms.

The present results agree with those of Wolf et al. (1946) in indicating ineffectiveness of glycols as aerosols against lactic streptococcus phage. The effectiveness of glycols dispersed by other means than by the DeVilbiss atomizer was not tested in the present investigation. Puck (1947) demonstrated that it was the vapor phase of glycols that was essential for aerial disinfection. However, Twort et al. (1940) found ethylene glycol and propylene glycol droplets 2 microns in radius evaporated in about 7 sec. and 4 sec. respectively. Baker and Twort (1944) concluded after bactericidal tests of propylene glycol, including various means of dispersal, that "near the boiling temperature and possibly beyond the results with the hot plate are as good as those obtained by mechanical atomization".

In evaluating the results of viruscidal treatments in this study, inactivation of the phage has been considered as being accomplished when there were no active particles present, rather than when 99 per cent inactivation occurs. In many cases, the latter criterion would have shown the viruscides to be more effective. The more rigid standard than is common in bactericidal studies probably is advisable because of the

greater rate of proliferation of bacteriophages than of bacteria under favorable conditions.

The dispersal of approximately 0.61 g. of available chlorine per 1,000 ft.^{3} of air space apparently represents the minimum effective treatment (See Tables 19-21). Even the dispersal of the 0.75 g. of chlorine per 1,000 ft.^{3} 3-2 min. before infection of the room was slightly less effective than dispersal simultaneously or following infection of the room. This demonstrates the importance of dispersing an effective concentration of viruscide in the air at all times that active phage may appear in the atmosphere. Air changes in the ventilation of rooms being treated might also require heavier dosages and/or more continuous treatment.

In order to obtain the most rapid and complete mixing of hypochlorite aerosols with the air and also to avoid too rapid settling of the mist droplets, atomizers which produce relatively fine mists should be used. The use of the cheaper insect sprayers or similar dispensers would probably give unsatisfactory results.

The results of corrosive action of the calcium hypochlorite aerosols upon exposed iron and copper parts of equipment in the test room were observed. Stainless steel is highly resistant to corrosion but care should be taken to protect some other metals from aerosols of this kind.

Chloramines generally are considered as reacting more slowly than hypochlorites. Although less destructive action of chloramine-T was apparent in the present tests, continuous dispersal of this compound over

longer periods might present more favorable results. Also, provisions for keeping the pH of the chloramine-phage mixture at a level comparable to that obtained with the hypochlorite probably would change the effectiveness of the chloramine-T significantly.

The presence of organic matter in liquids very definitely reduced the viruscidal action of solutions of either calcium hypochlorite (cf. Tables 28-30) or alkyldimethylbenzylammonium chloride (cf. Tables 32-34) of a given concentration. Because of the filtration methods employed in obtaining the filtrates used, the filtrates probably would contain a lower percentage of organic matter than the usual whey from cheese. The few tests made on filtrates containing 10 per cent added culture (Table 28) indicate that ordinary cheese whey may require heavier dosages of chlorine than are indicated in the results of this investigation.

Since lower concentrations of the viruscides inactivated the phages when the filtrates were diluted 10^{-1} or 10^{-2}, it is important that rinse solutions be kept as nearly free as possible of milk and whey or other milk derivatives. Thorough washing, followed by a preliminary rinse before the use of viruscidal solutions is highly recommended.

Wide variations in buffer actions of the phage preparations and of the viruscidal products were observed. Therefore attention should be given to this characteristic of viruscides. The kinds and amounts of cleansing agent residues which may be present on surfaces to be treated or which may be carried over into the viruscidal rinses may affect very markedly the efficiency of the viruscidal treatment. Increased alkalinity

would be advantageous if a quaternary ammonium compound were to be used but would reduce the activity if available chlorine were the virus-cidal agent.

The tap water used in the two trials in Table 28 contained relatively little impurity. More impurity, especially hardness, in many water supplies would very likely produce results more divergent from those obtained when distilled water was used as a diluent for the viruscides (cf. McCulloch, 1945; Lawrence, 1950).

The smaller numbers of phage particles present in the test filtrates reduced to some extent the numbers of active phage particles surviving (See Table 30). This factor should be kept in mind in practical applica-tions.

