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Research and Development Department

SMALL SCALE WATER PURIFICATION WITH  
SILVER ASBOLOXANES

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SMALL SCALE WATER PURIFICATION WITH  
SILVER ASBLOXANES

INTRODUCTION

This report deals with the application of the newly developed Silver Asboloxanes to the purification of small amounts of water. Supplying the individual soldier, or small groups of men in the field with a ready source of safe drinking water is a constant military problem. Men cut off from sources of purified water must be provided with simple methods by which they can sterilize whatever naturally occurring waters are available. Not only must the pollution ordinarily found in such waters be dealt with, but also it must be kept in mind that in warfare the waters may be deliberately contaminated.

The present methods of treating water either with Halazone tablets for canteens or with calcium hypochlorite in Lyster bags are not altogether satisfactory. With large amounts of organic matter or high bacterial counts, sterilization is not achieved unless great excesses of the chlorine compound are used. Moreover, sediment is not removed, and an objectionable taste is imparted to the water, so that even when the water treated by such methods is safe to drink, it may still be unsightly and unpalatable.

The Silver Asboloxanes, developed by Dr. Alexander Goetz at the California Institute of Technology as an outgrowth of theoretical studies on the oligodynamic effect of heavy metals, offer a possible alternative to these chlorine treatments. Two pieces of military equipment utilizing Silver Asboloxane were developed by Dr. Goetz during the war at the request of the Mexican Army. They were a portable filter pump capable of treating two liters of water a minute, and a canteen capable of treating one quart of water per treatment. These

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items are of considerable interest, because in them the water is filtered as well as sterilized and no objectionable flavor is imparted.

The Asboloxanes themselves have been the subject of considerable study in this laboratory. This report covers that information which is of interest in water purification, and particularly, that obtained from practical trials with the pump and canteen. Data and information gathered by Dr. Goetz at the California Institute of Technology are included.

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ACKNOWLEDGEMENTS

The Asboloxanes and the special equipment used in this study were furnished by Dr. Alexander Goetz and the Shellmar Corporation, who are handling the commercial development of these products. We gratefully acknowledge their supplying us with many products still in the experimental or developmental stage and with freely supplying us with information concerning the products. Dr. Goetz has also supplied us with results from his own experimental work at the California Institute of Technology and in the field in Mexico. Where we have used this material in the text of this report we have quoted it directly from his reports with the necessary acknowledgements.

The experimental work at Camp Detrick was largely the result of the combined efforts of most of the personnel in the Decontamination Branch. Particular credit is due to Dr. Ruth A. McKinney, especially for the studies on bacterial enzyme systems, and to the Misses Mary F. Goffin, Dorothy M. Fortner, and Helen F. Irminger, who performed most of the bacterial and chemical assays.

Thanks are due to Dr. B. Warshowsky and the Chemistry Branch, Basic Sciences Division, and to Dr. J. B. Bateman and the Physics Branch, Physical and Chemical Division, for some of the chemical and physical data.

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SMALL SCALE WATER PURIFICATION WITH  
SILVER ASBOLOXANES

A. SILVER ASBOLOXANES - GENERAL DESCRIPTION

The term "Asboloxane" is derived from the Greek, asbolos-soot, oxys - acid. It has been used by Dr. Goetz as a generic term for surface compounds with metals, where the radical is represented by the acid oxidation compounds on the surface of colloidal carbon particles. Dr. Goetz has given the following general description of the nature of the Silver Asboloxanes:

"The Silver Asboloxanes are an entirely new type of silver compound substantially different from the customary chemical silver compounds such as silver nitrate, silver picrate, and the like, or the preparations of colloidal silver such as argyrol, collargol, etc. In the Asboloxanes the silver is chemically coupled with oxygen by the strong adsorptive bonds existing on the surface of colloidal carbon and certain inert metal oxides. These physico-chemical forces which characterize chemisorption render it possible to couple silver with other elements such as oxygen to an extent and to a degree which is non-existent in normal silver compounds.

"Since the forces mentioned exist only on surfaces, carrier materials of extremely fine particle size must be employed. Such substrate materials, capable of great adsorptive power and available in sub-microscopic particle sizes, 20 to 100 millimicrons, are the colloidal forms of carbon. These colloidal carbons, while physiologically neutral on application to skin and mucous membranes, expose surface areas of as much as 10-20 acres per ounce.

"Such materials can be made to carry large quantities of oxygen in the form of oxidation layers upon the particle surfaces and by means of newly discovered

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processes it is possible to "build" into such adsorption layers substantial quantities of atomic silver. In contradistinction to normal silver compounds of colloidal silver preparations, the silver is bound to and held at the surface of the carbon particle and is only very slightly soluble.

"The advantage of such compounds lies in the combination of a highly germicidal and fungicidal property and an absence of toxicity and denaturation of proteins, because the silver atom can go only very sparsely into solution and its stability in its oxidation state on the surfaces protects it against the reducing qualities of the proteins."

The germicidal effectiveness of the Asboloxanes has been explained as being due to their ability to catalyze the decomposition of peroxides, probably by forming an intermediary oxide of silver on the surface of the carbon. In the presence of the Asboloxanes, peroxides become much more destructive to bacteria than are ordinary peroxide solutions. The developer of the Asboloxanes has also given the following description of this general property:

"It has been known for a long time that silver-oxide is a very efficient catalyst and based upon this experience a series of silver-containing catalysts have been developed which have in common the property of being practically insoluble, in as far as they do not send silver ions into solution, and at the same time render available a maximum of silver per total mass of silver employed. This condition can be realized by adsorbing silver on the surface of colloidal carbon in the presence of the oxygen adsorbed on the carbon so that one can assume that the carbon surface exposed and acting as a catalyst consists of adsorbed monomolecular layers or polymolecular layers of an atomic mosaic of Ag and O. In other words the catalyst consists of a surface compound representing an unknown oxidation state of silver. Colloidal carbon appears to be particularly suitable for this purpose, partly on account of its surface per unit mass, partly because of the strong adsorptive affinity of carbon for oxygen."

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Two particular applications have been made of the Asboloxanes for the sterilization of water. In one case, a special Asboloxane has been developed in which sterilization of water can be achieved prior to filtration. Another, the Asboloxane S-Filter Powder, is designed so that filtration and sterilization are achieved at the same time. These special Asboloxanes have been developed respectively for use with a specially designed military canteen and portable filter pump, called the S-filter by its developer, and likewise designed for military use. These two pieces of apparatus, the particular Asboloxane charge designed to be used in them, and the results of trials conducted on their performance both at California Institute of Technology and at Camp Detrick are described in the following sections.

## B. THE CANTEEN; UTILIZING ASBOLOXANE STERILIZATION PREVIOUS TO FILTRATION

### 1. Description of The Canteen

The canteen developed for use with the Asboloxane treatment is an ordinary Army Canteen with a specially designed filter head in place of the usual screw cap. The filter head consists of a filter set into a combined cap and drinking spout. This assembly and the filter head are illustrated in Figures 1 and 2.

The filter itself consists of a cylinder of cellulose material reinforced by impregnation with a thermosetting plastic. This unit, which is replaceable, is cemented into the metal cap. The liquid in the canteen must pass through this filter unit to empty through the spout in the filter head. No valve is involved. The filter unit holds back the solid Asboloxane particles (1) as well as suspended matter such as mud, sand, algae and protozoa. Bacteria would for the most part pass through the relatively coarse filter, but as mentioned previously the Asboloxane charge is designed to achieve sterilization before filtration.

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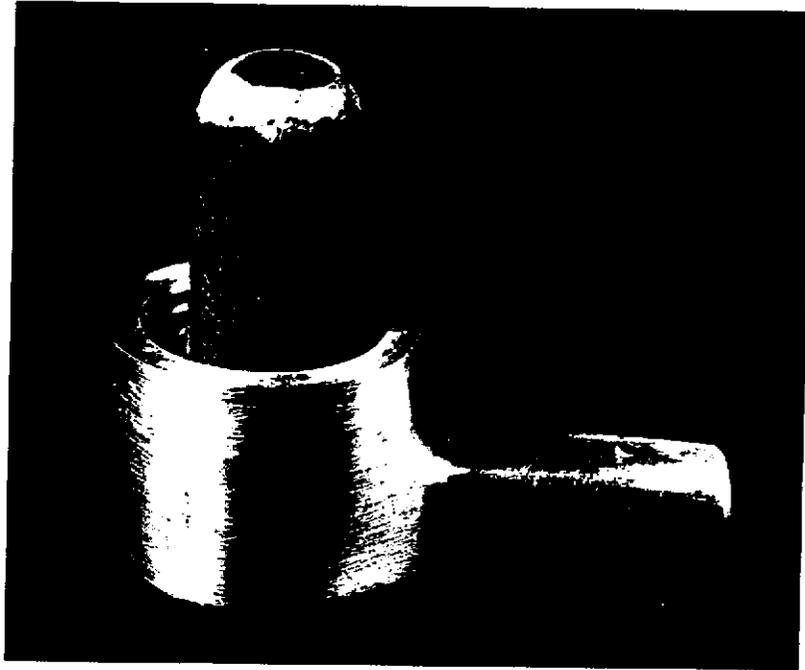
(1) Asboloxane particles, as we have observed them, have a particle size very similar to that of bacteria. There appears to be, however, an agglutination of Asboloxane and other particles in the canteen, so that the particle size is effectively increased after sterilization, and the particles are withheld.

FIGURE 1



Assembled Canteen

FIGURE 2



Canteen Filter Head

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In use, the canteen is filled with water and the special Asboloxane canteen charge is added. The filter head is then screwed on. The canteen is shaken for 15 to 30 seconds, and then allowed to stand for 15 minutes before using. The pressure developed within the canteen will force a few milliliters of water through the filter head and out through the drinking spout. This serves to sterilize the filter cylinder and the inner surfaces of the head at the same time as the liquid inside the canteen is being treated.

