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POSSIBLE APPLICATIONS OF BACTERIOLOGICAL WARFARE TO PUBLIC WATER SUPPLIES

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PREFACE

Epidemic diseases arising from natural causes have plagued military forces for centuries and have exacted heavy tolls as nonbattle casualties. Often, the outcome of wars has been decided by this third party. Contaminated water has played a major role in the spread of infection throughout troop concentrations. Therefore, the deliberate introduction of pathogenic agents into a water supply could cause untold casualties and havoc among military and civilian populations.

From this standpoint, it is of interest to the Navy to consider the possibilities of bacteriological warfare as it would relate to water supply and to protective measures that could be instituted to lessen the danger.

The Office of Naval Research, through the Naval Research Reserve, sponsors a training program to maintain the mobilization readiness of reserve officers with scientific and technical backgrounds. As a part of this program, members are encouraged to participate in projects which bring their talents to bear on some of the current problems in their respective fields.

LCDR John R. Maloney, USNR, of Naval Reserve Research Company 9-3, Des Moines, Iowa, was assigned such a project. During a period of approximately six months, under appropriate duty orders issued by the Commandant Ninth Naval District, LCDR Maloney conducted an exhaustive search of unclassified literature available on the subject of bacteriological warfare and water supply. His report, submitted as Research Reserve Project number 7, was considered worthy of publication. It is therefore being reproduced in this issue of "Surveys of Naval Science."

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POSSIBLE APPLICATIONS OF BACTERIOLOGICAL WARFARE
TO PUBLIC WATER SUPPLIES

INTRODUCTION

During World War II and later during the Korean Action there was considerable discussion, particularly in the public press, concerning the use of pathogenic organisms as a means of warfare. A search of available literature fails to reveal any authenticated instance in which Bacteriological Warfare was used in military acts, either offensively or defensively. It is conceivable that local commanders in the field have contaminated or have simulated the contamination of a stream or other source of water with the purpose of denying the use of such source to the enemy. It has been unofficially reported by troops that German forces did this in World War I. Paris, France, during World War I made arrangements to color the city water supply with a harmless blue or green dye as a warning to the citizens if the supply was believed to have been contaminated (1). It is the purpose of this review to evaluate the various means of bacteriological contamination of water supplies by an enemy, detection of this type of contamination, and action necessary for disinfection.

Bacteriological Warfare, for the purpose of this paper, is defined as follows: the introduction of living organisms, their spores or toxins, into a water supply with the purposeful intent of causing illness, disease, or death to individuals. Unless otherwise specified, the term BW in this paper will imply this limited definition.

The discussion will be limited largely to possibilities of contamination of municipal supplies, or parts of such systems, and large individual supplies (large military bases or industrial plants either served by city supplies or by independent sources). An extensive search of available literature applicable to this area of interest has been made. The author has deemed it his privilege to draw on his experience to interpret or qualify, and in a few instances to advance thoughts which he believes to be original. An honest effort has been made to credit all sources used.

For convenience, an objective approach to this subject has been chosen. We have not dealt with the moral issue of BW, as we do not believe such discussion is appropriate. In a total war, there is a very definite probability that BW may prove to be more humane than the classical methods of warfare. The civilian population has great fear of the use of BW and perhaps the greatest deterrent to the use of BW is the fear of retaliation. In the following discussion, we cannot escape the conclusion

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that the so-called "have not" nations can produce and use the weapons of BW just as effectively as the "have" nations.

CONTAMINATION OF PUBLIC WATER SUPPLIES

Historical Survey

The water works industry has for many years been seriously concerned with the potential hazards created when a physical connection between a potable supply and a supply of doubtful purity has been made. Such a connection is commonly known as a "cross-connection." Regulations concerning cross-connections have been promulgated by all state departments of health.

Numerous instances of illness, gastro-intestinal in nature, and presumed to have been caused by such cross-connections, have been cited in the literature. The outbreak in the Congress and Auditorium Hotels in Chicago in 1933 is probably the most outstanding example in recent years. This incident was caused by accidental pollution of the water supply of the Hotels and resulted in an epidemic of amebiasis (2). A total of 1409 cases of illness with 98 deaths involving individuals from over 400 cities from 43 states and 3 Canadian Provinces resulted and it is generally conceded that not all cases were reported (3). During 1945, a number of major outbreaks of gastroenteritis aboard ships in the Pacific Ocean area were investigated. The use of polluted sea water in the vegetable peelers was responsible for at least two of these outbreaks, resulting in a total of 500 cases, two deaths and the loss of the services of two ships for a total of three months (4). The classical case of "Typhoid Mary," found in practically every elementary text on sanitary bacteriology, emphasizes very well the dangers encountered from casual contamination. In Canada at St. Jerome de Matane, there was an outbreak of bacillary dysentery in which the causative organism was Flexner's bacilli. This was a very severe epidemic consisting of 2000 cases and resulting in 40 deaths. It was presumed that the causative organism from fecal material was carried on the boots of workmen (5).

Since it is improbable that the causative agent of an epidemic would be detected by the existing methods in routine use, Wolman in his discussion of the Canadian outbreak said, "The question still remains as to whether a more or less precise bacterial index for the determination of the sanitary quality of water is desired." In a discussion of BW it cannot be overemphasized that the existing practices in the United States and some other countries is one which aspires primarily to produce a water which is devoid of coliform types.

In the few cases cited and in many others which have occurred in the United States and other countries it has been demonstrated conclusively that illness can be caused by casual contamination - careless workmen, backsiphonage, etc. When we consider the practicality of BW, it seems obvious that if illness can be caused by accident it can also be caused by design. Also, with "contamination by design" the pathogenic organisms would be present in high concentration and would be placed in locations which would be strategically important. Any nation which plans to engage in BW will not depend on one single causative organism but will have an "arsenal" of various organisms which may be used to attain specific objectives and to make detection more difficult. It is well known that an organism may have its virulence modified by laboratory techniques. It is perhaps not so widely known that there is evidence to show that the transmission of infection from one continent to another may be accompanied by an increase in virulence due to the lack of native immunization against a particular strain.

One cannot omit the psychological implications of BW. The nation which first uses BW on a large scale (that is in several areas simultaneously) will gain considerable advantage from the element of surprise. Many people in several areas would become ill. There would be delay in the identification of the organisms and many laboratories would not have the appropriate culture media if the organism was one which they did not encounter routinely. If, at the same time, rumors could be started that the department of health, hospitals, and others responsible were not capable of dealing with the problem, the panic among the population could develop into a very serious problem for the community immediately involved. This panic could be spread to other communities. In his own experience, the author has noted this effect many times. At the time of the Chicago epidemic mentioned above many queries were received relative to possibilities of the same event happening in Des Moines, or if it had ever happened and had been "hushed up." This type of gossip is very difficult for a municipality or other authority to combat.

Generally speaking, one must conclude that the public prefers to believe the worst. Government officials from Japan, Germany, England, and Turkey have informed the author in private conversation that the same fact is true in their respective countries. In the consideration of the psychological aspects of BW, the author has noted that as a result of gossip a number of individuals always become "ill" due to the consumption of the city supply of water. It is suggested that psychological BW could be quite successful for at least a short time if the following rumor were started: "The enemy has discovered a new agent for the contamination of the water supply. It is without taste or odor and the only symptom is a tired feeling with aches in various parts of the body (in short, symptoms which every person can detect in his body with a little suggestion)."

In almost any military action, the production of panic in the civilian population is in many cases as beneficial as the immediate military objective. This panic would be easier to produce in the early days of a war than after the population had become inured to the hardships of war.

In the consideration of actual BW, possibilities have roughly kept pace with the advances in the fields of biology, bacteriology, and medicine; we must assume other nations have at least as much knowledge as we have. It is of particular importance to emphasize the increased knowledge in the art of developing strains of organisms which will remain viable under environmental conditions which are normally not favorable for that particular organism. It is unfortunate, at least for the areas which might be subjected to BW, that detection and identification procedures have not kept pace. With the routine methods in use in nearly all laboratories forty-eight to ninety-six hours are required for detection. This assumes that the BW agent will be detected by the APHA Standard Methods, but this is not a safe assumption. If we ever employ biological warfare against Russia, we would not, advisably, use an agent which would be detected by methods used to detect the coliform organism, which is reported to be the index used by Russia (6).

It should be noted that the person using the agent need not have highly specialized knowledge for the actual introduction of the organism into the water supply. An individual with only a basic knowledge of plumbing could easily introduce the pathogens into the system by any of several means.

Characteristics of an Ideal BW Agent

One must consider the characteristics of an ideal agent for BW and then, depending on the specific objective, decide which of the characteristics may be sacrificed to achieve the purpose. The characteristics of an ideal BW agent are as follows:

1. The agent should have an incubation period of five to ten days after it enters the body, with the exception of occasions when immediate effect is desired. Since a delay of several days would make the definite ascertainment of sabotage rather difficult, contamination of several locations in a community, or even several communities, could be accomplished and yet permit the escape of the saboteur before illness had occurred.

2. The organism should cause a long, disabling illness, requiring care and medication. This would not only disable the patient but deny the services of members of his family or others to the defense effort. Physicians, nurses, drugs, and other supplies might

becomes critical. The defense, or essential civilian production of an area could be caused to lag.

3. The illness should be one against which persons are not normally immunized and should, preferably, be an illness which confers, at the most, doubtful immunity to the victim.

4. The BW agent should be one which would not be detected by the methods routinely used for bacteriological analyses of water in the country concerned.

5. The organism should remain viable at pH's and temperatures encountered in a water supply and should suffer no loss of virulence.

6. The agent should be capable of maintaining its viability in the dehydrated or condensed state and/or one which saboteurs can grow at the area of their activities. If it is to be grown in foreign areas, the organism should grow on materials readily available, i. e. milk, potato, cereal, etc., or compounded from simple substances which can be obtained without arousing suspicion.

It is rather obvious that an organism which meets all of these criteria probably can not be found. However, organisms meeting most of these requirements can be cultured and adapted for the purpose of contaminating water supplies.

Practicable BW Agents

The classic diseases spread by water - cholera, typhoid, and the related Salmonella - cannot be considered as good agents for BW (7) since there are some uncertainties in the infectivity of these agents. Also, vaccination is quite effective in the case of cholera, typhoid, and the paratyphoids Type A and Type B. Theoretically, it would be possible to use a dosage high enough to over-ride these uncertainties, but we believe there are more suitable agents. Amoebic dysentery is not a practicable agent due to the difficulty in the propagation of the agent in sufficient quantity.

Some agents which we consider to be practicable are those causing botulism, bacillary dysentery, Weil's disease or leptospirosis, Brucellosis, tularemia, and melioidosis. There are probably others which could be used. Also, two or more organisms could be used as a mixture to confuse the diagnosis of the disease by producing a false syndrome and making the bacteriological identification of the causative agent quite difficult. In this report it is assumed that these agents would be used against civilians to disrupt production and destroy morale.