Exposure times over 300 sec. in liquids were not included in the present tests because this limit was considered to cover the extent of most treatments under practical conditions.

Chloramine-T, in comparison with calcium hypochlorite, was even less effective in liquids than in the air (cf. Tables 31, 28-30; 23, 19-21). The higher alkalinity of the chloramine-T product as revealed in the higher levels of pH in the treated filtrates (Table 31)may have been a disadvantage.

The data in Tables 28 and 32 clearly indicate a decrease in effec-tiveness of chlorine and an increase in effectiveness of alkyldimethyl-benzylammonium chloride at higher pH levels within the acid range. These results are in agreement with those given on the germicidal activities of these chemicals by McCulloch (1945) and Lawrence (1950).

The properties of quaternary ammonium compounds have been well reviewed by Lawrence (1950). They have the advantages of being only very slightly corrosive, practically oderless and relatively stable. Information on the toxicity of the compounds is rather meager considering the number of kinds. This subject also has been reviewed by Lawrence (1950). In view of this fact, health and pure food authorities who have jurisdiction over the dairy plants considering the use of the compounds should be consulted in advance.

The tests made on the different kinds of quaternary ammonium compounds did not reveal any outstanding differences in their actions. Some differences might be observed by making a larger number of trials and more nearly standardizing the numbers of active particles present in the liquids tested.

The results of the action of quaternary ammonium compounds on lactic streptococcus bacteriophage obtained in this investigation are far from agreement with those reported by Prouty (1949). Since Prouty did not use an inhibitor to stop the action of the compounds at the end of certain periods of exposure, the action observed by him probably was partly static, rather than entirely viruscidal. Lawrence (1950) has reviewed several reports on this point.

In many cases chemical viruscidal agents may be more conveniently and/or effectively applied in the inactivation of lactic streptococcus bacteriophage than heat can be. The use of effective chemicals is particularly advantageous in destroying air-borne phage. Calcium hypochlorite was the only effective aerosol for the purpose among the

agents tested in the present investigation. Although 0.61-0.75 g. of available chlorine per 1,000 ft.^{3} completely inactivated the phage in the air at the time of dispersal in these trials, the use of 1 g. per 1,000 ft.^{3} in a fine mist is recommended to allow a margin of safety. Such treatment is suggested to clear the atmosphere where milk or cultures are exposed to air suspected of carrying phage. The use of such an aerosol during the dispersal of a mist of phage-carrying liquid from an open discharge separator or other equipment would also be a good practice under circumstances where corrosion would not make such use impractical.

A final rinse containing 100 ppm. of available chlorine from hypochlorite or 200 ppm. of an approved quaternary ammonium compound equal in effectiveness to alkyldimethylbenzylammonium chloride is recommended for all utensils, equipment, lower walls and floors. These amounts make reasonable allowances for variations in pH and other factors. This treatment should always be preceded by thorough cleansing and by rinsing with clear water, due to the deleterious effect of organic matter and the possibility that the pH might be influenced adversely by a residue of washing powder and/or milk solids.

SUMMARY AND CONCLUSIONS

The persistence of air-borne infection with three lactic streptococcus bacteriophages and the action of certain viruscidal agents against these phages suspended in both air and liquid have been studied in this investigation.

Most of the Streptococcus cremoris phage carried in whey filtrates atomized into the air settled or otherwise disappeared in 1 to 2 hr., but some phage was recovered in certain of the trials 7 to 8 hr. after dispersal.

It was possible in some cases to resuspend the phage in the air after settling. Phage was recovered on surfaces in the room 2 days after dispersal into the air.

Glycols were not found sufficiently effective to recommend their use either as aerosols or liquids in control of lactic streptococcus phage.

Aerosols of calcium hypochlorite supplying a minimum of 0.61 g. of available chlorine per 1,000 $ft.^3$ of air completely inactivated air-borne phage under the conditions of this investigation.

Alkyldimethylbenzylammonium chloride aerosol had a highly astringent effect upon the respiratory tract of persons in the room where it was dispersed. A concentration of the compound which still failed to inactivate the phage was intolerable.