To drink from the canteen, the filter head is not removed. Rather it is loosened about a quarter turn, and the user drinks by inverting the canteen and sucking through the spout. Air passing along the loosened threads to the cap prevents the formation of a vacuum within the canteen which would make it difficult to suck out the water. The Asboloxane catalyst and whatever solid matter was originally present is held back mechanically by the filter. Not until the canteen is empty is the filter cap removed. Then the canteen can be rinsed, refilled with water, and the process repeated using a new charge of the Asboloxane.

The treatment, aside from its sterilization action, actually renders the water more palatable in that a mechanical filtration is included, and the odors and tastes present in the natural water are often destroyed by the oxidizing action. No objectionable chemical tastes or odors are added by the Asboloxane treatment.

## 2. Special Asboloxane Powder for Use in Canteen

A special charge, designed to sterilize water prior to filtration has been developed which can be used for treatment of small quantities of water collected in containers such as canteens, portable water tanks and the like. The charge for an Army Canteen, holding 850 cc. of water, or approximately one quart is as follows:

750 mg. Asboloxane catalyst [REDACTED]  
500 mg. Urea Peroxide  
180 mg. Sodium Carbonate

Such a charge can be packaged so that it weighs no more than 2-3 grams. The developers claim that the materials involved, if packed to exclude moisture, have a storage life of at least one to two years and do not have a corrosive action on their packaging containers. Other soluble peroxides could be used in place of the urea peroxide.

The charge is added directly to the water. Initial shaking or stirring is desirable, but not absolutely necessary because the rapid catalytic destruction of the peroxide and subsequent gas evolution provide enough agitation for a small quantity of liquid such as a quart. The bactericidal action takes place as a result of catalytic decomposition which is usually complete within 15 minutes at which time only a few parts per million of peroxide remain. It is recommended that the water stand for this full 15 minutes before drinking, although it is usually safe before this time.

### 3. Performance Tests on the Canteen Conducted at California Institute of Technology

The following statements are quoted from a report prepared by Dr. Goetz at the California Institute of Technology:

"The development of the S-canteen treatment was largely guided by bacteriological laboratory tests on artificially infected waters. These tests were conducted at the bacteriological laboratory of the Rare Metals Institute at the California Institute of Technology.

"The tests were conducted with various types of waters artificially inoculated with E. coli and mixtures of E. coli, staphylococci and streptococci. Inhibitory agents such as organic and inorganic nitrogen in the form of peptone and ammonia were added in certain tests in order to establish the limitations of the action of the preparation.

"After the filling of the canteen with the test water, the chemicals were added, and after the lapse of 15 and 30 minutes, water samples were withdrawn

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through the filter head with a vacuum pump under sterile conditions simulating as closely as possible the action of drinking through the filter head. The samples were distributed in the customary manner: five 10 cc samples, one 1 cc sample and one 0.1 cc sample in lactose broth, with subsequent incubation for 48 hours at 37°C. In case gas was observed, confirmation for E. coli was carried out in Brilliant Green Bile broth.

"The following are typical results of such tests:

a. "Tap water with the addition of 120,000/cc E. coli. Tested after 20 minutes. Result: one confirming gas tube of five 10 cc tubes.

b. "Tap water with the addition of 62,000/cc E. coli. Tested after 10 and 20 minutes. No gas.

c. "Lake water, very rich in algae, with the addition of 30,000/cc E. coli. Tested after 15 and 30 minutes. No gas.

d. "Well water, mixed with sewage (1/2000) plus 250 parts per million peptone, with the addition of 140,000/cc E. coli. Tested after 15 minutes. No gas.

e. "Same, with 500, 1000 and 2000 parts per million peptone and same coli inoculum. Tested after 15 minutes. No gas.

f. "Same, with 500 parts per million peptone and 40 parts per million NH<sub>3</sub>. Tested after 15 minutes. No gas.

"For the sake of comparison, the last mentioned substrate with the same inoculum was tested with the U.S. Army C.D.C. (emergency over-chlorination) preparation: after 20 minutes, confirming gas was observed in all tubes down to 0.1 cc

"The same was found to be true with an iodine-potassium-permanganate preparation and with Halozone tablets.

"In all these tests the S-canteen water was found to be tasteless, colorless and complying with rigorous sanitary standards.

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"In view of the fact that infected waters tested under laboratory conditions may not represent the performance of method and equipment in the field, a number of field tests were performed on various types of waters in the States of Sinaloa, Guerrero, Morelos and Distrito Federal (Republic of Mexico), in collaboration with the Dep. de la Salubridad Publica. In order to test the water after a short lapse of time, ambulant laboratory equipment was taken along to the site of the test. Thus the conditions in the water, when collected, treated and consumed under actual conditions, could be studied as closely as possible. These tests are enumerated as follows:

a. "Location: Rio Humaya, 5 km north of Culiacan, Sinaloa. Temperature: 22°C; pH: 7.5 - 8.0. Control: gas in 1 cc sample, confirmation questionable. Canteen water, withdrawn after 17 minutes: no gas in any sample.

b. "Location: faucet outlet in Public Health Laboratory in Culiacan. Temperature: 23°C; pH: 7.5. Control: 2 confirming gas tubes in five 10 cc samples. Canteen water, withdrawn after 18 minutes: negative throughout.

c. "Location: Indian water hole in river bed of Rio Siqueros near Pena Hueca, about 40 km inland from Mazatlan. Temperature: 23.5°C; pH: 7.5. Control showed confirming gas down to 0.1 cc. Canteen water, withdrawn after 30 minutes: one confirming gas tube out of five 10 cc samples, other tubes negative.

d. "Location: Rio Tepecua, Chatitla, Guerrero. Temperature: 29°C; pH: 7.5 - 8.0. Control: confirming gas down to 1 cc sample. Canteen water, withdrawn after 20 minutes: no E. coli throughout (but non-confirming gas formers in two 10 cc samples after 48 hours incubation).

e. "Location: Tixtla, Guerrero, water ditch leading from a native adobe swimming tank through a section used by local washer-women and subsequently under the cadaver of a burro, the latter in the beginning stage of putrefaction. The water was highly turbid and evil smelling. The sample was taken about three meters

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downstream from the cadaver. In view of the highly putrid condition, 2 capsules of the chemical were taken. The temperature of the water was 27°, the pH 8.5 (!). The control showed confirming gas in 0.01 cc. The canteen water, withdrawn after 15 minutes showed no gas in any of the samples. It was also observed that the water had lost its putrid odor.

f. "In order to test the nature of an infected water after prolonged storage in the canteen under field conditions, a sample of a stagnant water in a ditch near the highway 12 km east of Acapulco was filled into the canteen, the chemicals added, and the water stored for 48 hours in the canteen before withdrawal for testing. The temperature of the sample had been 28°C, the pH 6.5 - 8.0, but the canteen, lying in an open truck for two days was exposed to temperatures exceeding 40°. The control water showed confirming gas down to 0.1 cc. The canteen water was free of E. coli in all samples, but showed non-confirming gas in three 10 cc samples.

g. "Location: canal of Cuautla, Morelos, at Km 1. This test was run in the presence of Lt. Col. Dr. Granillo, as representative of the War Department and Sr. Ing. Acosta as representative of the Dep. de la Salubridad Publica. The latter took independent samples of the water, which was withdrawn 2 hours and 15 minutes later. The temperature was 20.5°C, the pH 7.5. The control water showed confirming gas down to 0.1 cc. The official E. coli content was estimated to be 40,000 per liter. The canteen water proved to be negative throughout in the official and in our tests.

h. "Location: stagnant water at the highway bridge in Xochimilco, D.F. Temperature: 20.5°C; pH: 8.0. This test also was run in duplicate by the writer (Dr. Goetz) and the Public Health Department. The control water showed confirming gas down to 0.001 cc, and the official estimate gives a probable content of E. coli of 4 million per liter. In view of the high algae content and general turbidity of

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the water, two capsules were used. The water was withdrawn after 1 hour and 35 minutes. In the author's test, the water was completely negative throughout. In the official test, two out of five 10 cc tubes showed gas after 48 hours, which did not confirm as E. coli. The water is therefore considered sanitary.

"The Laboratories of the Dep. de la Salubridad Publica tested the contents of the canteen of the last test (containing two capsulea) chemically, and found no toxic substance present.

i. "In analogy to test (g) (see above) an army canteen (with normal cap) was filled at the same location as the previous test and one capsule of the U.S. Army C.D.C. preparation was added. Subsequently the water was withdrawn after 3 hours and 15 minutes. After 24 hours incubation, the 1 cc samples showed confirming gas. It can therefore be estimated that the coli content was about 4000 per liter. This water is therefore definitely unsanitary.

"The above enumeration of field tests includes all tests made. From it, it is obvious that in every case sanitary drinking water was obtained, with the possible exception of test (c). For this test, the local conditions for the sterile withdrawal of the water from the canteen (in a dust storm) were definitely unfavorable and may have caused an error."

#### 4. Performance Tests on the Canteen Conducted at Camp Detrick

Two types of tests were run at Camp Detrick in evaluating the Asboloxane Canteen treatment. In the first runs, regular army plastic and metal canteens were used together with the special filter head and the Asboloxane charge. The full charge of 750 mg. Asboloxane catalyst, 500 mg. urca peroxide, and 180 mg. sodium carbonate was used. This was added to the canteen filled with approximately 850 ml. of heavy suspensions of various test organisms. The special filter head was screwed on and the contents well shaken. After the indicated times, samples were syphoned out aseptically without removing the filter head, and plate counts

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were made in the usual manner. Data obtained from these trials are presented in Table I.

Physical measurements made upon the samples removed from the canteen showed that the water had become alkaline as one would expect from the presence of sodium carbonate in the charge. For example, the suspensions of *E. coli* in distilled water exhibited a pH of 10.1 after being treated in the canteen. The water, however, was quite palatable in spite of this change in pH, and in spite of the fact that it had contained originally 35 million organisms per milliliter. No objectionable tastes or odors were noticed.