Clostridium botulinum

The toxin produced by this organism has been discussed widely in the press, e. g., the spectacular announcements: "one gram of toxin contains 7,000,000 lethal dosages" and "350 pounds would contaminate a ten million gallon reservoir." Such dosages have been estimated on the assumption that toxicity is on an equivalent weight-for-weight basis from experimental animals to man. While this may or may not be precisely true, it may be safely said that botulinus toxin is one of the most toxic materials known to man. Botulinus toxin has been identified as a complete and simple protein (8). It gives a delayed effect (roughly the equivalent of an incubation period) of from nine hours to as long as three days. Death may occur from within 24 hours to ten days, or there may be a slow recovery in two to four months. Reports have placed the mortality from 16% in Germany to 66% in the United States. High temperatures (boiling point of water) will inactivate the toxin. It is unfortunate that there is apparently no data on the effect of the various water treatments on the botulinus toxin. This area should be investigated.

Since the toxin is not a growing organism, it is necessary that any detection be made by purely chemical means or by a biological assay (five distinct botulinus toxins are known: Types A, B, C, D, E). Due to the very complex structure of the toxin, a chemical test specific for the toxin is not likely to be developed. Biological tests would seem to give the most rapid result. Animals such as white mice which are sensitive to the toxin or chickens which will develop symptoms in 3 to 4 hours and generally die in 24 to 36 hours could be used. The spores of Clostridium botulinum are extremely resistant. The toxin is easily produced (9), the crystalline toxin has been produced, and the lethal component has been identified as "Alpha toxin."

Considerable attention (10, 11) has been given to the question of the detoxification of the botulinus toxin for the purpose of preparing a toxoid. Immunization of man was first reported by Velicanov (12) in 1934 and 1936. Toxoids were first used on experimental animals in 1924 and it was the purpose of Nigg et al. (13) to develop methods for the production of a toxoid for human inoculation.

Both fluid and alum-precipitated toxoid have been used for the purpose of immunizing man against botulinus toxin. No cases of botulism developed in immunized individuals. A few individuals received botulinus antitoxin after known gross exposures. An antitoxic level of 0.02 unit per ml of serum was selected as a presumptive protective level and was the objective in the immunological program of persons exposed (14). A schedule of four doses of fluid toxoid

at two weeks was used. Within five months over 90 percent of the individuals had protective levels. Booster injections were given each six months. Using the alum-precipitated toxoid it was found most satisfactory to give three injections at three to four week intervals. Only a few of the individuals gave marked reactions to the injections.

From the above reports, it would appear that it is feasible to both produce the botulinus toxin and a toxoid which will immunize persons against this powerful toxin. Due to the extreme toxicity of this bacterial toxin, it would be advisable that any agent who would handle this material should be immunized.

Bacillary dysentery organisms

In modern times the epidemics of bacillary dysentery due to contamination of drinking water outnumber all others. Normally, these are attributed to the Shigella and Salmonella groups. It is also believed that some strains of Staphylococci, usually associated with food poisoning are responsible for some of these incidents (15). In many of the epidemics, neither the agent responsible nor the mode of entry into the system has been definitely determined because of the incubation period involved and the fact that the causative agent disappeared from the water before the epidemic came to the attention of the public health authorities. Therefore, this type of agent is greatly favored for the conduct of BW. Weil reports (16) that infections from agents other than Shigella are comparatively rare in this country. He has compiled a table of types, including synonyms found in the literature, which are definitely connected with human diarrheal disease.

The many types of Shigella, the difficulty of identification, the worldwide occurrence of dysentery in epidemic proportions from time to time, and the possibility of at least temporary protection of our armed forces by vaccination would seem to indicate the suitability of the Shigella for use as an agent of BW. The use of a type not normally present in a country would in all probability be quite successful.

The production of the Shigella organism in large quantity should present no great difficulty. Barnes and Dewey (17) reported on the development of a method of continuous culture of the virulent type III(Z). With the continuous culture method (see page 11), the bacteria were multiplying as well at the end of 36 hours as at the start. The average yield was about three grams of bacteria (dry weight) each three hours. This is many times the rate of growth with the use of ordinary methods. Barnes and Dewey also reported the successful growth of a Sonnei strain of Shigella by this procedure.

The Salmonella group of organisms has been responsible for many incidences of food poisoning. Curbelo et al. (18) have reported that an infection with a typhoid-like syndrome can be caused by Salmonella gatuni. Vaccination with TAB vaccine is effective against A, B, and D groups of the Kauffman-White Schema but does not protect against members of the C group to which belong S. gatuni and other widely known members of the Salmonella group. With massive dosages, symptoms can be exhibited in as little as two hours and death may result in as little as thirty-six hours.

Gorelick et al. (19) have described a technique which they used for the production of high concentrations of bacteria in small volumes. Their method was suitable for the cultivation of the Salmonella. Since the method is unique, a description is presented herewith:

This system consists of a thin-walled cellophane sack containing the nutrient medium immersed in a simple fluid containing activated charcoal or some other solid adsorbent. Cultivation of micro-organisms in a charcoal-cellophane system resulted in counts as high as 200×10^9 viable cells per ml. The capacity to increase the number of viable cells per unit volume appeared to be due to a synergistic effect between the cellophane membrane and the adsorbent.

To prepare the culture flasks, 3/4 inch dialyzer tubing from Fisher Scientific Co. was cut in lengths of approximately 20 inches and was completely wetted by being soaked for three to five hours in distilled water. A water-tight knot was made in one end of the tubing and 100 ml. of the media was poured into the other end. The open end was then tightly knotted leaving a liberal space over the medium to prevent rupture of the tubing during sterilization. One cellophane sack containing medium was placed in a 500 ml. Erlenmeyer Flask and 50 ml. of 0.5 percent sodium chloride in distilled water was added. The completed "cellophane culture flasks" were then autoclaved at 15 pounds pressure for 20 minutes. The adsorbents were usually added to the saline in a concentration of 0.2 percent. The ratio of broth to saline (2:1) appeared to be quite critical.

It would seem that by enlarging the apparatus and making some changes in technique that this type of procedure could be used as a method for continuous culture or, by the use of inhibitors in the culture media, that this method could be used for field cultivation of pathogens by agents in or associated with underground activities.

Leptospira icterohaemorrhagiae

Weil's disease, also known as infectious jaundice, is common to troops and agricultural workers who work in wet soils as rice planters. In the trench warfare of World War I, it was very common among both the German army and the Allied troops. The causative agent, Leptospira icterohaemorrhagiae, is normally present in rats on all continents. This organism rapidly dies when dried but will grow at room temperature and could be maintained in the field without special equipment (7). In BW it would be useful for the contamination of swimming pools and also drinking water. One great advantage for BW is that frequently the diagnosis of the disease is missed or is wrong. The organism produces a long, disabling illness and in Japan the mortality rate is 32 to 50 percent (20). The disease is not known to be contagious and if troops followed the use of this organism, their infection could be prevented by the use of disinfection and other sanitary measures. Vaccination has been used with some success but much work needs to be done in this regard. If an individual recovers from the disease, he has a lasting immunity. We have not found any information in the literature regarding the cultivation of this organism. Rosebury (7) reports that cultivation presents no difficulty.

Brucellosis organisms

Brucellosis (undulant fever) may be caused by either Brucella abortus, Brucella melitensis, or Brucella suis. The illness produced by Brucella abortus is considerably milder than that produced by Brucella melitensis or Brucella suis which produce an illness of long duration (perhaps several months or more). The organism is readily available and will live in tap water or sea water for thirty days (7). This illness is very difficult to diagnose and occurs in nearly all countries. The method previously cited of Gorelick, Mead, and Kelly under the discussion of the Salmonella culture was also used for the cultivation of the Brucella organisms with satisfactory results. It has been determined that Brucella will grow in chemically defined media with ammonium salts, asparagine, glutamic acid, or histine as the sole nitrogen source (21). A multiple synthetic amino acid medium has been formulated which is reported to give comparable growth of all strains (22). Media for the cultivation of the Brucella are available on the commercial market (23).

Pasteurella tularensis

Tularemia (rabbit fever) is a disabling disease prolonged over a period of at least several weeks and when not given specific treatment results in about 5 percent mortality. The causative agent, Pasteurella tularensis has an average incubation period of five days. Water-borne outbreaks caused by this organism have been definitely established as

the result of an investigation of an epidemic which occurred in Russia, Turkey, and perhaps in Czechoslovakia (7, 24). Although for many years this organism has been thought to be very difficult to cultivate, there is evidence that under proper conditions it can propagate in nature outside an animal host (25). This fact was demonstrated after an epizootic of tularemia among beavers in the northwestern United States. A prolonged heavy contamination of river waters which could not be attributed to animals was demonstrated. It was also found that the tularemia organism would live in refrigerated marsh mud for as long as 12 weeks. The authors of this reference state that there is little doubt that the organism could live in waters in the absence of any living host. They noted that even a salinity due to two percent sodium chloride was not inhibitory. From the limited data available (24) it is shown that the organism has rather a long survival time in the presence of chlorine, thus indicating that this organism would be very good as a BW agent.

There have been numerous reports in the literature of the infection of laboratory workers by the tularemia organism in the course of research. Over a period of years, laboratory infection approached 100 percent of those who came in contact with the organism. Therefore, to protect laboratory workers at Camp Detrick, Maryland, prophylactic vaccination was adopted and only about 14 percent of the persons exposed contracted the disease (26). This would seem to be a good margin of safety when one considers the hazards of handling high concentrations of organisms and the work with experimental animals in that laboratory. Foshay determined the duration of the disease (total period during which any symptom was shown) for four infections among vaccinated laboratory workers and found the average to be 25 days compared to an average duration of disease of 141 days among 18 infections in unvaccinated laboratory personnel.

The experiences reported at Camp Detrick indicate that inoculation against tularemia is accompanied by no more hazard than would be anticipated from the use of typhoid vaccine, provided that precautions are taken to eliminate hypersensitive individuals by means of a skin test and clinical history. The data suggest that all who may be exposed to tularemia as a result of an occupational or recreational hazard should be protected by vaccination.

Malleomyces pseudomallei

Melioidosis (Whitmore's disease) is a glanders-like disease of rodents which is transmissible to man. In one survey only two of 83 known cases survived and another survey only five of 95 cases survived. Death occurs in a period of ten days to four weeks. The causative organism, Malleomyces pseudomallei, is reported to be readily available; it grows best at a pH of 7 but is not sensitive to changes

in pH of the media(7). The organism has survived at room temperature for 44 days in drinking water. It is readily killed by heat and also by disinfectants. Diagnosis of this disease often is not made until after death. No specific method of cultivation has been found in the literature, although the organisms could probably be cultivated on a potato-veal medium. If a highly fatal agent were desired for the achievement of a specific purpose, use of this organism would be indicated.

Production of BW Agents by Continuous Culture Methods

If use of a BW agent is considered, a means must be used whereby the organism or its toxin can be mass produced after the desired strain has been developed. The method of continuous culture is the most efficient, if large quantities of material are desired, and has been used for many years in industry. Golle (27) has discussed the theory of this operation in some detail; the discussion which follows is a brief account of his conclusions.