When the tests were carried out on bacteriophage suspended in a 1:2 dilution of whey with an original pH of 5.0, 400 ppm. of available

chlorine from calcium hypochlorite inactivated the three phages used in this investigation in 15 sec. More than 500 ppm. were found necessary to inactivate the phages in 300 sec. in 1:2 whey dilutions at pH 6.4-6.6. The phages when suspended in a 10^{-1} dilution of whey with an original pH of 5.0 were inactivated in 60 sec. by 100 ppm. of available chlorine from calcium hypochlorite but survived in small numbers an exposure of 300 sec. at pH 6.9-6.95. The phages when suspended in 10^{-2} dilutions of whey at pH 5.4-6.0 were inactivated in 15 sec. by 25 ppm. of available chlorine from calcium hypochlorite but were not inactivated in 300 sec. by the same concentration of chlorine at pH 7.2-7.4.

There was some evidence of differences in susceptibility to available chlorine between the three phages but the data were not generally consistent on this point.

Available chlorine from chloramine-T was much slower in action and was very noticeably less effective even after 300 sec. exposures than available chlorine from calcium hypochlorite. A higher pH level in the former may have been a factor.

A concentration of 1,000 ppm. of alkyldimethylbenzylammonium chloride inactivated the phages in 30 sec. when they were suspended in 1:2 dilutions of whey at pH 6.6-6.65 but failed to inactivate them at pH 4.6. Six hundred ppm. of the compound inactivated the phages in 120 sec. in a 10^{-1} dilution of whey at pH 4.8 and 300 ppm. inactivated the phages in 15 sec. at pH 6.9. When the phages were suspended in 10^{-2} dilutions of whey, 50 ppm. of the compound inactivated the phages in 15 sec. at

pH 5.1 but 25 ppm. was insufficient to inactivate them even at pH 7.0-7.05.

Presence of organic matter decreased the effectiveness of either available chlorine or the quaternary ammonium compounds studied in the destruction of the phages.

Available chlorine was more effective in the destruction of the phages at the lower pH levels than at the higher levels within the range of pH 4.6 to 7.4. The opposite was true of alkyldimethylbenzylammonium chloride.

No outstanding differences in the actions of the five quaternary ammonium compounds on lactic streptococcus bacteriophages were observed.

The use of 1 g. of available chlorine per 1,000 $ft.^3$ from hypochlorite in a fine aerosol is recommended for the inactivation of airborne lactic streptococcus bacteriophage. A final rinse containing 100 ppm. of available chlorine from hypochlorite or 200 ppm. of an approved quaternary ammonium compound equal in effectiveness to alkyldimethylbenzylammonium chloride is suggested for the destruction of the phage on cleaned surfaces.

LITERATURE CITED

Anderson, E. B. and Meanwell, L. J. 1942. The problem of bacteriophage in cheese making. Part I. Observations and investigations on slow acid production. Jour. Dairy Res. 13:58-72.

Babel, F. J. 1946. Factors influencing acid production by cheese cultures. II. Influence of bacteriophage on acid production in the manufacture of cheddar and cottage cheese. Jour. Dairy Sci. 29:597-606.

_____ 1947. Studies on the susceptibility of certain cheese cultures to the action of bacteriophage. Jour. Dairy Sci. 30:507-516.

Baker, A. H. and Twort, C. C. 1941. The effect of humidity of air on the disinfection capacity of mechanically atomized and heat-volatilized germicidal aerosols. Jour. Hyg. 41:117-130.

_____ and _____. 1944. Germicidal mists and vapours in air disinfection. Jour. Hyg. 43:382-394.

Beckwith, T. D. and Rose, Blythe, J. 1930. The bacteriophage content of sewage and its action upon bacterial organisms. Jour. Bact. 20:151-159.

Bigg, Edw., Jennings, B. H., Olson, F. C. W. 1945. Epidemiologic observations on the use of glycol vapors for air sterilization. Amer. Jour. Pub. Health 35:788-798.

Buchanan, R. E. and Fulmer, E. I. 1928. Physiology and chemistry of bacteria. Vol. 1. Baltimore, Md., Williams and Wilkins Co.