After these first preliminary tests had confirmed that the canteen was indeed quite effective, a second series of trials was started in Erlenmeyer flasks, using only 85 ml. of bacterial suspension and one tenth the charge indicated above. Using small volumes of liquids in laboratory glassware, it was possible to control better various factors such as time of contact and to evaluate the action of the Asboloxane charge itself against various test organisms in containers more easily cleaned and sterilized between trials. As with the trials run in the canteen, the Asboloxane charge was added to the suspension and shaken well. The suspensions were then filtered, this time through coarse sterile filter paper, and plated out immediately. In this way, quick dilution into nutrient media was counted upon to eliminate any possible lethal effect caused by the filtrate itself after the Asboloxane catalyst had been removed by filtration. The Asboloxane canteen charge was evaluated in this manner against a spectrum of eight bacterial test organisms and two bacteriophages. The data obtained are given in Table II.

The action of the Asboloxane charge is exceedingly rapid on vegetative organisms. Most of these organisms were completely killed within 1/2 minute, and all such suspensions were rendered sterile within 5 minutes.

TABLE I

PERFORMANCE OF ASBOLOXANE CANTEEN CHARGE

(Trials Made Using Army Canteen  
with Special Filter Head)

Test Organism	Original Suspension	Organisms per ml. after Various Exposure Times
B. globigii	532,000	36,000 ( 3 min.)
6-Hour Cultures (Vegetative Form)	30,000	230 ( 3 min.)
E. coli	35,000,000	4 (15 min.); 1 (24 min.)
	710,000	44 ( 5 min.); 3 (15 min.); 0 (24 min.)
	475,000	5 (1/2 min.); 0 ( 5, 10, and 15 min.)
E. typhosa	21,700,000	0 ( 3 min.)
	8,260,000	0 (15 min.)

TABLE II

## PERFORMANCE OF ASBOLOXANE CANTEEN CHARGE

(Trials Made Using Glass Equipment)

Test Organism	Original Suspension	Organisms per ml. after Various Exposure Times			
		1/2 min.	5 min.	10 min.	15 min.
<i>B. globigii</i> (spores)	62,000,000	-	-	-	1,560,000*
<i>E. coli</i>	257,500	2,200	0	0	0
<i>E. coli</i> phage T-2	640,000	TNTC	-	149,000	56,000
<i>E. typhosa</i>	7,500	0	0	0	0
<i>G. tetragena</i>	11,050	0	0	0	0
<i>K. pneumoniae</i>	1,515,000	0	0	0	0
<i>M. phlei</i>	275,000	0	0	0	0
<i>M. phlei</i>	6,850	0	0	0	0
<i>S. aureus</i>	750,000	1,700	0	0	0
<i>S. aureus</i> phage P-209	TNTC	TNTC	TNTC	TNTC	TNTC
<i>S. marcescens</i>	245,000	0	0	0	0
<i>S. marcescens</i>	5,100	0	0	0	0

\* 2 Hours - 3,400

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Within 15 minutes, the time which the developers of the Asboloxane treatment recommend that the contents be allowed to stand before drinking, 97.5 per cent of the spores of *B. globigii* had been killed. At two hours 99.995 per cent had been killed. These spores are the most resistant of the bacterial test organisms which have been studied here.

It is quite interesting to note that the two bacteriophages tested displayed resistance of the same order of magnitude as this resistant bacterial spore. Whether pathogenic virus organisms would be as difficult to disinfect is an open question.

Trials were also made, using *E. coli* as the test agent, in which the bactericidal activity of the various ingredients of the Asboloxane canteen charge were measured separately. The trials were run in exactly the same manner as the Erlenmeyer flask trials with the complete charge which were just described and reported in Table II. The weights of the ingredients were the same, and in one case an equal weight of activated carbon was substituted for the Asboloxane catalyst. As the data which are given in Table III show, the Asboloxane catalyst alone is a fairly effective bactericide but by no means as active as it is when urea peroxide and sodium carbonate are present as is the case in the full charge. The urea peroxide alone is not too effective, nor is its effect increased by the addition of sodium carbonate and activated carbon. No loss, but rather a gain in the *E. coli* count is observed in contrast when the original suspension is shaken in distilled water for 15 minutes and filtered. This was often noted in control runs with this and other organisms, and probably is due to the breaking up of groups of clumped cells, present in the original suspension.

TABLE III

PERFORMANCE OF THE VARIOUS INGREDIENTS IN THE

ASBOLOXANE CANTEEN CHARGE

(Trials made using Glass Equipment)

Test Organism - E. coli in all cases

Ingredients of Canteen Charge Present	Original E. coli Suspension	Organisms per ml. after various Exposure Times			
		1/2 min.	5 min.	10 min.	15 min.
All. Full Charge	258,000	2,200	0	0	0
Asboloxane Catalyst Only	285,000	11,250	1,800	50	0
Urea Peroxide Only	258,000	TNTC	TNTC	TNTC	28,000
Activated Charcoal, Sodium Carbonate and Urea Peroxide	428,000	69,000	42,000	64,600	61,800
None. Shaken in Distilled Water and Filtered.	285,000	-	-	-	840,000

## C. THE S-FILTER - SIMULTANEOUS FILTRATION AND STERILIZATION WITH SILVER ASBOLOXANE

### 1. Description of the S-filter

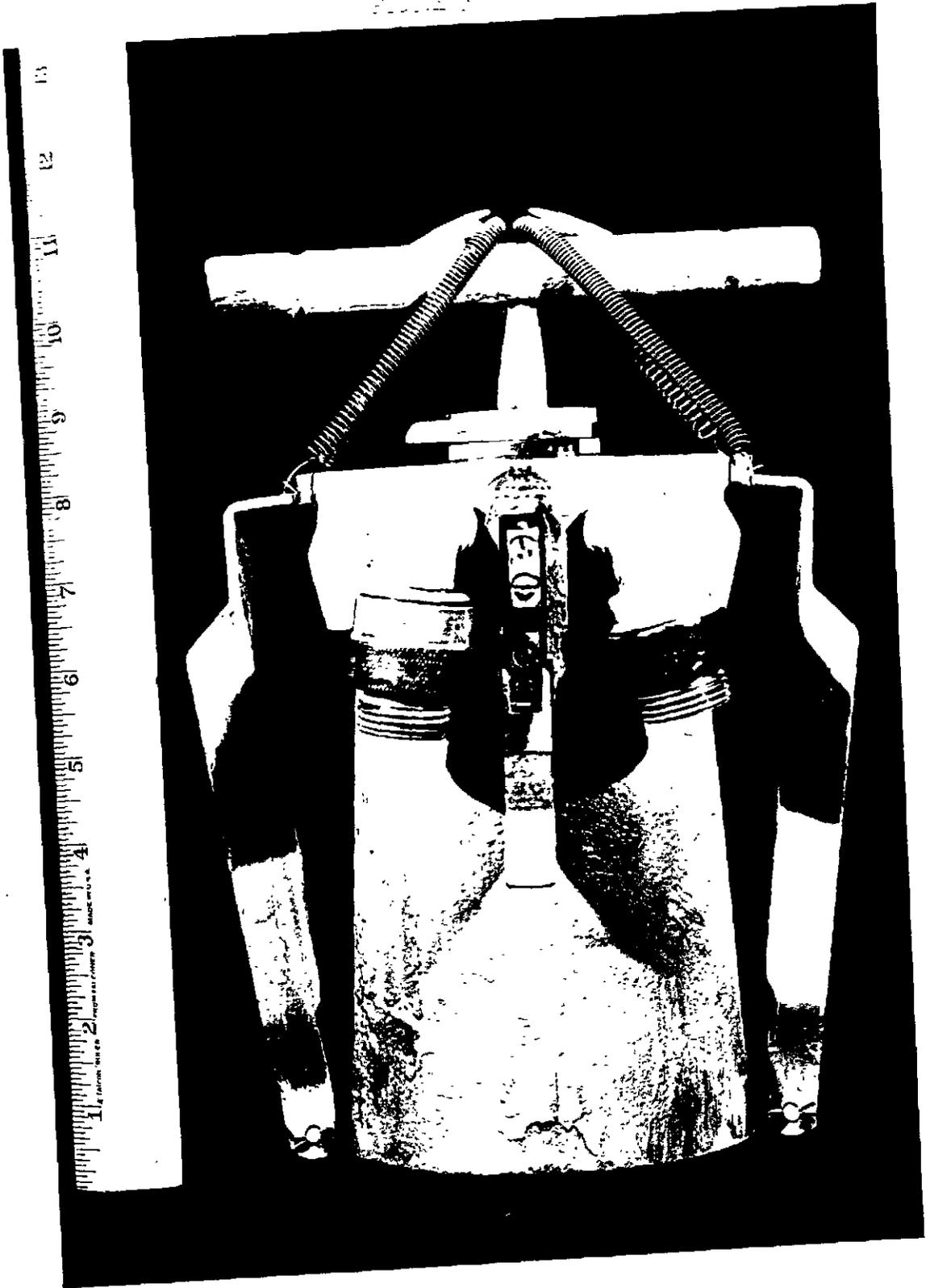
The S-filter is a light-weight apparatus, weighing under 10 pounds; requiring one man for operation. Water is drawn into the S-filter and forced through a cake of Asbолоxane S-filter powder so that it is filtered and given a sterilization treatment simultaneously. The purified water is delivered at a rate of 2 liters per minute. Each charge of Asbолоxane will treat about 50 liters of water.

The S-filter itself is shown in photographs in Figures 3 through 6 and diagrammatically in Figure 7. The photographs do not show the two hoses or rubber tubes which are connected to the inlet and outlet valves during operation. The inlet hose is dipped into the stream, pond, or other water source, which is being utilized. Through the outlet hose, the purified water can be delivered directly to canteens, water cans, or other containers.