There is a distinct advantage in using a second and possibly a third culture vessel in tandem to achieve a higher concentration of product at some fixed medium rate. It is necessary that contamination be avoided in the operation of the system and conditions must be such that growth of variants be kept to a minimum.

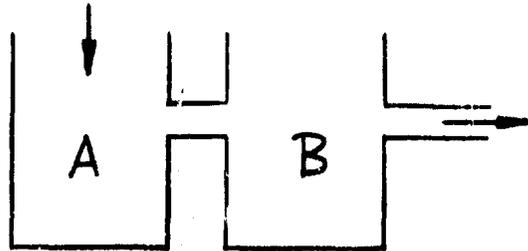
In continuous culture the organisms are grown under conditions where sterile medium or a culture of low cell concentration is added continuously to a culture vessel while product having a higher concentration is removed at the same rate, leaving no net change in the volume of culture in the vessel. Continuous culture has several advantages over a batch process:

1. A steady load on the utility requirements is realized, with no peak loads.
2. Continuous culture processes are more adaptable to automatic control.
3. A more uniform product is obtained.
4. Increased product for a given culture vessel is obtained.

In practice there are three major problems:

1. Optimum growth of the desired organism.
2. Replacement of the desired organism by genetic variants.
3. Replacement of the desired organism by contaminating organisms.

The following diagram illustrates the arrangement of vessels for a continuous culture:



For this discussion, it is assumed that vessel A will be filled to the overflow with liquid having a number of organisms when the operation starts and that the culture in each vessel will be kept homogenous at all times.

Golle developed the theoretical considerations for continuous culture and arrived at some useful conclusions. He feels that in some processes there is an advantage in having vessel B larger than vessel A. Most rapid production of organisms is usually realized in the logarithmic growth phase.

For many organisms the log growth phase ends with a concentration which is considerably below the maximum concentration attainable. So the decision must be made whether the effluent should contain a large number of organisms at a low concentration (large volume of product) or a lesser number of organisms at a higher concentration (small volume of product). Usually a combination of a large number of organisms at high concentration is the most desirable. While this combination cannot be obtained with one vessel, additional vessels can give the desired result. The limit of the number of vessels used would be determined by the growth rate of the organism and by economic considerations, as the point would be reached where the cost of adding another vessel would outweigh the advantage of the small increase in the concentration of the product.

Genetic variants cannot be prevented since mutations occur randomly as a function of growth. The average mutation rate probably lies between one per million and one per one hundred million gene duplications. The number of vessels would have no effect on mutation. If the growth of undesired types, either mutants or contaminants, should occur, it would be necessary to discontinue the operation, sterilize the equipment, and recommence operations with a fresh culture. By means of formula developed by Golle, steady-state conditions can be predicted.

Possible BW Targets

It is important to remember that the addition of a given pathogen to a water supply does not insure that all persons who drink the water will become ill. Successful BW must provide for the intake of sufficient quantities of the agent to overcome individual resistance.

There are many obvious places where BW agents could be applied to a water supply. However, due to the rather complex branching of the modern city's water supply with the usage in various districts causing great variation in flows to various areas, the contamination of a water supply at treatment plants and pumping stations would not be a good calculated risk from the standpoint of a saboteur. Pumping stations, treatment plants, standpipes, and other large installations can be effectively destroyed by use of explosives, abrasives, or corrosive chemicals. Each water plant has its own security measures which are dictated in large part by the physical characteristics of the particular plant. Generally speaking, utility employees have an outstanding loyalty to their plant and this is the strongest weapon against sabotage.

Defense plants or military establishments would be very good targets for BW since it is quite common practice to have very good security at the treatment plant and rather lax security in other areas insofar as the protection of the water supply is concerned. The routine collection of samples for bacteriological analysis from various locations on the reservation should be done at varying times so that the time of sampling cannot be predicted. The armed forces of some other countries pay less attention to their water supply than we do in the United States due to the lack of appreciation of the need for proper care, not to the lack of knowledge. This statement is based on private conversations with personnel from English, German, Japanese, and Russian armed forces during and after World War II.

Hotels, dormitories, and housing developments would be a very favorable field for the practice of BW. Large numbers of individuals could be infected and morale in these areas could become a serious problem. Due to the urgent needs of wartime, only a cursory security inspection, at most, is permitted of persons living in such areas. One can realize that it would not be too difficult for a saboteur to have access to such developments.

The public school system would be a particularly vulnerable target. Since there is no care taken against strangers entering the buildings, a saboteur would have easy access. Probably nothing would destroy morale and cut down on production so much as would an epidemic among children. Such destruction of morale would be emphasized even more if there were delay in the identification of the illness. If parents were working in areas distant from their normal residence, there would be

a very strong tendency to leave the area and return to their homes unless detained by force. This tendency would be strong even though their children were not ill. With this fact in mind, the general ease of access to schools in this country is regrettable. In his experience, the writer has entered many schools and has never been asked to produce any identification. He has operated valves, taken measurements to determine if back-syphonage exists and in most cases the custodian has assisted without any understanding of the purpose of the work. In many cases, the school custodian, if given a rather glib explanation, would actually assist a saboteur in the contamination of the water supply of the institution!

Contamination can be accomplished in a number of ways. The precise method, of course, is determined by the particular situation and the area to be affected. The simplest manner is the direct introduction of the contaminant into an open section of the pipe. This, usually, will not be feasible except during new construction, repairs, or alterations. Allied closely to this method is the introduction of contaminants into bypasses placed in lines for the purpose of adding water-treatment compounds to the system. Usually, a "pot" is installed in the bypass which has an easily removable head. This pot could be emptied or partially emptied and a considerable quantity of material could be placed in it. If the contaminant were to be prepared in pellet form, using a material of slight solubility as a binder, little or no evidence would be left after the material had dissolved. Such bypasses are used widely for the introduction of complex phosphates of limited solubility (Micromet, etc.) for the control of corrosion in many establishments. It is possible that a quantity of material which would absorb a suspension of organisms such as hemp, untarred jute, or even rags could be impregnated with the culture, tightly bound, and placed into an open pipe or a pot as mentioned above.

The principle of back-syphonage could be used in many cases, particularly in multistory buildings. For each foot of elevation 0.433 pound pressure per square inch is required to raise the water, so if a heavy flow of water occurred in the lower level there would be a noticeable drop in pressure at the upper level. If the mains and service entrance pipes are not adequate, this drop in pressure may be such that negative pressure will exist in the upper level. To achieve this effect, valves entering the building or parts of the structure may be closed, or fittings loosened or broken to cause heavy flows of water. In older buildings, it is often true that plumbing is undersized for modern requirements and for additional requirements caused by enlargements of the building and increased personnel. This is particularly noticeable in wartime.

It seems to be entirely feasible, and in most cases advantageous, to introduce contaminants against the pressure of the water in the building. The simplest application of this idea is by the use of a pump

to force a suspension of the bacterial agent into the water. Such pumps could be improvised from materials available in the locality. For some experimental work, we have improvised pumps from automobile oil pumps driven by electric motors. It would seem possible that miniaturized equipment could be developed for this purpose. Reports in the press of miniature "cyanide guns" shaped to resemble cigarette cases suggest that perhaps a similar "gun" could be constructed which could be connected to the pipe and could discharge a pellet or several pellets of organisms into a water supply against pressure. It would also seem that a pressure bomb could be constructed which could be connected to a supply and discharge a culture of organisms into the water. The source of the pressure could be air or a water miscible gas such as carbon dioxide. A device could be constructed which could be recharged with the organism and pressure generated chemically by the familiar acid-carbonate reaction. Such a bomb could even simulate an instant lather shaving soap preparation!

Once contamination occurs, even with the most modern procedure, a minimum of sixteen hours is required to detect the fact. This assumes that the organism will be detected by the routine procedure, and this is not a valid assumption. It is evident that the BW agent would have several hours to infect persons before it was detected even if bacteriological tests were made several times daily, which in most cases would not be practicable. Thus, the disability of many persons would be the first sign of contamination.

The act of contamination would normally be done inside a building or in a secluded place. Physical security of plants and buildings is the greatest safeguard against BW. Where possible "open type" construction with secluded areas kept to a minimum is advisable. Adequate lighting in areas of buildings where only utility lines are located is very important.

For security reasons there should be an accurate map showing locations of all valves, all hydrants, all lines in the building, and all apparatus. Valves and hydrants should be inspected periodically to insure proper operation and to insure, to some extent at least, that there has been no unauthorized operation.

In the case of public distribution systems, the general public should be alerted to report unusual activity in the vicinity of fire hydrants, valves, or isolated components of the system. Every city supply has had many experiences with unauthorized operation of its facilities without any interference, even by the police. However, the general public is most cooperative in reporting events which seem improper or unusual. Therefore, every community and every establishment can consider their good relations with their consumers or employees as a strong weapon against sabotage.

From a defense standpoint, it is particularly important that rigid control of purification processes be exercised during a war and in periods when war may be imminent. The relaxing of sanitary measures due to the press of other activities in wartime should not be permitted.

Contamination Detection

It is difficult to conceive of a disinfecting agent which cannot be made ineffective by a skillful act of sabotage. Therefore, several disinfectants should be available for use and the disinfection method should be alternated in a more or less random fashion. However, this is not at all practicable as it would make the surveillance of the supply very difficult and would introduce undesirable operational complexities, particularly in times of emergencies.

All water plants have adopted the procedure of collecting and analyzing a certain number of samples from the distribution system. These specified numbers are determined by factors of supply and treatment. Cognizant health authorities specify a minimum number of samples which usually corresponds to that recommended by the Joint Committee of the United States Public Health Service and the American Water Works Association (28). These samples must be taken in such locations that they will be representative of the entire city water supply. Although we do not wish to minimize the importance of this sampling procedure in normal peacetime, we do question the rationality of blindly accepting the results. In a large community, it frequently is a long time between taking samples in a particular location.

Perhaps even a greater hazard is the blind faith of many in the bacteriological test itself. Since the bacteria in water were recognized to be of importance the water works industry has placed great emphasis on the elimination of coliform types from the supply. * The Standard Methods test (29) has come to mean to many people an absolute assurance of a water which is bacteriologically safe, although this certainly has no basis in fact or in the intent of those who have developed the method. Many organisms capable of causing disease would not be detected with the tests routinely used in water laboratories. Pure cultures of cholera, typhoid, and the paratyphoid organisms, for instance, would not be

*This country has probably the highest standard for municipal water supply in the world, since the United States definition of the coliform group includes both the fecal and non-fecal members. In Europe the non-fecal members, such as Aerobacter aerogenes, are disregarded (30).

detected. Obviously, if a saboteur used an organism which was outside the experience of the technician, the detection would be made only after the occurrence of illness in numerous victims.