Diénert, F. 1934. Le bacteriophage dans les eaux. Rev. Microbiol. Appl. Agric., Hyg., Indust. (Paris) 1(3):153-162.

Dunham, Wolcott B. and MacNeal, Ward J. 1943. Inactivation of vaccinia virus by mild antiseptics. Jour. Lab. and Clin. Med. 28:947-953.

Hammer, B. W. 1933. The germicidal property of milk. In Report on agricultural research for the year ending June 30, 1933. Ia. Agric. Exp. Sta., 1933+63.

Harriman, L. A. 1934. Causes of slow acid production in butter cultures. Unpublished Ph. D. Thesis. Iowa State College Library, Ames, Iowa.

Henle, Werner and Zellat, Joseph. 1941. Effect of propylene glycol aerosol on air-borne virus of influenza A. Proc. Soc. Exp. Biol. and Med. 48:544-547.

Hunter, G. J. E. 1944. The influence of bacteriophage on the cheese making process. Jour. Dairy Res. 13:294-301.

_____ 1949. The characteristics of streptococcal phages. Interaction between different races. Jour. Dairy Res. 16:374-376.

_____ and Whitehead, H. R. 1940. The action of chemical disinfectants on bacteriophages for the lactic streptococci. Jour. Dairy Res. 11:62-66.

_____ and _____ 1949. Phage-carrying cultures as cheese starters. Jour. Dairy Res. 16:368-373.

Jensen, T. M. 1943. Report for year ended 30th June, 1943. Jour. Dept. Agric. Victoria 41:504-509.

Johns, C. K. 1943. Further studies on bacteriophage in relation to cheddar cheesemaking. Jour. Dairy Res. 13:119-122.

Kalter, S. S., Mordaunt, V. and Chapman, O. D. 1946. The isolation of Escherichia coli phage by means of cationic detergents. Jour. Bact. 52:237-240.

Klein, M., Kalter, S. S. and Mudd, S. 1945. The action of synthetic detergents upon certain strains of bacteriophage and virus. Jour. Immunol. 51:389.

_____ and Stevens, D. A. 1945. The in vitro and in vivo activity of synthetic detergents against influenza A virus. Jour. Immunol. 50:265-273.

Krueger, A. P. 1930. A method for the quantitative determination of bacteriophage. Jour. Gen. Physiol. 13:557-564.

Lange, Norbert Adolph. 1946. Handbook of chemistry. Sandusky, O., Handbook Publishers, Inc.

Lawrence, Carl A. 1950. Surface active quaternary ammonium germicides. N. Y., Academic Press, Inc.

Lester, William Jr., Kaye, Saul, Robertson, O. H. and Dunklin, Edward W. 1950. Factors of importance in the use of triethylene glycol vapor for aerial disinfection. Amer. Jour. Pub. Health, 40:813-820.

Maier, Eugene. 1939. Preservation of biological fluids (bacteriophage, vaccines and venom solutions) with alkyldimethylbenzylammonium chloride. Jour. Bact. 38:33-39.

Mazé, P. 1941. I. Origine des bacteriophages des ferments lactiques normaux du lait. Compt. Rend. Soc. Biol. 135:807-808.

_____ 1941. II. Origine des bacteriophages des ferments lactiques normaux du lait. Compt. Rend. Soc. Biol. 135:1332-1335.

McCulloch, Ernest C. 1945. Disinfection and sterilization. Phila., Lea and Febiger.

Miller, D. D. and Elliker, P. R. 1950. A test for quaternary ammonium compounds in milk and in detergent sanitizers. Ore. Agric. Exp. Sta. Circ. of Inf. 472.

Nelson, F. E., Harriman, L. A. and Hammer, B. W. 1939. Slow acid production by butter cultures. Iowa Agric. Exp. Sta. Res. Bull. 256:219-287.

Nichols, A. A., and Hoyle, Margery. 1949. Bacteriophage in typing lactic streptococci. Jour. Dairy Res. 16:167-208.