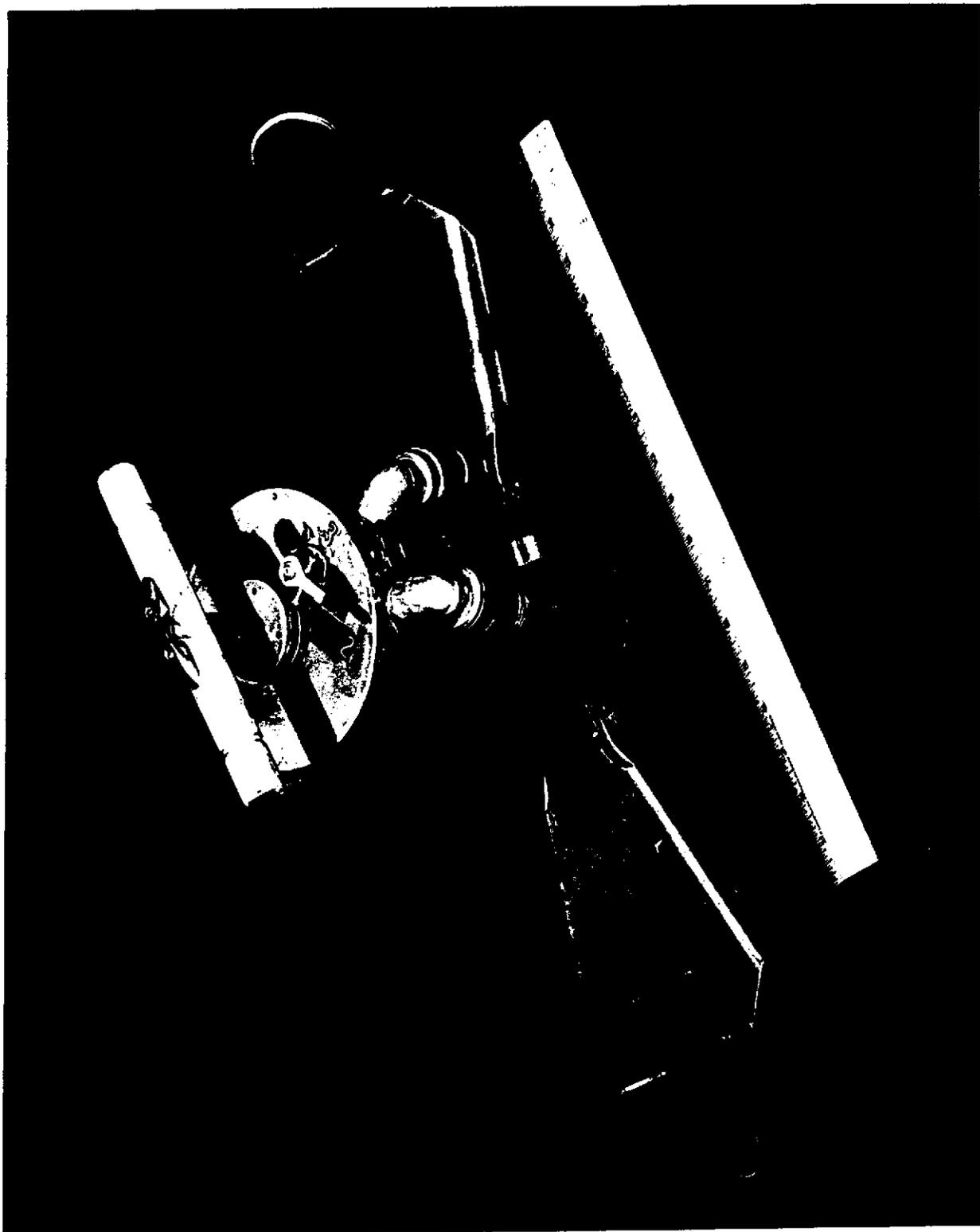
As shown in Figure 5, the outer portion, or base, of the S-filter is nothing more than a tank. The central portion, which contains the pump, the filter, the inlet and outlet connections, and the control valve, rests inside this outer base, and is attached to it by three clamps. The Asbолоxane S-filter powder charge is added directly to the outer tank. At the end of a run, the S-filter is opened as shown in this photograph, and the spent charge is discarded.

The pump in the S-filter is a hand-operated piston type, with a bore of 30 mm. and a stroke of 75 mm.; delivering 53 cc. with each down-stroke.

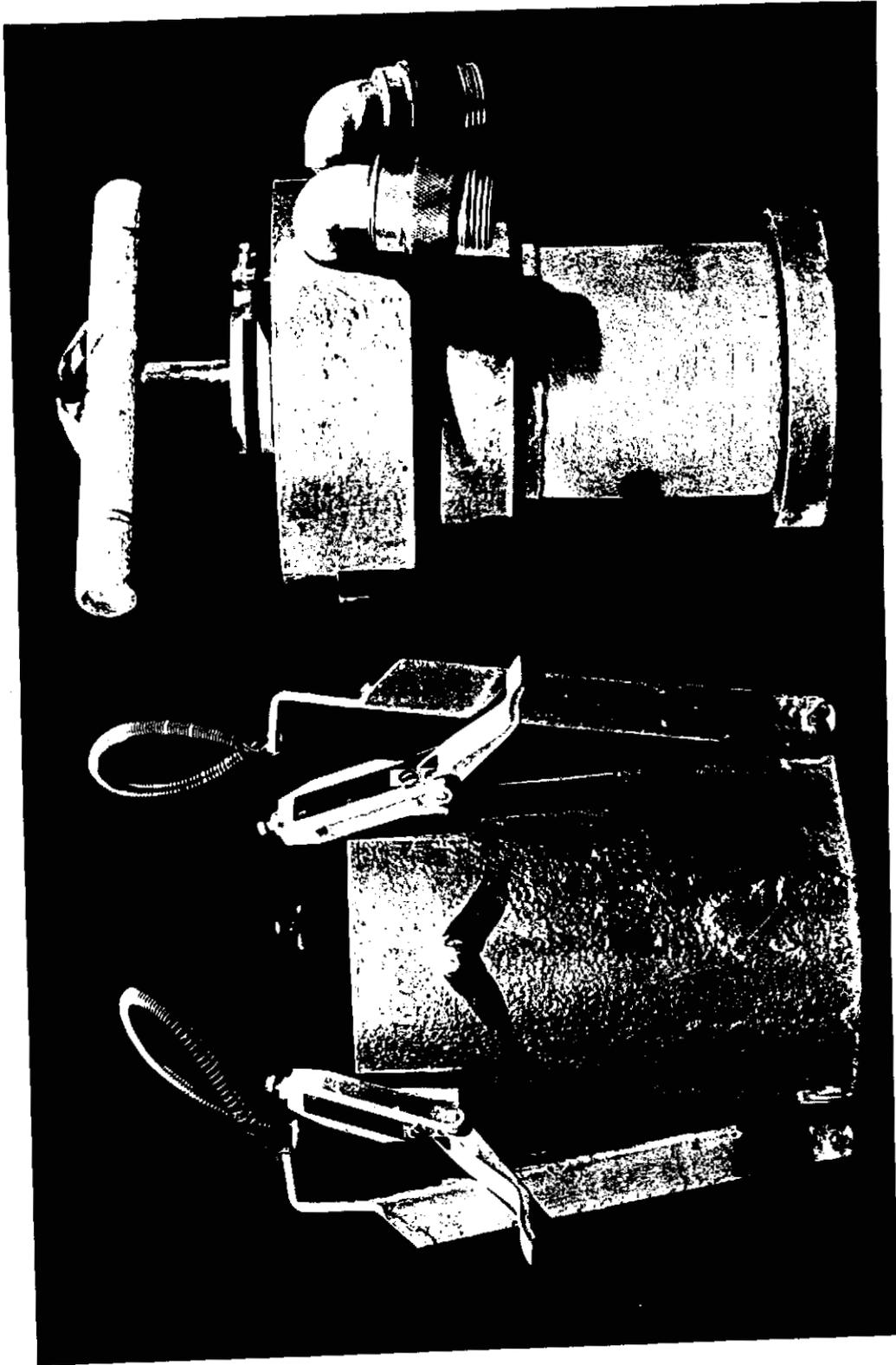
The disperser, shown in Figure 6, which fits below the filter, consists of a series of baffles and a metal screen which breaks up the flow of water and causes it to flow evenly about the filter. At the beginning of a run when dry Asbолоxane powder is placed below the disperser, this turbulence causes a thorough mixing of incoming water and filter powder, and an even deposition of the filter cake about the filter screen.



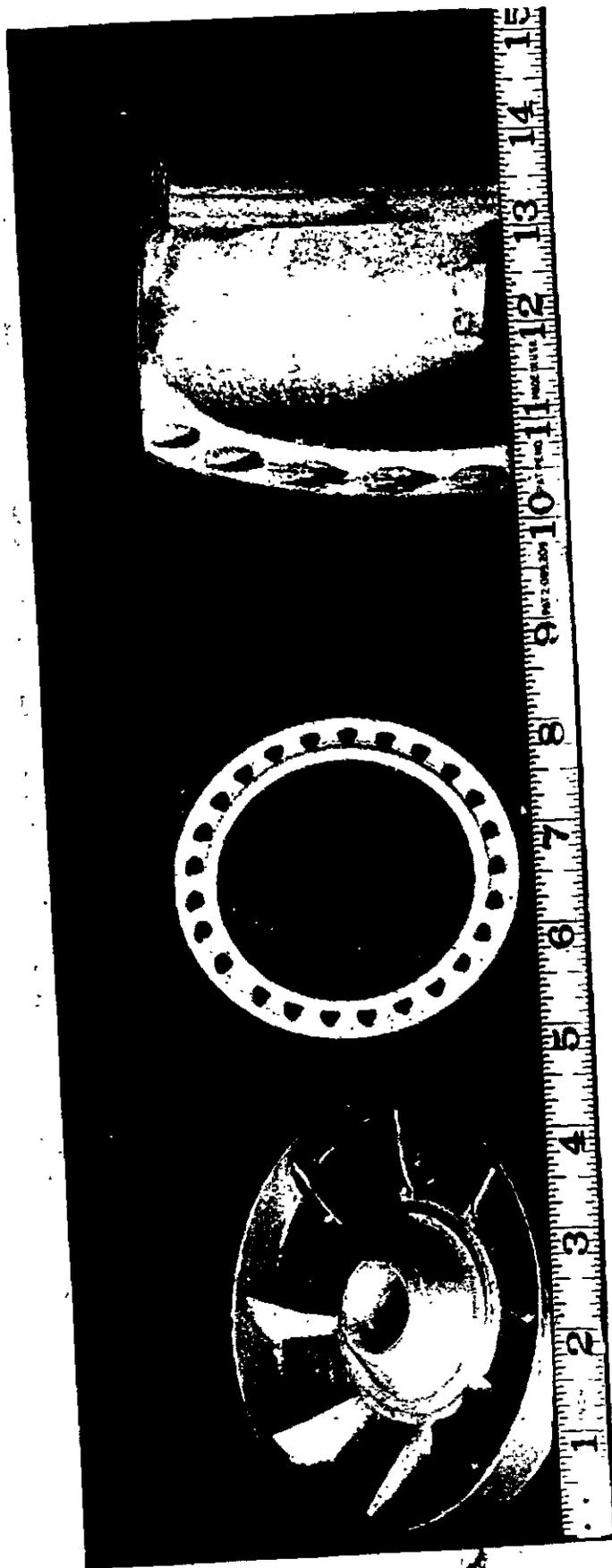
Assembled Carburetor - De-icing Position