Even if we assume that the disease-producing organism would be detected by the Standard Methods procedure, we must consider the time-lag involved in detection. In this test, as normally employed, five ten-milliliter portions are placed in tubes of sterile lactose broth and incubated for forty-eight hours at 37° C with observations made at the end of twenty-four and forty-eight hours. If the broth is fermented with the production of gas, a liquid media such as Brilliant Green Bile Lactose broth, or a solid media such as Levine's Eosin Methylene Blue agar is inoculated from the lactose broth tube(s) and incubated for forty-eight hours with observations at twenty-four and forty-eight hour periods. In normal operation, the completed test is not often used; the confirmed test described is deemed sufficient information. Thus, to verify the fact that a water is free of coliform organisms requires a minimum of forty-eight hours and may require as long as ninety-six hours with this procedure.

Much has been done in the field of the detection of various groups, such as, Salmonella, Shigella, and others, but to make tests on the large number of samples which must be collected periodically for the many groups of organisms and with the limited training of many technicians this is obviously an impossibility.

The rate of survival of the contaminant is quite important, both to the one who contaminates the water and to the person who has the responsibility for countermeasures against BW. It has been suggested (29) that an investigation be made of the relationship between virus pollution and coliform pollution of water and the relative rates of survival of each. Such a study is being made by the Division of Laboratories and Research of the New York Department of Health. Presumably, some data will be available in the near future. The area of lengths of survival of various organisms in varying conditions is a field of research which is far from exhausted.

Since the discovery of numerous cases of illness may be the first indication of an act of BW, it is important to realize that the particular disease may not display the classical symptoms. One important variable in the production of disease is the mode of entry of the infectious agent into the body. For instance, an organism ingested may give certain classical symptoms, whereas that same organism entering the respiratory system may give symptoms of an entirely different nature. Another variation which may be employed is the use of a mixture of pathogens which when ingested will complicate the diagnosis. Present clinical practices which do not essentially attempt to identify the organism but merely try to find the appropriate antibiotic which will destroy the illness-producing organism have much to commend them.

In an effort to detect chemical poisons rapidly, it has been suggested that mountain trout could be cultivated in an artificial brook and a portion of the supply passed through this stream. Since these fish are very sensitive to changes in their environment, they would react very quickly by coming to the surface and breathing heavily (31). There are possibilities of similar tests for at least some BW agents. For instance, the effect of botulinus toxin on poultry is well known, causing the disease known as "limber neck." The use of such procedures should be investigated. Considerable investigation on the detection of pollution has been done and is underway at many places in colleges and in the Environmental Health Center at Cincinnati, Ohio. Much of this investigation has followed, essentially, the classical methods of bacteriology. The modern idea of research by teams of various specialists could be used to great advantage.

In recent years the membrane filter has been widely discussed. This method presents a substantial saving in time compared to the bacteriological tests. However, there still remains a period of hours before an act of BW can be detected, and this presupposes that the organism will be detected by the particular media used and that the samples will be taken shortly after the act of contamination has occurred. Neither of these is a safe assumption. It would seem, therefore, that the nation who engages offensively in acts of BW has the advantage. BW has all of the advantages of a purposely delayed explosive bomb and yet none of the factors which make detection possible with certainty.

Membrane Filter

Development

For many years man labored to separate bacteria from water and other liquids by the process of filtration. Eventually, the membrane filter appeared. The history of its development, as presented by Kruse (6), may be briefly summarized as follows:

- 1874 - Chamberlain first acquainted Pasteur with the unglazed porcelain filter.
- 1892 - Berkefeld prepared his filter from kieselguhr and magnesium silicate.
- 1915 - The seitz filter appeared.
- 1917 - A membrane filter was prepared.
- 1935 - The glass filter was prepared by Scott in Jena.

During the development of these various filters, there were several interesting applications. Hesse (32) attempted to overlay a Berkefeld filter with a layer of kieselguhr and after filtration remove this layer by a short backwash and determine the organisms present. Other workers scraped the top layer of kieselguhr and then determined the bacteria in the scrapings. Citron (33) in 1919 was able to establish the presence of tuberculosis bacteria in the urine from a case of tuberculosis of the kidney. Eichoff (34) in 1921 recovered typhoid bacteria artificially placed in water. Escher investigated milk and sputum in 1927 to determine their content of tuberculosis bacteria.

Kruse in his article says, "Looking back it strikes one as peculiar that no one had the idea to lay the filter on top of a bacteria nutrient and allow the nutrient to come up through the filter and grow a visible colony." It was not until 1933 that the Russian women Rachlina (35) and Rosso-wskaja (36) placed the filter directly on the nutritive base and achieved success. The Russians used this method especially in water investigations. The method became known to Kruse in Germany through Feldmann (37) and various authors (38, 39, 40) after the fall of Kiev.

Kruse describes the production of the membrane filter in the Sartorius Works in Göttingen, as follows:

The process is, roughly, as follows: Nitrocellulose is dissolved in certain organic solvents - usually acetone - and then poured in a very careful manner, casting on a perfectly level glass plate and evaporated. The solution is vaporized gradually and a thin white film with many small pores remains. The size of the pores can be changed by altering the humidity and the temperature of the air by the evaporation of the solvent as well as the varying of the average polymerization products of the original Cellulose nitrates. In this manner it is possible to prepare Membrane Filters with pore sizes of two to three microns. Each filter has a number on the rim. This indicates, however, not the pore size but the Z value. A "Z Value", for example, means that 100 cc. of water will pass through 100 square centimeters of this filter in five seconds with a pressure of one atmosphere. The relationship between pore size and Z value are shown in the following table.

| Type of Filter | Z Value in Sec. | Pore Size in Microns |
|----------------|-----------------|----------------------|
| Large | 1 - 10 | 3.00 - 0.75 |
| Medium | 10 - 30 | 0.75 - 0.50 |
| Fine | 30 - 100 | 0.50 - 0.20 |
| Finest | Over 100 | Under 0.20 |

The Sartorius Works, also prepares a very fine pore filter, the so called ultrafine filter having a pore size of 200 microns down to 5 microns. Due to the very low rate of passage of distilled water through this filter the Z value will be given in minutes. A survey of the pore size and the Z value of the ultrafine filter is shown in the following table.

| Type of Filter | Z Value in Minutes | Pore Size in Micromicrons |
|----------------|--------------------|---------------------------|
| Large | 1 - 10 | 200 - 80 |
| Medium | 10 - 30 | 80 - 50 |
| Fine | 30 - 100 | 50 - 10 |
| Finest | 100 - 500 | 10 - 5 |
| Very Finest | Over 500 | Under 5 |

The membrane filter comes dry in trade, but the ultrafilters come in a moist condition and are ruined if they become dry.

Since the Z value is largely proportional to the total area of the pores the Sartorius Works constructed an apparatus to determine the maximum pore size. This apparatus is described by Kruse as follows: It consists of a pot-like understructure with a supply line to the supply of compressed air and a manometer as well as a lid with long slits and ribs, with a single gauze base underneath. Before starting the test the lid will be removed, a piece of ordinary filter paper placed on the gauze base, thereon is placed the membrane under test, and finally a rubber ring. Now the pot-like part is placed on top of the rubber ring and inverted. The apparatus is closed with the aid of the wing nuts. Water is placed in the slits of the cover and now compressed air is passed into the understructure until the first bubbles are observed through the slits of the cover. The manometer value is read and converted into atmospheres. The maximum pore diameter can be calculated from the formula

$$D = \frac{4a}{1.033 P (10^2)}$$

where a is the manometer reading and P is the air pressure in atmospheres. With this apparatus only pore sizes to 0.3 micron can be calculated. With smaller pores the pressure would be too high. The pore size of the ultrafilter can be determined by Gold hydrosol and other methods.

The "bacterial tightness" of the filter is determined by the process of filtering dilutions of Escherichia coli suspensions and then examining the filtrate on Endo's medium.

Bechhold (41) observed that for the bacteria to pass through the filter the pore diameter of the filter must be from two to fifteen times the diameter of the bacteria and that the length of a pore passage of 100 microns exceeds the size of the bacteria thirty to one hundredfold.

Kruse mentions that in his investigations a membrane filter with a 5.5 cm total and a 3.5 cm utilizable diameter with a Z value of 5 (pore size about 1.5 microns) is used for the determination of Escherichia coli content. For several days before use the German filters must be washed in several changes of distilled water and after this washing must be kept wet. To sterilize the filters, they are arranged in a circle alternating a filter paper and a membrane filter with a paper filter on the bottom and on the top of the stack. This stack is placed on an inverted petri dish and held down with a weight. The filters are sterilized for 30 minutes in streaming steam. Filters not to be used immediately are stored in a tight sterile container.

Kruse found that iron or manganese in quantities of about 2 parts per million did not disturb the development of Escherichia coli colonies and in even larger quantities interfered only slightly.

Kruse used solid lactose - fuchsin according to Endo for his work although he mentioned Müller's work (42) with the use of agar-free lactose absorbed on a paper disc. Kruse mentions that the membrane filter has been very successful in the separation of typhoid and paratyphoid bacteria from water samples because of the fact that larger quantities of water may be used and the bacteria concentrated.

The German apparatus varies in form from the American apparatus but is identical in operation. The only difference in preparation is that the American filter needs no soaking before use.

In the United States, the two companies which market the membrane filters are:

Carl Schleicher and Schuell Co., Keene, New Hampshire
Lovell Chemical Co., Watertown, Massachusetts.

The Carl Schleicher and Schuell Co. (43) states that their membrane and coli types of ultrafilters are produced by them from raw material supplied by the original Göttingen manufacturers. The Lovell company has followed quite closely the work of Goetz, Clark, Kabler, and others (44-52) and can be regarded as essentially an American development of the membrane filter technique of manufacture, after the German success (50).

Description

Essentially, the apparatus consists of two sections - an upper section comprising a modified funnel which can be fastened to a lower section by a suitable clamping device. In one American apparatus (Lovell Chemical) an all-glass apparatus uses a metal clamp to fasten the two glass parts together. Another Lovell model and the Schleicher and Schuell model, which appears to be identical with the apparatus described by Kruse (6), use an all metal apparatus with a bayonet coupling. The supporting element in the Schleicher and Schuell apparatus uses a fritted glass disc, while the Lovell equipment uses a disc of porous carbon for the supporting plate.

The type of material designed for bacteriological analysis of water has a flow rate of 1000 to 1200 ml per minute through an area of approximately 10 square centimeters at a vacuum of 20 to 30 inches of mercury. The pore volume is 75 to 80 percent of the volume of the leaf. The leaf is about 150 microns (6 mil) thick and theoretically 500 million pore openings in the area used for filtration. Membranes are 50 mm (about 2 inches) in diameter (44). An important characteristic is that the pore size is very small on one side and much larger on the other side (anisomorphous pore structure). The flow should always enter the leaf at the side of narrow pore openings. In practice this side is usually marked with a grid which facilitates in the counting of the numbers of organisms.

At the present time the so-called "Aibimibroth" is used as the medium for obtaining total count and also for the preliminary enrichment of a sample before transferring to the confirmatory media for coliform organisms. Colonies visible to the naked eye develop in as little as four hours. Better counts are obtained if plates are incubated for twelve hours.