_____ and Wolf, J. Z. 1942. A note on the recovery of bacteriophage. Proc. Soc. Agr. Bact. (Gr. Britain) 1942:19. (Original not examined. Abstracted in Jour. Dairy Sci. 26:A124. 1943).

_____ and _____ 1944. The persistence and recovery of bacteriophage in cheese. Jour. Dairy Res. 13:302-307.

Prouty, C. C. 1949. Inactivation of bacteriophage of the lactic acid streptococci of starters by quaternary ammonium compounds. Jour. of Milk and Food Tech. 12:214-218.

Puck, T. T. 1947. The mechanism of aerial disinfection by glycols and other chemical agents. Jour. Exp. Med. 85:729-757.

Pulvertaft, R. J. V., Lemon, G. C. and Walker, J. W. 1945. Atmospheric and surface sterilization by aerosols. Lancet, 236:443-446.

Robertson, O. H., Bigg, Edward, Puck, Theodore T. and Miller, Benj. F. 1942. The bactericidal action of propylene glycol vapor on micro-organisms suspended in air. I. Jour. Exp. Med. 75:593-609.

_____, Loosli, Clayton, G., Puck, Theodore T., Bigg, Edward and Miller, Benjamin F. 1941. The protection of mice against infection with air-borne influenza virus by means of propylene glycol vapor. Sci. 94:612-613.

_____, Puck, Theo. T., Lemon, Henry F., and Loosli, Clayton G. 1943. Lethal effect of triethylene glycol vapor on air-borne bacteria and influenza virus. Sci. 97:142-144.

Rosebury, Theodor, Meiklejohn, Gordon, Kingsland, Lawrence C., and Boldt, Martin H. 1946. Disinfection of clouds of meningopneumonitis and psittacosis viruses with triethylene glycol vapor. Jour. Exp. Med. 85:65-76.

Stokes, Joseph Jr., and Henle, Werner. 1942. Studies on methods of prevention of epidemic influenza. Jour. Amer. Med. Assoc. 120:16-20.

Sutton, W. S. 1939. Bacteriophage in a pure strain culture of Streptococcus cremoris in New South Wales. Jour. Australian Inst. Agric. Sci. 5:168-169. (Original not examined. Abstracted in Biol. Abstr. 14:16550. 1940).

Tilley, F. W. and Anderson, W. A. 1947. Germicidal action of certain chemicals on the virus of Newcastle disease. Vet. Med. 42: 229-230.

Twort, C. C., Baker, A. H., Finn, S. R. and Powell, E. O. 1940. The disinfection of closed atmospheres with germicidal aerosols. Jour. Hyg. 40:253-344.

Weber, George R. and Black, Luther A. 1948. Laboratory procedure for evaluating practical performance of quaternary ammonium and other germicides proposed for sanitizing food utensils. Amer. Jour. Pub. Health 38:1405-1417.

Whitehead, H. R. and Cox, G. A. 1935. The occurrence of bacteriophage in cultures of lactic streptococci. A preliminary note. New Zealand Jour. Sci. Tech. 16:319-320.

_____ and _____ 1936. Bacteriophage phenomena in cultures of lactic streptococci. Jour. Dairy Res. 7:55-62.

_____ and Hunter, G. J. E. 1941. Starter cultures for cheese manufacture. Further attempts to eliminate failures due to bacteriophage. Jour. Dairy Res. 12:63-70.

_____ and _____ 1945. Bacteriophage infection in cheese manufacture. Jour. Dairy Res. 14:64-80.

_____ and _____ 1947. Bacteriophage in cheese manufacture. Contamination from farm equipment. Jour. Dairy Res. 15:112-120.

Wolf, J. Z., Nichols, Agnes A. and Ineson, P. June. 1946. Mists containing hypochlorites as germicides in the destruction of air-borne bacteriophage attacking lactic streptococci. Jour. Dairy Res. 14:291-315.

ACKNOWLEDGEMENTS

The writer wishes to express deep gratitude to Dr. F. E. Nelson for directing this investigation and for guidance in the preparation of this manuscript.

Sincere appreciation also is expressed to Dr. C. E. Parmelee for many helpful suggestions and for assistance with a number of the details of the laboratory work.