Assembled 6-Filter - Operating Position

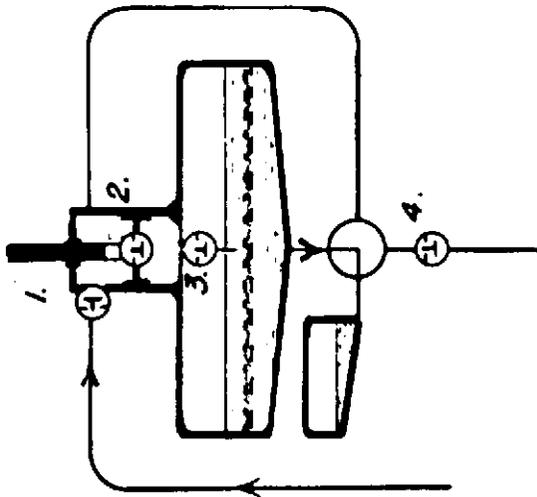


3-Filter - Opened for Recharging



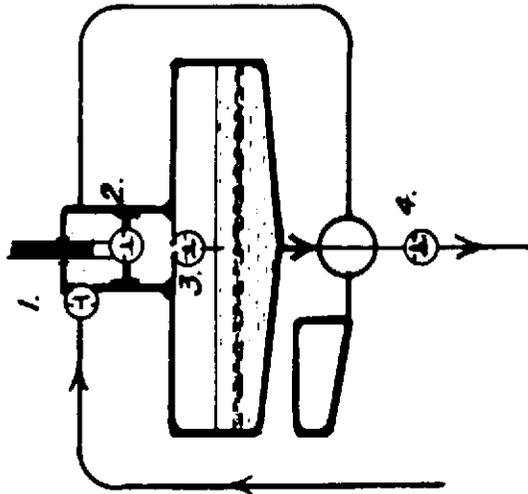
Dispersed ... of the ...

FIGURE 7



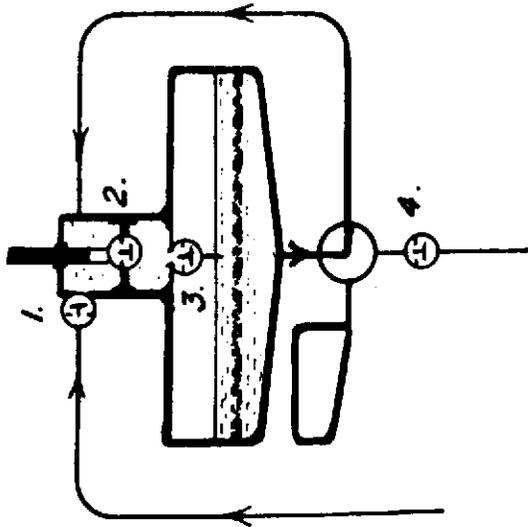
POS.3.

BLOWOFF.



POS.2.

FILTER.



POS.1.

PRECOAT.

The filter screen, also shown in Figure 6, is a plastic bonded cellulose cylinder. The actual filtration is accomplished by the cake of filter powder, which is formed on both sides of this cylindrical screen. Water passes through the cake into the screen, and comes out through the holes bored down the center of the screen. The total filtering surface is about 450 cm<sup>2</sup>. The Asboloxane S-filter powder charge used in each cycle weighs from 35 to 40 grams. This forms a cake approximately 2 mm. thick about the filter screen.

The circulation of water through the S-filter is controlled by an operating valve which can best be seen in Figure 4. The three numbered positions for this valve adjust the S-filter for the pre-coat cycle, filter cycle, and blow-off position respectively. The flow of water through the S-filter in these three positions is shown diagrammatically in Figure 7.

Here, the pump, the filter cake, and the operating valve are shown, for the sake of simplicity, one above the other, rather than in the positions which they actually occupy in the apparatus. The check valves (1) and (2) are so arranged that the pump operates on the down stroke, syphoning water through the inlet line and forcing it through the filter cake in the same operation. Check valve (3) causes positive pressure to be maintained over the filter cake even during the upstroke of the pump, resulting in a more even flow rate through the filter and preventing a sluffing off of the cake due to a drop in pressure. After passing through the filter cake, the water goes through the operating valve which can be set in any one of three positions.

Position 1. (Pre-Coat Position). When the valve is in this position, water is re-circulated through the pump and filter cake. This accomplishes two purposes. The Asboloxane S-filter powder is picked up and deposited as an even cake over the filter screen. Secondly, all inner parts of the S-filter are sterilized by the continued flow through them of freshly filtered and treated water.

Position 2. (Filter Position). In the filter or operating position, water is drawn into the S-filter through the inlet hose and passes directly through the filter and into the outlet hose. A check valve (4) on the outlet line is set so as to operate at a higher pressure than the other check valves. In this way, the positive pressure on the outlet line is always higher than the pressure of contaminated water at the operating valve. Thus, any leaks or cross connections, which might take place at this valve, consist of sterilized water flowing into the untreated water and not the other way around. In addition to this safety factor, the check valve (4) also prevents any back flow and consequent loosening of the filter cake, which might occur when the outlet hose was lifted above the level of the pump.

Position 3. (Blow-Off Position). When the operating valve is set at position 3, the water, after passing the filter, is forced into a separate small tank compressing the air already present in that tank. When the front clamp on the S-filter (not shown in the diagram) is released, the compressed air forces the water backwards thus removing the Asboloxane cake from the filter screen. The removed cake can then be pushed out with the water, when the S-filter is opened to change the Asboloxane charge. The water, which is forced back through the screen, is water which has already been filtered and purified.

## 2. Instructions for the Use of the S-filter, Two Liter Model

The following instructions are those given by the developer for the use of the Two Liter Model of the S-filter:

"Prior to use, the top assembly (lid) of the filter should be removed and the inside inspected for cleanliness; that is, absence of sand or major particles.

"The inlet and outlet hoses should be connected with their respective unions and care should be taken that both units are tightened in order to avoid air leaks.

[REDACTED]

"A sack of filter powder is opened and all of its contents put into the tank, subsequently the lid is put on the tank and the two rear clamps are closed while the front clamp (red) is left open. The foot pedals are then put on the ground. The valve handle is put into position '2,' the suction hose is inserted into the water supply, and the pumping is started in regular strokes (about 30-40 per minute).

"After about 30 strokes (depending upon the priming condition for the pump), blackish water squirts out under the lid of the pump which is a sign that the pump is now filled and dispersion of the powder has begun. The front clamp (red) is now tightened and a few more strokes may be given to the pump until water squirts out of the outlet hose.

"The valve is now turned into position '1' and pumping is continued at a fairly vigorous rate for 30 to 40 seconds in order to complete the pre-coat cycle. While continuing to pump, the valve handle is put quickly into position '2' and filtration begins.

"The pumping action should be continued for the next 25 to 35 minutes; that is, until the pump has delivered approximately 50 liters of water, yielded at the rate of about 2 liters a minute.

"Care should be taken that the pumping is not interrupted during this procedure for any appreciable length of time.

"In order to remove the cake from the filter screen at the completion of the filtration cycle the valve is put in position '3' and about 3 to 5 strokes exerted with the pump, at the completion of which the red clamp is quickly opened. By so doing the air in the filter tank will expand and a backwash action through the screen will result which deposits the filter cake in the tank. Should inspection show that the cake has not been completely blown off, the lid should be put on again, the clamp tightened and the operation (compression and expansion) with the valve in position '3' is repeated.

██████████

"The top is then entirely removed and the disperser unscrewed. If no fresh water is available, the latter as well as the filter screen can be flushed in the water contained in the tank.

"This tank is subsequently emptied and the disperser is put back in position. The filter is then ready to receive another supply of filter powder for the next filtration cycle.

"If the water to be filtered contains a considerable quantity of suspended matter (clay, etc.), the filter cake will become clogged prior to the completion of the filtration cycle of 50 liters. This is indicated by the fact that the pumping action becomes somewhat difficult and water spurts out from below the lid. In this case it will be necessary to blow the cake off (see above) and start a new filter cycle with a new supply of filter powder.

"Under no conditions should the filter screen be used as filter without the insertion of filter powder, or with a quantity which is less than that packed in a single bag.

"If the operation of the pump becomes difficult after about 12 to 15 cycles, representing 600 to 750 liters of water, the filter screen has to be cleaned with an acid carrier provided in a separately marked pack.

"The operation in this case is the same except that only the pre-coat cycle position '1' is used for about 4 to 5 minutes, after which the powder is blown off and the contents of the tank are poured out. It is desirable that the tank and filter screen be rinsed after this operation."