The filter assembly of this apparatus is such that manufacture in large quantities would pose no particular difficulties. The preparation of the discs is known only in a general manner. Kruse (6) has given the most complete account: The filter is prepared by casting a mixture of nitrocelluloses on a perfectly flat surface and the porosity of the filter may be determined by controlling the rate of evaporation of the solvent. He also describes in some detail the apparatus for the determination of the pore size of the filter leaf. The procedure is in essence the measurement of the resistance offered to the passage of air through the leaf under controlled conditions. Improved methods of manufacture are in effect since his article and we assume also that improved methods of testing have been developed.

Analysis Procedure

Sterilization may be accomplished by autoclaving, or by flaming all parts of the apparatus coming into contact with samples by an alcohol torch, or gauze swab dipped in alcohol and ignited and rinsing with sterile water. Another method is the use of a "sterilization base" supplied with the Lovell apparatus. This base is equipped with a well in the center containing a porous material held in place with wire gauze. A small amount of methyl alcohol is poured into the porous material and ignited. The assembled apparatus, less the membrane, is inverted on the base. Due to a limited supply of air, formaldehyde is generated and accomplishes the sterilization. Ethylene oxide may be used for the sterilization of the entire equipment and also separately for the filters. It is very important that sterile air be used to flush all traces of the sterilizing agent from the apparatus and the filters before use.

To perform the actual analysis, the membrane filter is placed on the supporting grid, care being taken that the proper side is up. The upper and lower parts of the apparatus are clamped together, placed on a vacuum filter flask, and the sample placed in the funnel. Suction is applied at a rate so that the sample flows in a series of connected droplets. After all of the sample has passed, the apparatus is disassembled and the membrane is transferred, aseptically, and placed on a pad saturated with the desired medium. In water analysis, the modified agar-free Endo's medium is generally used for confirmation of coliform organisms. If the medium is prepared in the laboratory, the standardization is quite troublesome. A dehydrated formula is available (Difco Laboratories) which gives results comparable to Standard Methods and to the freshly prepared modified Endo's medium as recommended by Clark and others (47). To eliminate the transfer of membranes from the enrichment medium (Albimi broth) to the confirmatory medium, dehydrated nutrient schedules have been developed (53). The nutrient schedule consists of two pads - Pad A is a thin leaf of an absorbent porous paper overlying a heavier disc of blotting paper (Pad B). Pad A is impregnated with a dehydrated enrichment medium, whereas Pad B contains the dehydrated inhibitory agents of the differentiating medium. Upon the rehydration of the pads with distilled water, the nutrient in the upper leaf diffuses through the membrane filter leaf and provides an immediate stimulus for all of the organisms. At the same time, the differentiating medium in Pad B begins to diffuse into Pad A and gradually saturates it with the inhibiting agent. By the time Pad B is saturated, the organisms have reached the period of active growth. The author has not had experience with the nutrient schedule, but has been informed (54) that such pads do not give results comparable to conventional procedures. It would appear, however, that such schedules do have considerable promise, particularly for work in the field.

To this date, no satisfactory explanation of the precise mechanism by which the nutrient passes through the membrane has appeared. All who have worked with this technique agree that the mechanism is somewhat complex.

During the incubation of the membrane filter, it is imperative that no condensed water come in contact with the membrane surface. At the same time, it is very important that the atmosphere in contact with the membrane be saturated with moisture. Goetz has suggested that the incubation be performed after the membrane has been carefully "rolled" onto the absorbent pad (to exclude air bubbles between the medium and the filter leaf), which is contained in a petri dish of suitable size, and the half petri dish inverted on a wet towel contained in a larger dish (45). He recommends a glass tray approximately 8-1/2 by 13 inches in which several of the small petri dishes be incubated simultaneously. These larger trays and the wet towels need not be sterile since they do not come into contact with the sample. An expendable dish made of plastic and having a tightly fitting cover is also available.

The filter is usually grid marked. Each square of the Lovell filter represents 1/100 of the area used for filtration. Standard Methods plate count with overlap is 8 colonies per square centimeter or 500 in a standard plate. With the membrane filter, colonies can be identified with a density of 500 per square centimeter or 5000 over the area of the disc. Short incubation periods are not only possible but desirable to prevent the converging of the colonies.

The American Water Works Association Task Group (53) states that with a drinking water which is expected to comply with USPHS standards, and thus contain an average of not more than one coliform organism per 100 ml, the filtration of a volume of 300 to 500 ml should provide an ample margin of detection for coliform types. One such quantity should be equivalent in precision to presumptive tests in lactose broth with 30 to 50 tubes of 10 ml each. For the analysis of drinking water and relatively clear water, the 47-mm-diameter filter disc is generally adequate. For water which is high in suspended solids and low in bacterial content, the 86-mm size may be preferable, since its area is approximately five times the area of the 47-mm filter disc, and as a result the surface coating of solids will be only one-fifth as thick. The discs can be preserved as a permanent record by removing, drying, and moistening them with dilute glycerol and mounting them in a cellophane or glassine envelope. At the California Institute of Technology the discs have been kept several years without the glycerol treatment.

Many technicians prefer the coliform confirmation since the results are obtained as a direct number of coliform types per unit volume instead of the statistical approximation (most probable number).

If it can be assumed safely that there is equal distribution of organisms on the filter, the membrane may be cut into two or more sections with sterile scissors and each section placed on different media. Specifically, this could be of considerable advantage in the bacteriological analysis of water, since in the total count, the coliform, and perhaps other types of organisms could be detected at the same time.

Preparation of media

In this country the formula and the procedure recommended by the Environmental Health Center, Cincinnati, Ohio, are generally preferred. For the preliminary enrichment, the membrane is cultivated on the Albimi broth for two hours and then transferred to the modified Endo's medium (EHC Endo). The Endo's medium is incubated for 14 hours. All dark colonies showing a metallic sheen are coliform colonies; pink or colorless colonies are not coli. It should be noted that the classical description of coliform types on solid Endo's medium **DOES NOT APPLY** to this procedure. All of the Escherichia coli and most of the Aerobacter aerogenes develop the sheen over the surface of the colony. A few of the Aerobacter aerogenes may be missed but the increased accuracy of the procedure compensates for this. The membrane filter method with the EHC Endo's medium gives results comparable to Standard Methods, although the most probable number fluctuates over a much wider range. It appears that the membrane filter has a greater degree of reliability than the most probable number with also a shortening of time from 96 to 16 hours.

Bismuth sulfite broth is satisfactory for the isolation of Shigella typhosa. Kabler and Clark (48) have reported that recovery rates of S. typhosa from water using the membrane filter technique are superior to the standard methods used in most laboratories. They found that bismuth sulfite (Wilson-Blair) broth, double strength, is a satisfactory medium for use with the filter disc in the isolation of S. typhosa. On this medium S. typhosa grow as shiny black colonies with narrow white borders. A black metallic sheen may surround the colonies if they are not too crowded. With the proper selection of differential media and development, it may become possible to differentiate the Shigellas as well as other pathogenic bacteria.

It has recently been reported (55) that the addition of oxine (8-hydroxyquinoline) to the modified Endo medium is quite effective in the inhibition of the non-coliform types; 8 ml of a solution containing 100 ppm of oxine is used. With this additive, the authors state that 81 percent of the sheen formers and 22 percent of the non-sheen-producing colonies produced gas in lactose broth. Medium with oxine added has been used in the Des Moines laboratory with comparable results.

Advantages

The membrane filter has several advantages over the methods in routine usage. Possibly the most important in regard to its use in detection of BW agents is the saving of incubation time. Also, much larger samples can be used; therefore smaller quantities of contamination can be detected than is possible by the present Standard Methods of water analysis (29). The bacteria are removed by the filtration from their previous environment, which is important if any inhibiting substances or bacteriophages are present. Experimental studies on the original German membrane show that the uniformity of pore structure was not always reproducible to the degree required for water bacteriology. New production methods improved the flow rates and uniformity, thus making treatment by soaking prior to use unnecessary.

Experience has proved that the membrane filter method is useful in clear water samples with either low or high bacterial content and in muddy waters with large numbers of bacteria. It has not been found satisfactory in the case of turbid water with low bacterial counts. While of some interest, the case of turbid waters with low bacterial content is of little importance in this discussion, since nearly all potable waters are relatively clear.

The advantages of the membrane filter may be summarized as follows:

1. This procedure permits the concentration of a small number of organisms from large quantities of water, increasing the accuracy of the determination.
2. It minimizes the cross diffusion and spreading of colonies and reduces the number of laboratory dilutions and duplicate incubations.
3. It permits the separation of organisms from the nutrient and permits a change of nutrient with ease or the total inhibition of development of the organisms.
4. A direct count of coliform organisms is obtained instead of the most probable number.
5. It permits a considerable saving of time in obtaining results, and a considerable saving of laboratory time used in the preparation of conventional culture media.
6. It requires considerably less laboratory space and very little glassware.

7. It permits better and faster differentiation of bacteria.
8. It could be adapted to a field procedure with little difficulty.
9. A permanent record is obtained by the drying and preservation which could be forwarded to a laboratory by a technician for evaluation.

The membrane filter could be adopted as a standard of procedure by all countries and thus be of great benefit in an exchange of information. In case of hostilities allied forces could exchange information without the necessity of checking results against their own procedures and making a rather lengthy process of evaluation.

To many of us in the field of bacteriology of water supply, it hardly seems possible to detect, even with the above rapid procedure, an act of BW prior to the illness of individuals, and for perhaps this reason alone fear of retaliation may postpone the use of BW.

Disadvantages

In spite of the advantages there are several disadvantages in the membrane filter technique, when applied to samples containing unknown concentration of bacteria. Goetz (56) has recently discussed the following disadvantages:

1. The volume of water to be filtered can not be predetermined since the bacterial concentration required to get a reliable count can not be determined except by several filtrations with varying sample sizes. Filters are somewhat high-priced, resulting in an economic handicap as well as unnecessary time expended.
2. High concentrations of coliform organisms or of non-coliform organisms coexisting with small population of coliform organisms may prevent satisfactory growth development (that is, the development of the Endo sheen).
3. There prevails a general uncertainty on how to correlate the statistical value of the most probable number as defined in "Standard Methods" with the membrane filter count. (At the Des Moines Water Works the practice is to classify the sample unsatisfactory if it contains over 1 coliform organism per 100 ml.)

Centrometer Method

A centrometer method has been designed and according to Goetz has been able to cut substantially the cost of the filter materials needed for each test. Also, the method does not require an alteration of the already developed techniques of nutrition and incubation or of

the basic membrane materials available at present. The membranes differ only in shape and assembly from those in use at present. The procedure consists of a new physical application of the molecular filter membrane to the isolation of suspended matter and bacteria from water samples.

The concentrator uses a membrane area 3 cm high and 4 cm wide, and the total sample volume has been standardized at 300 ml. The container is a prismatic sealed unit with plane sides and a curved top surface. Permanently attached to the container is a face plate with a rectangular aperture which is accessible through the interior of the container. Hinged to the face plate is the filter plate assembly. Basically, the general method of operation is similar to the conventional operation of the membrane filter even though the filter is mounted in a vertical plane. The membrane is marked with guide lines. By virtue of the construction a different quantity of water flows through each section indicated by the guide lines. A very complete description of this apparatus appears in reference 56. Goetz mentions that the instrument has a range of 1 to 75,000 organisms per 300 ml.

The approach of this principle is unique and represents a rather unusual method. It deserves careful consideration. This type of procedure should have use in laboratories which deal with waters greatly fluctuating in their coliform content. In the case of most of the municipal water plant laboratories, the range of coliform variation is known, and it is believed that the membrane filter technique as now employed is satisfactory.

DISINFECTION OF PUBLIC WATER SUPPLIES

Common Methods of Disinfection

The classical method of prevention of illness resulting from accidental methods of contamination, such as might be introduced by cross-connections, is the maintenance of a fixed residual of free chlorine or in some cases of chloramines in the water supply. The residual deemed necessary is determined by the chlorine demand of the water. Wood and Pulham (57) report that British practice recommends that all plants should have chlorinators available to feed enough chlorine to provide a residual of one part per million of free chlorine and such chlorinators should be equipped for operation on reduced pressure of water supply in case of damage to the high pressure system. In the ordinary peacetime operation of a water supply this is satisfactory. However, if a saboteur so desired, it would not be a difficult procedure to add sufficient reducing material (organic or inorganic) to consume the chlorine in the water. A resourceful saboteur could even add materials which would simulate the yellow color

of orthotolidine which is almost universally used for the routine test for free and combined (chloramines) chlorines. Normally, the "Flash Test" is used for free chlorine, which is simply reading the intensity of the developed color immediately after addition of the orthotolidine reagent. Chloramines are determined by making another observation after five minutes. Iron, manganese, inorganic compounds and lignocellulose, organic iron compounds, and even certain types of algae will produce a yellow color with orthotolidine which is very similar to the color produced by chlorine. Over 0.3 part per million of ferric ion, 0.01 part per million of manganic ion, or 0.10 part per million nitrite ion will accentuate the yellow color and give an apparent determination of chlorine which will be misleading (58).

For several years, in many cities, the use of the Langelier Equation (59) for the prevention of corrosion has been used. Briefly, this theory considers the inter-relationship of pH, alkalinity, calcium concentration, total solids, and temperature. The equation is usually solved in rapid manner by a nomograph or from tables. Without going into further detail, use of this equation in treatment means, generally, the effluent is maintained at a higher pH than was formerly the case. With most waters a pH range of 8.5 to 10.0 is indicated. This high pH presents an environment which is unfavorable for the survival of many bacteria. This pH could be adjusted by an enemy agent, but it would require considerable skill on the part of the saboteur.

Ozone is used for the disinfection of water, but has not found wide acceptance primarily due to the cost of the installation. Also, ozone can not be stored (it must be generated as it is used) and it has not been found suitable for large-scale disinfection.

The oligodynamic properties of some silver compounds have been reported as satisfactory in small communities in Germany (60).

Whichever sterilizing agent is used, standby equipment and an adequate supply of the agent should be available. In the case of chlorine, which is most commonly used and the best agent for most installations, the Joint Committee on Chlorine (61) has published certain recommendations:

The rate of withdrawal for a 100 or 150 pound cylinder should not exceed 40 pounds per day and should not exceed 450 pounds per day if a one-ton chlorine cylinder is used. These rates may be exceeded for short periods (as much as fifty percent for periods not exceeding two hours). If an evaporator is used the rate of withdrawal may be considerably higher.

The Joint Committee defines three classes of inventory which are significant to any organization engaging in the disinfection of water.

These classes are:

1. **CRITICAL INVENTORY** - Equivalent to a number of unconnected full units which equals the number of units normally connected and in service. With this limited inventory, a plant is considered in **EMERGENCY OPERATION**.

2. **WORKING INVENTORY** - Equivalent to the duplication of connected chlorine units (Critical Inventory) plus a chlorine reserve to cover the length of time for delivery from the shipping point to the user's plant (varies from 2 to 15 days generally). To this must also be added a reserve for exigencies (strikes, local emergencies, etc.) equivalent to a 15-day supply.

3. **MAXIMUM INVENTORY** - Equivalent to a sixty-day supply of chlorine (small users of 5 pounds or less per day are excepted from this definition). Exceeding the Maximum Inventory ties up an unwarranted number of containers. **MAXIMUM INVENTORIES SHOULD BE CONSIDERED TEMPORARY.**

In all installations where disinfection is practiced standby chlorinators should be available without placing dependence upon the use of such equipment from local departments of health. Indeed, in peacetime the emergency chlorinators are usually in use by the various small communities which do not have standby equipment. Such standby equipment should be installed in such fashion that it is completely independent of electric power. It should utilize either gravity feed or water pressure for its operation. In case of units operated by compressed air, consideration can be given to the use of compressed inert gases (carbon dioxide or nitrogen). Care must be used in this event due to the high pressure of these compressed gases in the containers on the market.

For many years, the Des Moines Water System has chlorinated new installations using a simple device which would be quite useful in an emergency. A "corporation tap" was made in the main, connecting by a one-half-inch copper line to the bottom of a "trap" and connecting a one-quarter-inch copper line to the chlorine cylinder. The trap consisted of a two-foot length of water pipe four inches in diameter with a glass "sight gage" connected to the side of the pipe. The trap was mounted vertically on an integral tripod. The sight gage was used to insure against water entering the chlorine tank which could cause damage and possible explosion of the cylinder. The cylinder could be mounted on a platform scale to determine the rate of use of the chlorine, or the free chlorine in the water after passing the chlorine addition point could be determined. This setup would give satisfactory sterilization in an emergency.

Characteristics of an Ideal Disinfectant

An ideal disinfectant for water should meet the following requirements:

1. The agent should be in plentiful supply.
2. Physical, physiological, and chemical properties of the agent should be well known.
3. It should be in a form easily handled - gaseous for large-scale disinfection and liquid or solid for small-scale disinfection.
4. The disinfection agent should not deteriorate in storage nor should it attack the container under proper storage conditions.
5. It should not impair the palatability of the water.
6. Disinfection should be accomplished in a very short time.
7. The quantity of the agent should be readily ascertainable with simple equipment by persons with a minimum of formal training.
8. The agent should be one toward which organisms can not become resistant.
9. The agent should be one which can be used in very large dosages for achievement of instantaneous disinfection and a second agent used to neutralize an undesirable excess of germicide.
10. The agent should remain active over a long period.
11. The demand of the water for the agent should be readily ascertainable.

The general idea of the demand of waters for disinfectants will be discussed in some detail in the case of chlorine. It can not be overemphasized that all waters do not behave the same as to the effectiveness of a germicide. Temperature, pH, and organic material most frequently cause the demand. Inorganic material can also be a factor. In BW it is imperative that we have knowledge of this factor both from an offensive and a defensive standpoint.

From the above considerations, although it does not meet all of the criteria, chlorine appears to be the best all-around disinfectant.

The greatest disadvantages of chlorine are its impairment of taste and its dissipation from open containers. Chlorine appears to be the most universally effective agent against pathogenic organisms and is, incidentally, effective against some of the chemical warfare agents. Also, the properties of chlorine are very well known to all interested in disinfection.

Common Disinfectants

Chlorine

For many years chlorine in several forms has been used as a disinfecting agent in the water works industry both in the United States and abroad. This use has been both for purification and as a safeguard in the distribution system. The practice of carrying a definite residual of chlorine in the distribution system is practically universal in all major cities and in many small communities. In the larger installations, chlorine is purchased as a liquid under pressure in suitable tanks, ranging in size from 100-pound capacity to single unit tank cars. In smaller establishments, chlorine is often obtained in powder form as chlorinated lime or as a high chlorine content material or in the liquid sodium hypochlorite form. Since large water supplies are being considered our discussion will be confined to the field of elemental chlorine in compressed gas form.

The dosage required for the disinfection of a particular water can not be determined except by the study of the chlorine requirements of that water. Chemically, chlorine is a vigorous oxidizing agent and it is generally assumed that much of its disinfecting value stems from this chemical property. Therefore, any material which is capable of being oxidized by chlorine will decrease the quantity of chlorine available for the purpose of disinfection. This would include organic matter, nitrites, ferrous iron, etc.

In the study of an unknown water, a determination of the chlorine breakpoint is usually made. Figure 1 illustrates an ideal breakpoint curve, which quite frequently is not obtained. However, familiarity with the idealized curve does permit rather accurate judgement of an appropriate dosage of chlorine to achieve sterilization of the water under consideration. From the curve it will be noticed that between 0.0 and 0.5 part per million there is a somewhat proportional rise of the residual chlorine with increased dosage. At approximately 0.5 part per million an increase of dosage fails to give an increase of residual. This results in a plateau at B. Additional dosage of chlorine results in a precipitate drop in the residual to almost zero. A still further increase in the dosage will then give a gradual increase in the residual which