### 3. Asboloxane S-filter Powder

Asboloxane S-filter powder was developed for use in the special S-filter, just described, where water is filtered and sterilized simultaneously. This powder consists of diatomaceous earth, together with an Asboloxane catalyst and an inorganic peroxide. Mechanical filtration is achieved by forming a cake of this

[REDACTED]

powder over a rigid filter bed just as is done with ordinary untreated diatomaceous earth. Such untreated diatomaceous earth filters, however, if they are of such pore size as to produce adequate filtration rates, are by no means bacterial filters, even though they will retain a very large number of organisms from the suspensions passing through them. Sterility of the filtrate is achieved by the Asboloxane incorporated with the diatomaceous earth, which kills these organisms passing through the cake. Quoted below is the description given by the developer, of the way in which the mechanized filtering properties of diatomaceous earth and the sterilizing properties of the Asboloxanes are combined in S-filter powder.

"The diatoms are coated with an extremely thin layer of a thermo-plastic material (for instance, a polymer or co-polymer of vinyl-esters). It is characteristic of this plastic coat that it must be susceptible to water and neutral to fairly high alkali concentration. This plastic serves at the same time as vehicle for two colloidal or semi-colloidal suspended chemical agents. One of these agents is almost water insoluble peroxide such as, for instance,  $\text{CaO}_2$ ,  $\text{MgO}_2$  and the like. The other agent is a catalyst of the above mentioned type such as a surface-compound of silver and oxygen adsorbed on colloidal carbon.

"It is not necessary that the two agents (peroxide and catalyst) co-exist on the same particle although it is perfectly possible to incorporate both agents into the same vehicle prior to coating the diatoms. It is also possible to incorporate each agent separately into two batches of vehicle and coat half of the diatoms with one and the other half of the diatoms with the other. After drying, the two lots of diatomaceous earth have then to be mixed with each other intimately. It is assumed that the separation of the two agents may produce a filtration material which is less easily decomposed while in storage, in contradistinction to the other form in which both agents are in more intimate contact, being suspended in the same coating.

████████████████████

"This filter powder is then brought into water in order to form the filter cake in exact analogy to the customary procedure for the use of diatomaceous earth. The filter cake, built from filter powders so coated consists thus of particles which have the structural shape of diatoms. It exposes, however, to the passing filtrate a surface which can adsorb, by virtue of the water susceptibility of the plastic, a sufficient amount of water in order to start the catalytic decomposition of the peroxide due to the close juxtaposition of the two agents in the coating. The silver-bearing catalyst can be designed to yield a certain (minute) quantity of silver-ion if this is desirable in order to accelerate the killing action and for producing a certain retention of germicidal agent in the water.

"As the action of the filter powder is due to the decomposition of the peroxide and the catalyst, it is obvious that the activity of such a cake is limited. The weight ratio between the water to be treated and the quantity of powder necessary for its treatment, even in heavily infected states, is favorable indeed. For instance, 5-7 gallons of water can be filtered through a cake, the dry weight of which is 1/2 oz. or less.

"Powders of this type can be stored, are relatively temperature insensitive (below 150°F.) but have to be kept dry.

"In addition to the sterilizing action, filter powders of this type have, of course, the same retention qualities as untreated diatomaceous earth so that the filtration accomplishes mechanical retention of solids as well as the germicidal action. The layer of retained solids does not impair the action of the cake because of the fact that the retained solids remain on the top of the cake and do not penetrate into the latter."

██████████

4. Performance Tests on the S-filter and S-filter Powder Conducted at California Institute of Technology

The following is quoted directly from a report of Dr. Goetz of the California Institute of Technology:

"The development of the S-filter powder and the application of the latter to the S-filter was largely guided by bacteriological laboratory tests on artificially infected waters. These tests were conducted at the bacteriological laboratory of the Rare Metals Institute at the California Institute of Technology.

"The tests were conducted with various types of waters artificially inoculated with *E. coli* and mixtures of *E. coli* with staphylococci and streptococci. Inhibitory agents such as organic and inorganic nitrogen in the form of peptone and ammonia were added in certain tests in order to establish the limitations of the action of the preparation. The technique of these tests was as follows:

"After preparing 20 liters of test water with chemical and bacterial additions, filtration was started, and, in general, a sample taken from the first, seventh and fifteenth liter filtered. These samples were divided in two equal portions, one of which was plated immediately (i.e., within one minute) while the other portion was allowed to stand for 15 minutes before it was brought into contact with the nutrients. Each of these samples (i.e., 6 altogether) was divided into five 10 cc, one 1 cc and one 0.1 cc samples, inserted into lactose broth and subsequently incubated for 48 hours at 37°C. In case gas was observed, confirmation for *E. coli* was carried out in Brilliant Green Bile broth.

"The laboratory tests gave always sanitary water (i.e., no confirming gas in any tube) for coli concentrations up to approximately 200,000/cc (or 200,000,000/liter). This is similarly true for suspensions of streptococci and staphylococci. At higher concentrations of *E. coli*, occasional gas tubes

[REDACTED]

in the 10 cc samples occurred, but it is assumed that such concentrations do not occur in nature.

"As aqueous substrates, well water with sewage (1:2000), lake water rich in protozoa and algae, well water with the addition of up to 500 parts per million peptone as representative of organic nitrogen and up to 250 parts per million ammonia as representative of inorganic nitrogen, were used. Furthermore, as a representative of heavy organic contamination, up to 25,000 parts per million urine was used. To each of these substrates, artificial inocula of E. coli up to 100,000/cc were treated successfully and resulted in an effluent fulfilling the bacteriological sanitary standards, either immediately or at least after 15-20 minutes elapsed time.

"In view of the fact that infected waters tested under laboratory conditions may not represent the performance of method and equipment in the field, a number of field tests were performed on varying types of infected waters in the States of Sinaloa, Guerrero, Morelos and Distrito Federal in collaboration with the Dep. de la Salubridad Publica. In order to test the water after a short lapse of time, ambulant laboratory equipment was taken along to the site of the tests, in most cases. The results reported refer to an incubation time of 40-48 hours. Thus the condition in the water when filtered and consumed under actual conditions would be studied as closely as possible. These tests are enumerated as follows:

a. "Location: Rio Humaya, 5 km north of Culiacan, Sinaloa. Temperature: 22°C. pH: 7.5 - 8.0. Control: gas in 1 cc sample, confirmation questionable, growth till 0.00001 cc. Filtrate: sample after third liter, plated after 12 minutes: no gas in any sample, growth in three 10 cc tubes only.

b. "Location: Rio Culiacan, 3 km west of Culiacan at the point of confluence with the sewage canal (Canal Almada). Temperature: 25°C. pH: 7.5 - 8.0. Control: confirming gas in 1 cc sample, growth till 0.0001 cc. Filtrate:

sample after sixth and fifteenth liter, tested after 15 and 20 minutes respectively: no gas in any sample, growth in four 10 cc samples only.

c. "Location: Rio Tamazula near public laundries of Culiacan, Sinaloa. Temperature: 24.5°C. pH: 8.0 - 8.5. Control: confirming gas in 0.01 cc, no growth in 0.001 cc and lesser dilutions. Filtrate: sample after fifth liter, tested after 20 minutes: no gas in any sample, no growth in any sample.

d. "Location: faucet in Public Health Laboratory in Culiacan. Temperature: 23°C. pH: 7.5. Control: confirming gas in two of five 10 cc tubes. Filtrate: sample after fifth liter, tested immediately: no gas in any sample, growth in two 10 cc samples.

e. "Location: sewer canal (Canal Alameda) of Culiacan. Temperature: 28°C. pH: 8 - 8.5. (Water very turbid and odorous). No control taken. Filtrate: sample after fifth liter, tested after 15 minutes: confirming gas in one of five 10 cc samples, no growth in 1 and 0.1 cc.

f. "Location: stagnant water body in an arroyo near Ranchita "La Divisa," approximately 8 kilometers east of Culiacan. (Full of mosquito larvae, algae and with a very muddy bottom). Temperature: 24°C. pH: 8.0. Control: no coli but growth till 0.001 cc. Filtrate: sample after sixth liter, tested after 20 minutes. no coli and no growth in any sample.

g. "Location: Rio Tepecua near Xatitla, Guerrero. Temperature: 29°C. pH: 7.5 - 8.0. Control: confirming gas in 1 cc, growth till 0.0001 cc. Filtrate: sample after third liter, tested after 15 minutes: no gas in any sample, growth in one 10 cc sample only.

h. "Location: stagnant pool at the entrance of Tixtla, Guerrero. The water was extremely turbid from algae growth, so that the filter clogged after three liters. Temperature: 27°C. pH: 8.5 (1). Control: confirming gas till 0.1 cc, growth till 0.001 cc. Filtrate: sample after two liters, tested after 20 minutes: confirming gas in one of five 10 cc tubes, no growth in 1 and 0.1 cc.

i. "Location: Rio Sabana, 8 km east of Acapulco, Guerrero. Stagnant pool in river bed, clear water. Temperature: 36.5°C. (1). pH: 8 - 8.5. Control: confirming gas in 0.1 cc, growth till 0.001 cc. Filtrate: sample after seventh liter, tested after 15 minutes: no gas in any sample, no growth in 1 cc.

j. "Location: Rio Sabana, same location as (i.) but flowing river water. Temperature: 35°C. pH: 8.0. Control: gas in 0.01 cc., growth till 0.001 cc. Filtrate: sample after tenth liter, tested after 15 minutes: no E. coli. Two non-confirming 10 cc samples out of five, no growth in 1 and 0.1 cc.

k. "Location: Canal de Cuautla, Morelos, at Kilometer 1. Clear water. Temperature: 20.5°C. pH: 7 - 7.5. Control: confirming gas in 0.1 cc., growth till 0.001 cc. Filtrate: sample after fifth liter, tested after two hours and 15 minutes: no gas in any sample, no growth in 1 and 0.1 cc.

l. "Location: Arroyo near Yautepec, Morelos, at Km 30. Fast flowing, very muddy water. Temperature: 25°C. pH: 7.5 - 8. Control: confirming gas in 0.01 cc., growth till 0.0001 cc. Filtrate: sample after third liter, tested after 1 hour and 10 minutes: no gas in any sample, no growth in any sample.

m. "Location: Xochimilco, D.F. Stagnant canal under street bridge. Temperature: 22°C. pH: 8.0. Control: confirming gas in 0.001 cc. Filtrate: sample after third liter, tested after 2 hours and 40 minutes: no gas in any sample, growth in one 10 cc tube only.

n. "Location: Xochimilco, D.F. Same location as (m.) but two packages of filter powder used, in view of extremely turbid water. Filtrate: sample after third liter, tested after 2 hours and 35 minutes: no gas or growth in any sample.

o. "Location: Xochimilco, D.F. Embarcadero, San Cristobal. Temperature: 20.5°C. pH: 7.0. Control: confirming gas in 0.001 cc. Filtrate: sample after second liter, tested after 2 hours and 20 minutes: no gas in any sample, growth in four 10 cc. samples only.