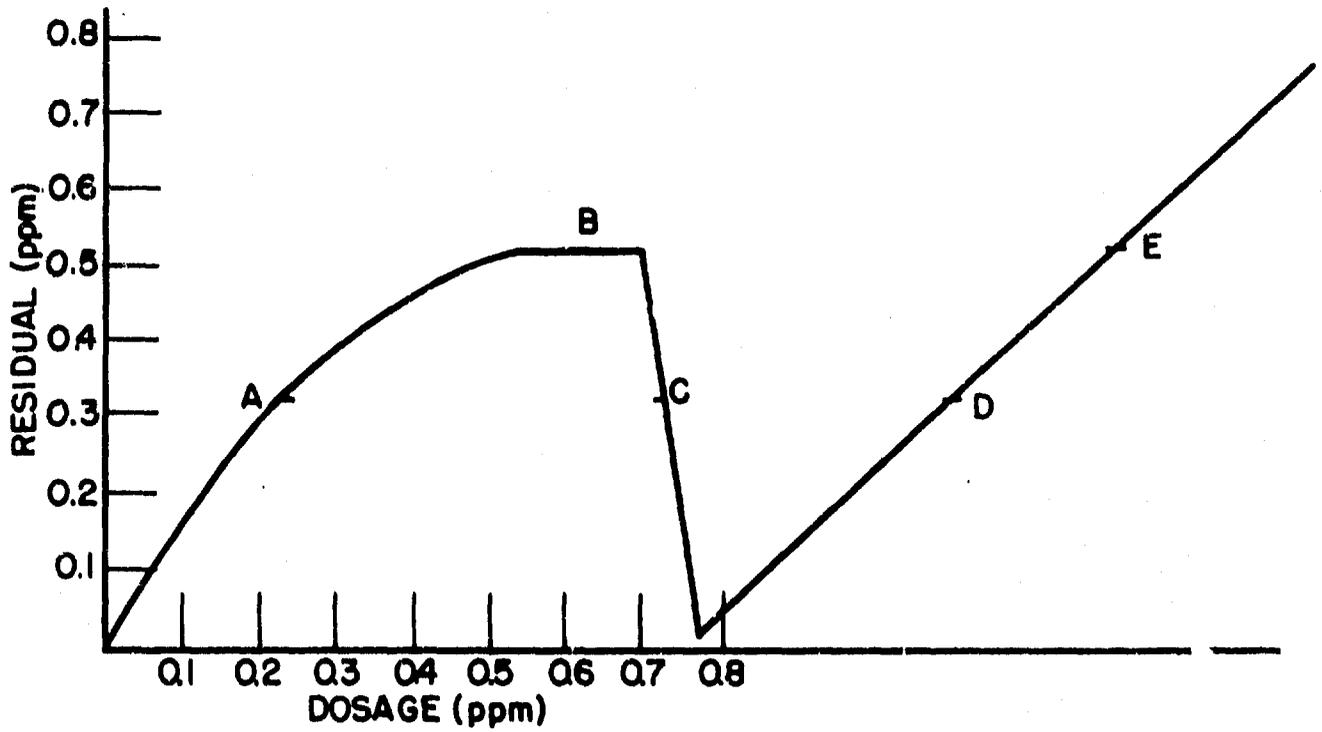


Figure 1 - Chlorine breakpoint, idealized curve

will continue to rise proportionally with the increase in chlorine dosage. It is readily observed that any residual at or below the plateau can cause uncertainty as to the completeness of the disinfection of the water. For instance, on this curve if we take the residual of 0.3 part per million, we might be obtaining a reading at either point A, C, or D. Obviously, only beyond point E is there complete disinfection, and the only safe residual which can be set is above the plateau, or in this case above 0.5 part per million. Many water plants can not normally carry a residual higher than the breakpoint since the water would not be acceptable to their consumers. In such cases, frequently the water is chlorinated beyond the breakpoint and then dechlorinated to a residual which will not cause complaints from consumers. To many people the taste of chlorine is very objectionable and it has been the practice for many years to add ammonia in addition to chlorine for the formation of chloramines. The use of ammonia in this manner permits a higher total chlorine residual without the objectionable taste of chlorine, although the chloramines do not disinfect as readily. However, in time of emergency the question of taste is a minor consideration; the public are perfectly willing to tolerate the additional chlorine if they understand their safety is involved.

Chlorine is, obviously, the most satisfactory disinfectant for water treatment in large-scale water supply. In the event gaseous chlorine is not available, calcium or sodium hypochlorite can be used. Of the latter two, calcium hypochlorite is to be preferred due to its better keeping qualities and its ease of handling since it is a solid. From the calcium compound, the sodium hypochlorite may be prepared if desired.

Chlorine is prepared by the electrolysis of a solution of sodium chloride or by electrolysis of the molten salt. The production of this material is adequate, the production having risen rapidly from 2,372,500 tons in 1950 to about 3,467,000 tons in 1953; approximately 50 percent is liquified. It has been estimated (60) that 89,000 tons, roughly five percent of the total production, were used in 1953 for sanitation purposes. Chlorine is shipped in the liquified gas state in either 100 lb, 150 lb, or ton containers for use in water purification. Single unit tank cars are available in three sizes - 16, 30, and 55 tons of chlorine - and in some cases shipment is made in 6000-ton tank barges. During World War II, the procurement of chlorine was quite difficult due largely to a shortage of shipping containers. During an emergency there should be rigid enforcement of a policy of rapid return of chlorine containers. In such times, the use of steel for other vital purposes and the almost total conversion of industry to the war effort would preclude the fabrication of chlorine containers. On the other hand, private industry should be encouraged to build a suitable stock pile of such containers in times when materials and labor are plentiful.

Since chlorine is handled in the larger plants in the liquified gas state, there are some hazards. This gas is quite poisonous and personnel should not enter the vicinity of a leak without the protection of a good gas mask containing a suitable canister. In common with all compressed gas containers, chlorine cylinders are equipped with a "fusible plug" which will melt at 158° to 165° F. There is a twofold reason for this plug in the case of chlorine:

1. For the prevention of explosion due to the greatly increased pressure which develops due to increased temperature.
2. Above 180° F., dry chlorine will attack steel and would cause possible corrosion of the entire tank.

Since infrequently the fusible plugs will blow out for no apparent reason, it is advisable to have long tapered wooden plugs which can be driven into the fusible plug opening. Heavy gloves should be worn when moving the container to an open area for the release of chlorine either slowly to the atmosphere or into one of the solutions mentioned above.

In the smaller water plants and in some industrial applications for purification, sodium hypochlorite is used. This product is made by feeding chlorine into a solution of sodium hydroxide under controlled conditions. Such a material is sold on the commercial market as household bleach, e. g., Chlorox, Hilex, and others. It is also locally produced in many areas for use in swimming pools, dairies, etc. Settled solutions of chlorinated lime e. g., HTH and Perchloron, are sometimes used as a source of chlorine and fed in a hypochlorite feeder.

Chloramines

Since free ammonia is present in most waters, chloramines will be formed in some degree when chlorine is added. Therefore, in most water plants tests for both free chlorine and the combined chlorine are made. Orthotolidine is almost universally used for this test.

The effect of temperature upon sterilization has long been known - disinfection being slower in cold water than in warm water. Also, the effect of pH has been observed. In 1946, Butterfield and Wattie (62) reported some interesting results which suggest the following conclusions:

1. The length of the time of exposure of the bacteria in water to chloramine and the amount of chloramine present are primary factors governing the rate of bacterial kills. Under favorable conditions, i. e., at pH 7.0 and a temperature of 20 to 25°C, 100 percent kills cannot be expected in less than 20 minutes with chloramine residuals of about 1.2 ppm.

2. The hydrogen-ion concentration has a pronounced effect on the bactericidal activity of chloramine, the activity being diminished with each decrease in hydrogen-ion-concentration. For instance, if under given conditions at room temperature, 0.6 ppm of chloramine at pH 7.0 produced a 100 percent kill in 40 minutes, then at pH 8.5 under otherwise identical conditions approximately 120 minutes would be required, and at pH 9.5, 240 minutes. Therefore, to produce a 100 percent kill in 40 minutes at pH 8.5, the chloramine residual would need to be increased to about 1.5 ppm.

3. A lowering of temperature retards the bactericidal activity of chloramine. A reduction of 20 degrees in temperature (e. g., 20-25°C to 2-6°C) requires 9 times the exposure period, or 2.5 times as much chloramine to produce a 100 percent kill. Thus, when the effect of a high pH water is superimposed on the effect of low temperature, very marked retardation of bactericidal activity must be anticipated.

4. Under certain conditions some strains of Eberthella typhosa and Shigella sonnei appear to be slightly more resistant than some strains of E. coli. However, they were not found any more resistant than the strains of A. aerogenes studied.

5. The presence of excessive amounts of ammonia nitrogen did not markedly reduce the bactericidal efficiency of the resultant chloramines.

6. The duration of the contact time (0 to 68 hours) of the chloramine components, chlorine and ammonia, did not alter the bactericidal properties of the chloramine.

7. Chloramines are much less efficient as bactericidal agents than free chlorine. Thus, to obtain a 100 percent kill with the same period of exposure required about 25 times as much chloramine as free chlorine, and to obtain the same kill with the same amounts of chlorine and chloramine under the same conditions required approximately 100 times the exposure period for the chloramine. Certain desirable minimum residuals which have been proposed (63) are shown in Table 1.

TABLE I
Minimum Residuals

| Disinfectant | Time (min) | pH | Residual (ppm) |
|---------------|------------|-------------|----------------|
| Free chlorine | 10 | 6.0 - 8.0 | 0.2 |
| | | 8.0 - 9.0 | 0.4 |
| | | 9.0 - 10.0 | 0.8 |
| | | above 10.0* | 1.0 |
| Chloramine | 60 | 6.0 - 7.0 | 1.0 |
| | | 7.0 - 8.0 | 1.5 |
| | | 8.0 - 9.0 | 1.8 |
| | | above 9.0† | ? |

*Preferable to keep in contact for 4 hours. The better solution for the high pH problem, when possible, would be to reduce the pH below 9.0 because at pH of 10.0 and above the taste of chlorine becomes accentuated due to the formation of hypochlorites.

†Preferable to reduce the pH below 9.0, when possible, or to extend the contact time to 4 hours, as in general 1.8 ppm of chloramine will produce a 100 percent kill in 2 hours of contact at pH 9.5 at either of the temperature ranges investigated.

If there is any doubt as to whether the chlorine present is in the form of chlorine or chloramine, as determined by the breakpoint procedure, then the safe assumption is that all of it is chloramine.

In general, the primary factors governing the efficiency of both chlorine and chloramine are:

1. The time of contact - The longer the more effective.
2. Temperature - lower the temperature the less effective.
3. pH - the higher the less effective, so, if low temperature and high pH are both encountered the poorest results are obtained.

With the most favorable conditions - pH 7.0 and temperature of 20° to 25°C, 100 percent kills cannot be obtained with chloramine residuals of 1.2 ppm in 10 minutes but may be obtained in 20 minutes. Under the same conditions with chlorine, 100 percent kills are obtained with 0.04 ppm residuals in 1 minute of contact.

Chlorine dioxide

Chlorine dioxide has been used for both disinfection and for the purpose of odor and taste removal. This compound has roughly two and one half times the oxidation capacity of chlorine. It has been found to be quite valuable in areas of slight contamination of water by phenolic wastes. Due to the vigorous oxidation afforded by chlorine dioxide, phenolic compounds are destroyed and the very objectionable chlorophenols are not formed as when elemental chlorine is used.

Chemically, chlorine dioxide is quite unstable and as a result is very dangerous to handle in the pure state. At Des Moines we have had some violent detonations of small amounts of this compound in the course of laboratory experiments. Because of this property, chlorine dioxide is produced in relatively low concentrations at the site where it is to be used. Customarily, the compound is produced by the action of gaseous chlorine on a solution of sodium chlorite. The resulting solution is then fed to the water supply. Since there should be an excess of chlorine, a ratio of one part of chlorine and two parts of sodium chlorite is recommended (64).

Ozone

In the past several years ozone has shown some merit in the reduction of tastes and odors in waters. The Ozone Processes Division of Welsbach Corporation has installed the largest capacity ozone plant in the U.S. at the Belmont Filtration Plant in Philadelphia, Pennsylvania. This plant has a capacity of 1250 pounds of ozone per day to treat 36 million gallons per day.

Since ozone cannot be stored due to its instability, it must be generated as required. Air which has been completely dried is passed through a high-voltage electric discharge. The resulting product should contain, under normal operating conditions, one percent by weight of ozone (64). The resulting mixture of air and ozone is passed through the water to be treated. The amount of ozone required to treat a supply depends on several factors:

1. Impurities present which would be oxidized by the ozone, such as organic material and reducing salts or gases.
2. The previous treatment undergone by the water.

3. The desired result - taste or odor removal or disinfection. Typical dosages of ozone range from 8 to 50 pounds of ozone per million gallons of water treated. A review of the use of ozone in water purification has appeared in the literature (65).