[REDACTED]

canteen trials, a larger series of tests was run with the Asboloxane powder in laboratory equipment, where its performance could be studied separately from the mechanical functioning of the S-filter pump, and where various factors influencing its action could be better studied. The Camp Detrick trials largely centered about investigating the performance of the S-powder when extremely heavy suspensions of various types of organisms were used. The data from the California Institute of Technology, already quoted, had shown what the S-filter and the Asboloxane S-powder could do towards handling the amounts of contamination ordinarily found in naturally occurring waters. The tests at this installation were designed more to indicate what the upper limits of performance were, and how broadly effective the apparatus was against all types of agents. For this reason, tests were not confined to the common water-borne organisms usually found in natural waters, nor were they usually run with concentrations as low as those which might be expected to occur naturally.

a. Trials Utilizing both the S-filter and Asboloxane S-powder

The first preliminary trials with the S-filter were run with 20-liter suspensions of *B. globigii* spores and of *E. coli* cells. Samples were taken for bacterial counts after approximately 2, 10 and 18 liters respectively of the 20-liter samples had passed through the filter.

The S-filter was operated according to the instructions already quoted, given by the designer. In each case 4 grams of the Asboloxane S-filter powder were used in the pre-coat cycle so that a filter cake approximately 2 mm. thick is formed. Through this cake the water was pumped, filtered, and sterilized at the rate of about 2 liters a minute.

The *B. globigii* spore suspensions were reduced in count from an original concentration of 200,000,000 organisms/ml. to 16, 26, and 12 organisms/ml. respectively in the three samples of the filtrate. *E. coli* was reduced in count from 35,000,000 to 13, 13 and 1 organism/ml.

These filtrates were pooled and then run back through the S-filter using a fresh charge of Asboloxane S-powder. The counts were about the same as in the first passage.

A set of experiments was then run utilizing in each case 50 liters of very heavy suspensions of typical bacteria. Samples of the filtrate were collected after 5, 15, 30, and 50 liters respectively had passed through the filter. In several of the experiments no collections were made after 30 liters. The data collected are given in Table IV. TNTC indicates that the colonies were too numerous to count at the dilutions used. In such cases it can be assumed that the filtrate contained over 500 organisms/ml.

It is evident that the S-filter can effectively remove very high concentrations of various types of organisms, and do this at the relatively rapid rate of 2 liters a minute. It is also evident that it is possible to exceed its sterilizing capacity especially when counts rise to the order of magnitude of  $10^6$  organisms/ml. This is apparent usually in increased counts toward the end of the 50-liter filtrations, or in at least one of these tests, in high counts throughout the entire course of the filtration.

Some evidence was obtained which indicated that, with Staph. aureus at least, bacteriostasis was occurring in certain cases. Additional colonies appeared on a few plates, which had not been destroyed after the usual reading at the end of 48 hours incubation. Five trials were set up, all with Staph. aureus as the test agent, in which counts were made of viable organisms in the filtrate after the usual 48 hours and again after the same plates or broth tubes had been incubated for 96 hours. Many plates recorded as sterile at 48 hours showed some growth at 96 hours, as indicated in the data in Table V from two of these trials.

TABLE IV

EXPERIMENTS WITH  
THE S-FILTER AND ASBOLOXANE S-FILTER POWDER

<u>Test Organism</u>	<u>Original Contamination</u> Count per ml.	<u>Bacterial Count per cc. in Successive</u> <u>Filtrate Samples</u>			
		<u>5 L</u>	<u>15 L</u>	<u>30 L</u>	<u>50 L</u>
S. marcescens	670,000	1	0	2	0
S. albus	750,000	1	0	0	0
B. globigii (Veg.)	1,760,000	7	4	TNTC	
B. globigii (Veg.)	1,000,000	8	2	2	62
B. globigii (Spore)	970,000	1	7	11	
B. globigii (Spore)	16,000,000	263	294	TNTC	TNTC
E. coli	15,000,000	10	13	TNTC	TNTC
S. aureus	145,000,000	TNTC	TNTC	TNTC	TNTC

TABLE V

## DIFFERENTIAL 48 AND 96 HOUR COUNTS ON FILTRATES

OBTAINED USING

ASBOLOXANE S—FILTER POWDER IN THE S-FILTER

(40 grams Asboloxane used in all tests)

Original Contamination	Volume Filtered Before Sample Taken	Ratio Number Organisms to Mg. Asbo.	Plate Count Organisms/ml.		Broth Count MPN/ml.	
			48 hr	96 hr	48 hr	96 hr
Staph. aureus 385,000/ml	5 liters	48 T	0	31	0	5
	15	144 T	0	730	0.2	8
	20	193 T	0	2950	0.2	8
	30	288 T	0	1040	0.4	18
Staph. aureus 136,000/ml.	5	17 T	0	4	0	5
	15	51 T	0	38	0	2
	20	68 T	0	15	0	160
	30	102 T	0	13	0.2	2

Since *Staph. aureus* is sensitive to high pH values, and the Asboloxane filtrates are alkaline, it was thought possible that this bacteriostasis or lag was a pH effect, and not due to the Asboloxane. To evaluate this factor, two sterile broth solutions were made alkaline, one by passing the solution through an Asboloxane cake; the other by adding NaOH until its pH value matched that of the Asboloxane filtrate. A third lot of broth kept at pH 6.8 served as control. These broths were inoculated with *Staph. aureus*, giving the theoretical counts indicated below, and after the indicated times, 1 ml. portions were removed and plated with nutrient agar in petri dishes. These plates were read both after 24 hours and after 56 hours of incubation at 37°C. The data appear in Table VI.

The data show that contact with alkali can definitely so affect *Staph. aureus* organisms that colonies develop much more slowly than usual from those organisms which survive the treatment. The time lag in the development of *Staph. aureus* colonies could thus be accounted for by the alkalinity of the Asboloxane filtrate. Of greater interest, however, is the evidence that the Asboloxane filtrates possess a definite anti-bacterial activity. There is a much greater killing action in the Asboloxane filtrate than can be accounted for by alkali alone. Not only are viable organisms killed in passage through the Asboloxane S-filter cake, but also a lethal property is imparted to the filtrate which, as shown in these tests, is effective against organisms added later to the filtrate. This residual anti-bacterial property of the Asboloxane filtrates has been called its "antibiotic potential," and will be further demonstrated in experiments done with laboratory equipment, rather than utilizing the S-filter pump.

TABLE VI

EFFECT OF pH IN ASBOLOXANE FILTRATE  
ON LAG PHASE OF STAPH. AUREUS

<u>Treatment Given Broth</u>	<u>Inoculum of Staph. aureus/ml.</u>	<u>Contact time</u>	<u>Plate Counts (Organisms/ml)</u>	
			<u>24 hr</u>	<u>56 hr</u>
Filtered thru Asbolexane pH 10.5	31,500	5 min.	0	12
		30	0	7
		60	0	0
Adj. with NaOH pH 10.5	31,500	5	0	TNTC
		30	0	652
		60	0	4
No treatment pH 6.8	31,500	5	TNTC	—
		30	TNTC	—
		60	TNTC	—

[REDACTED]

b. Trials with Asboloxane S-filter Powder on a Sintered Glass Filter Bed

While the trials with the Asboloxane S-filter powder were being conducted in the S-filter designed for military use, laboratory scale trials were inaugurated. Working on a small scale rather than with the 50 liters of bacterial suspension and 40 grams of Asboloxane S-filter powder required for each trial in the S-filter, it was possible to step up the tempo of the work. Besides, closer control was possible with the laboratory trials, when it was desired to vary certain factors in the filtration process and to use high concentrations of various pure culture suspensions, which necessitated sterilizing the total equipment between runs. The first of these laboratory trials was run with very simple glass equipment using vacuum suction flasks and Buechner type funnels of coarse sintered glass, which served as the filter bed against which cakes of Asboloxane S-filter powder were formed. The sintered glass alone retained very few organisms, as evidenced by a drop from  $1.30 \times 10^8$  organisms per ml. to  $1.03 \times 10^8$  organisms per ml., when a *B. globigii* spore suspension was passed through it.

The recommended charge of Asboloxane powder in the S-filter was one gram per  $15 \text{ cm}^2$  of filtering surface. A corresponding charge for the Buechner funnel which had a sintered glass bed approximately  $30 \text{ cm}^2$  in area was two grams. The filtering cake so obtained was about 2 mm. thick. Utilizing the service vacuum lines, however, it was not possible to filter bacterial suspensions through these cakes at the rate of 150 ml./minute which corresponded to the rate achieved in the S-filter pump. The 300 ml. suspensions used in most cases required from 3 to 4 minutes to filter.

The cake was formed by mixing the dry Asboloxane S-powder with a small amount of sterile water and drawing by vacuum practically all of this

water into a sterile suction flask. The bacterial suspension was then added and the vacuum turned on fully. After all the suspension had passed through the filter, the filtrate was usually allowed to stand for at least 15 minutes in the vacuum flask, then removed and plated out with the usual dilution technique into nutrient agar.

Representative data obtained from the use of the Asboloxane S-filter powder in this glass laboratory equipment are given in Tables VII through XII, presented in chronological order, as the tests were run in the laboratory. Variations in the techniques described above are either apparent from the headings, or explained in foot notes.