Ultraviolet

Sterilization can be effected by means of ultraviolet energy. Steritron is manufactured by the Hanovia Chemical and Manufacturing Company in units having a capacity of sterilizing 1600 gallons of water per hour (64). For many years, the objections to the use of this type of process have been the necessity of exposing the water in thin layers to the action of the ultraviolet energy and the tendency of the envelopes containing the sources to become coated with iron, manganese, or other scale-forming materials from the water under treatment.

Ultrasonic vibration

Some work on sterilization by ultrasonic vibration has been reported as a result of studies conducted at the Massachusetts Institute of Technology (64). Frequencies above 1800 cycles per second were used and were found to produce complete sterility after sonoration for 60 minutes at 90° F and almost instantaneously at 140° F. This field is only of academic interest at this time.

Silver compounds

In recent years the oligodynamic properties of silver compounds (65) have been investigated and some have been reported as quite satisfactory. Zimmermann (60) has presented an excellent review of this action mechanism as well as his experience with its long time use in the disinfection of a village water supply. Gesser (66) has stated that finely divided silver or silver compounds destroy bacteria but the action is so slow that the process is not generally practical. He electrolyzed water containing 50 parts per million sodium chloride in iron containers (the anode) with carbon or graphite cathodes and with a silver rod or strip near the cathode. He found the rate of destruction of bacteria was greatly increased. In this procedure destruction of the bacteria occurred in 15 minutes as compared to four to six hours in the absence of the electrolytic method. Gesser found no destruction of bacteria in the absence of the silver strips. In Gesser's work it is emphasized that the silver strips were not connected in the electrical circuit. The mechanism of this effect is not understood by us, unless by their particular location in the cell the silver strips serve as a secondary cathode. Braune (67) reported favorably on some exploratory observations with filtration through Sterilit, a granular material with a particle size of 0.3 to 1.0 mm containing

silver, as a sterilization substitute for chlorine. Only a portion of the water needs to be treated. It is then mixed with the remainder. Bactericidal effect decreased with increased amounts of organic material and with dilution. Tap water in contact with Sterilit for 4 minutes contained 7 gamma of silver per liter and 2.5×10^4 E. coli per liter were removed.

Zimmermann (60) reports that silver-coated sand was effective as a sterilizing agent.

The oligodynamic compounds have been used in Germany with quite good success and it has been reported (63) that silver compounds have been studied as water disinfectants. The complex sodium-silver chloride which decomposes in contact with water to give a fine colloidal silver chloride gave the best results. In another article János Páter (69) has recommended the application of small amounts of silver in quantities of 100 to 200 gamma per liter in water sources used by Hungarian railroads.

One of the greatest difficulties encountered in the preparation and use of the oligodynamic compounds has been the stability of the material. A preparation called Movidyn, marketed by the U. S. Movidyn Corporation is probably equal to or perhaps superior to other oligodynamic preparations. The Movidyn Corporation has an interesting background (66). Movidyn was originated in Czechoslovakia by Zdenek V. Moudry and was manufactured and marketed in that country for the disinfection of potable water supplies, swimming pools, and other industrial uses. In 1947 the Czech government ordered the use of Movidyn for the disinfection of nearly 10,000 wells in 200 villages in a typhoid area. After the disinfection had been accomplished there were no new cases of typhoid. In 1948, Moudry learned through the underground that the Russians planned to capture him. He, his wife, and parents escaped to the United States.

The U. S. Movidyn Corporation makes many claims for another material which they market under the name of Algaedyn. Some of these claims are: "Quickly eliminates scum and slime, water odors, and plant growth; guarantees lasting protection; proved to kill algae while not harming human tissues or membranes; will kill all algae growth; will not harm any aquatic plants, fish, turtles, snails, or any water animals; it is equally effective in hard, soft, acid, or alkaline waters." It is very difficult to accept these claims at face value. Although we believe their advertising claims to be extravagant, the material does have merit from all evidence.

The Environmental Health Center tested two hand-prepared batches of Movidyn, prior to the organization of the U. S. Movidyn Corporation (70). It is assumed that the commercial material does not differ ap-

preciably from this material. With a pH of 8.5 to 9.0 and a temperature of 22°C, 99.9 percent of the test organisms may be killed by as little as 0.1 ppm of Movidyn in six to eight hours. Under the same conditions the killing time may be reduced to two hours by using 0.4 ppm of Movidyn. At a pH of 6.0 to 6.5 higher concentrations of Movidyn were required. It should be noted that this is just the opposite of chlorine in regard to the effect of pH. Similarly to chlorine, Movidyn has a reduced disinfection action at low temperatures. A combination of low pH and low temperature requires both a high concentration of Movidyn and relatively long contact time to produce a satisfactory kill of organisms.

In December 1949, samples of Movidyn became available in experimental lots for evaluation in the Chemical Corps Biological Laboratories at Camp Detrick, Maryland. This product is described (71) as a finely dispersed colloidal silver stabilized by the addition of a protective agent such as gelatin to prevent re-coagulation. The tests reported in the reference were made on a laboratory sample prepared by the Czech developer and contained about five percent by weight of silver (expressed as silver nitrate). The tests were satisfactory with the following organisms being used: Escherichia coli, strain B; Serratia marcescens; Salmonella typhosa; Micrococcus pyogenes, var. aureus 209; and Bacillus globigii spores.

Possibly the best review of the subject of oligodynamic action is the excellent article by Zimmermann (60). He regards the mechanism of the oligodynamic silver as adsorption of the silver by the bacterial membrane. He reports varying resistances by various bacteria to this type of compound. It was also found that by serial cultivation a tolerance can be developed by some organisms. Zimmermann also reports the use of a silver preparation in a small reservoir. He used a silver chloride-sodium complex containing 1.0% silver (Micropur). Satisfactory treatment was given with the maintenance of 2 to 3 micrograms of silver per liter. In reporting the results of this experiment he again stressed the possibility of the occurrence of the development of silver fastness. He stresses the advantages of long continued activity between dosages and no effect on the taste, odor, or appearance of the water. The silver compounds of this type have one unusual property - that of maintaining a bactericidal effect on the walls of a container.

Chambers and Kabler (70) reported that this activity was rather difficult to destroy permanently. It was found that removal of the bactericidal effect could be obtained temporarily by boiling the container in cysteine hydrochloride and permanently by treating the glassware for 15 minutes in saturated sodium chloride containing 1 gram of copper sulfate and 2 milliliters of hydrochloric acid per liter. L-cystine in amounts of one percent would neutralize up to 20 ppm of the silver compound and 10 percent of cystine would neutralize up to

500 ppm. The compound which they tested was composed of roughly 30 percent of silver (the average particle size was approximately 75 angstrom units) with gelatin present as a stabilizing agent.

Specific Applications for Disinfectants

The oligodynamic silver compounds seem to be impracticable for use in the disinfection of municipal supplies, at least in cities of appreciable size. There are possible applications of this and other compounds as disinfectants for special purposes. Many times the need arises for disinfectants for specific applications in the construction of new facilities, such as additions to existing mains and treatment or collection facilities. Individual cases dictate, in many circumstances, the conditions of treatment. In some instances time may be of the essence; introduction of the disinfectant must be done prior to enclosure. In time of hostilities, breaks in the system may be caused by bombing or by use of explosives by enemy agents. Then, repairs must be made and the facility placed back in service in the shortest period of time possible. Prompt repair is essential from the standpoint of continued public service and the doubly needed fire protection. In times of emergency, water distribution centers for the public can be set up at various locations, such as schools, filling stations, grocery-stores, and other convenient locations (72). Probably some of this water would come from private wells if the municipal system were damaged severely. Water would be delivered to the several locations in tank trucks which are normally used for hauling milk, gasoline, and other products. Of course, these tanks would be suitably cleaned with steam and detergents. The silver compounds would be very useful in this case. Sterilization could proceed during the transportation of the water. Even the containers used by the public to secure water could be sterilized to prevent the inevitable contamination in the casual handling of water after withdrawal.

When new mains are extended - which in time of emergency is always needed in defense establishments or from existing city mains to defense plants or military areas - it is imperative that such mains be disinfected before being placed into service. This writer has always insisted that the water in a new facility be rendered free of coliform organisms before the consumer can obtain permission to tap the main. In work extending from 1930 to the present time, many agents have been tried at the Des Moines Water Works for the purpose of achieving this disinfection. Tests have been run with chlorine, both gaseous and in the form of hypochlorites, various heavy metal compounds; mixtures of various agents with wetting agents and detergents; and quaternary ammonium salts.

During the course of the investigation, many workers have observed that one great source of contamination was the material used

as a packing material in bell and spigot pipe. This packing material is usually jute or hemp which is highly contaminated with many types of organisms when received, and is usually not carefully handled by the workmen. It was found that soaking this material in a mercury phenol compound overnight effectively disinfected it. The hemp was passed through a wringer, stored in a tight box, and then used while still damp. This damp material could be used because "cement joints" were used instead of the customary joints of caulk and lead. Search for a more suitable packing material led to a braided paper material which is quite free from contamination and according to the manufacturer actually contains a disinfecting agent. Due to the impervious nature of this material it is rather easy to avoid gross contamination with reasonable care in handling.

During this research many special-purpose disinfectants were tested for stability and disinfecting ability. After the use of the paper material as a jointing material, the question was one merely of finding an agent which would satisfactorily disinfect cast-iron pipe which had become contaminated in storage. A quaternary ammonium salt was found to be most suitable for the purpose. There is some question of the toxicity of this compound, but in our application this is not a factor due to the tremendous dilution in the flushing of the installation. The quaternary salts could be used in the initial sterilization of temporary containers for water in times of emergency. It would seem that the oligodynamic silver compounds would be ideal for this purpose, although there might be some question as to their universal availability. Other materials which could be used for this purpose are the familiar commercial laundry bleaches, as well as chlorinated lime and the more modern high test hypochlorites. The chlorine materials have the advantage of normally being readily obtainable.

A polyiodide tablet which will disinfect drinking water in ten minutes has been reported in the literature (73). The authors claim these tablets contain 20 milligrams of tetraglycine hydroperiodide, which is the germicide, and 90 milligrams of disodium dihydrogen pyrophosphate, which serves as an extender. Five milligrams of talc is included in the preparation of the tablet. These tablets will dissolve in less than one minute and will treat one liter of most natural water in ten minutes. They are recommended by the authors as suitable for field disinfection of canteens because of the marked convenience of use and the palatability of the treated water. In the emergency treatment of water in municipal supplies the acceptability of the water is an important consideration, especially in the case of children. The polyiodide tablets are reported to be very stable when properly packaged.

CONCLUSIONS

1. Municipal water supplies may be contaminated with bacteriological agents but due to the intricacies of such systems the effect would be localized.
2. Even with the most modern techniques for detection, the first indication of an act of BW probably will be the illness of a large number of individuals.
3. Certain psychological factors greatly favor the aggressor and very definitely handicap the one attacked.
4. Either an organism or several organisms may be chosen to achieve a specific purpose.
5. Disinfecting agents may be neutralized by skilled saboteurs.
6. Physical security of plants and buildings is the greatest safeguard against BW.
7. Fear of retaliation is the greatest deterrent to Bacteriological Warfare.

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