The data obtained with the Asboloxane S-filter powder on the sintered glass filter beds showed, as had the trials in the S-filter itself, that the treatment was effective against widely different types of bacteria present in very large numbers. Suspensions up to the neighborhood of  $10^6$  organisms per ml., varying of course with type of organism and other factors, could be effectively sterilized by a single passage through a cake of the thickness recommended for use in the military S-filter. As might be expected, repassage through an Asboloxane cake of this thickness or single passage through cakes of greater thickness caused increased reduction in suspensions so high in count that normally appreciable numbers of viable organisms would be found in the filtrate. Parallel filtrations through Asboloxane S-filter powder and diatomaceous earth showed that something more than mechanical retention of organisms was operating here, although large percentages of organisms from heavy suspensions are trapped on the filter cake.

When viable organisms do appear in the filtrate, their count drops as the filtrate stands. This is another indication of the "antibiotic potential"

TABLE VII

ASBOLOXANE S-FILTER POWDER ON SINTERED GLASS

FILTER BED

(2-gram Cake)

Repassage of B. globigii Suspension Through

The Same Filter Cake

Treatment	B. globigii spores/ml.
Original Suspension	160,000,000
1st Passage thru cake	3,000,000
2nd Passage thru cake	76

NOTE: The B. globigii suspension made by resuspending dried spores contained many lumps or clusters of spores and non-viable organic material.

TABLE VIII

ASBOLOXANE S-FILTER POWDER ON SINTERED GLASS

FILTER BED

Single Passage of B. globigii Suspension Through

Cakes of Varying Thickness

Treatment	B. globigii spores/ml.
Original Suspension	10,000,000
Passed thru 2 gram cake	86,000
Duplicate	37,000
Passed thru 4 gram cake	0
Duplicate	3
Passed thru 6 gram cake	5

NOTE: B. globigii suspension made by resuspending dried spores.

TABLE IX

## ASBOLOXANE S-FILTER POWDER ON SINTERED GLASS

## FILTER BED

Comparative Trials with Asboloxane S-Filter Powder  
and Diatomaceous Earth

Treatment	Bacterial Counts/ml.	
	Plated Immediately	Plated after 24 hours
Original Suspension	10,000,000	
Thru 2 grams Asboloxane	45,000	8,500
Thru 4 grams Asboloxane	2	3
Thru 6 grams Asboloxane	2	3
	(All above colonies B. globigii)	
Thru 2 grams Diatomaceous Earth	41,000 (21,000 B.g.)	1,300,000
Thru 4 grams Diatomaceous Earth	6,700 (29 B.g.)	TNTC in dilutions plated
Thru 6 grams Diatomaceous Earth	120 (5 B.g.)	Over 5,000

NOTE: The same crude resuspension of dried B. globigii spores was used. Only B.g. colonies appeared in the filtrate which had passed through the Asboloxane. Many small white colonies were found in the filtrate which had passed through diatomaceous earth. These were counted differentially. Apparently, a contaminant was present in the B. globigii suspension which was killed by the Asboloxane but which passed through the diatomaceous earth more easily than did the B. globigii spores, and showed up when the large excess of B. globigii spores were removed from the suspension. Since the filtrates had not been discarded, duplicate plates were made from them the next day. Then interestingly enough, the Asboloxane filtrates still contained only B. globigii spores in lesser number than when first filtered, while the filtrates from the untreated diatomaceous earth showed greatly increased counts of the contaminating organism.

TABLE X

## ASBOLOXANE S-FILTER POWDER ON SINTERED GLASS

## FILTER BED

(4-gram Cake Used)

Action Against Various Organisms and Drop of Count  
in Filtrate Upon Standing

<u>Test Organism</u>	<u>Original Contamination</u> Count per ml.	<u>Time Lapse</u> After Fil- tration Be- fore Plating	<u>Bacterial Count per ml.</u>
Staph. albus	560,000	0 - 5 min.	26
		15 min.	8
		30 min.	2
Staph. aureus	1,100,000	0 - 5 min.	TNTC
		15 min.	TNTC
		30 min.	386
Staph. aureus	930,000	0 - 5 min.	238
		15 min.	155
		30 min.	70
Staph. aureus	620,000	0 - 5 min.	207
		15 min.	137
		30 min.	11
Staph. aureus	338,000	0 - 5 min.	0
		15 min.	0
		30 min.	0
E. coli	288,000	0 - 5 min.	1
		15 min.	0
		30 min.	0
E. coli	272,000	0 - 5 min.	0
		15 min.	0
		30 min.	0
B. globigii (Veg.)	960,000	0 - 5 min.	4
		15 min.	3
		30 min.	5
B. globigii (Veg.)	623,000	0 - 5 min.	0
		15 min.	0
		30 min.	0

TABLE XI

## ASBOLOXANE S-FILTER POWDER ON SINTERED GLASS

## FILTER BED

Action Against E. coli Bacteriophage

Test Organism	Original Contamination		Filtrate Count per ml.	
	Count per ml,		4 Gram Cake	6 Gram Cake
T <sub>3</sub> Phage	18,000,000		below 100	below 100
T <sub>3</sub> Phage	6,800,000		300	below 100

NOTE: Phage counts are made by plating out dilutions in agar heavily seeded with E. coli and counting the clear plaques where the phage is destroying the host cell. When the filtrate through the Asbолоxane was added to the seeded agar directly and in a 1:10 dilution, the E. coli growth was so inhibited that it was not possible to determine whether or not phage was present. At dilutions of 1:100 or greater the E. coli cells developed, and in one of these plates, three clear plaques were found indicating a count of 300 ml. for that filtrate. The other filtrates could have had any count, ranging from 0 to 100 phage particles per ml.

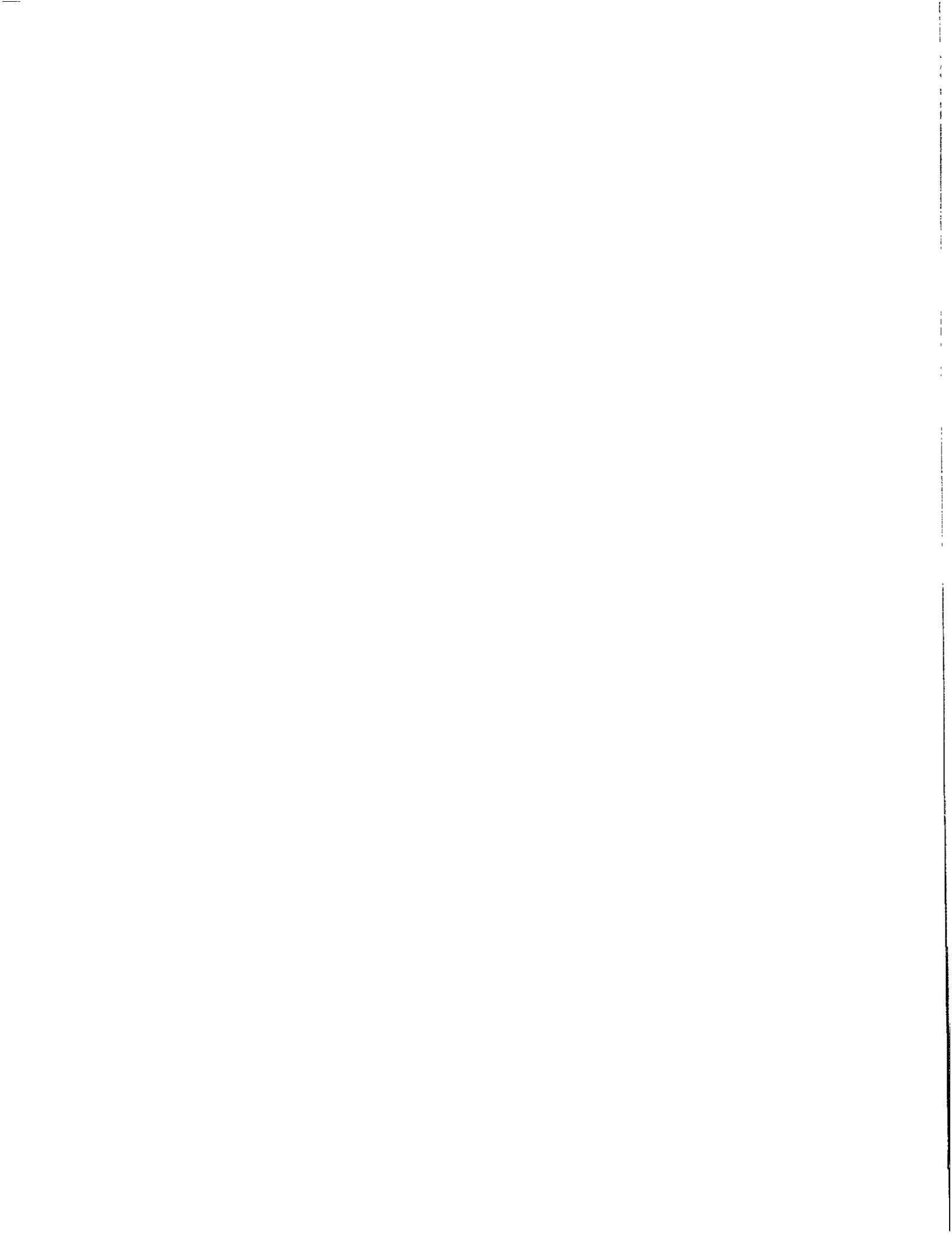


TABLE XII

ASBOLOXANE S-FILTER POWDER ON SINTERED GLASS

FILTER BED

(4-gram Cake Used)

Effect of Successive Filtrations and of Temperature

Upon Action Against E. coli

Original Suspension Count per ml.	Temp. °C.	Bacterial Count per ml. for Successive One Liter Lots of the Filtrate				
		1st	2nd	3rd	4th	5th
67,200	3°	-	100	150	100	-
	22°	0	0	0	10	135
45,500	2°	0	0	0	10	TNTC
	37°	0	0	0	10	20
48,000	2°	20	0	0	50	50
	37°	0	0	0	0	0
42,120	2°	0	0	0	100	0
126,000	3°	0	0	250	1950	1400
	22°	20	200	200	200	400
	37°	0	0	650	250	200
86,800	3°	0	1	5	7	56*
	37°	0	4	6	13	13*

\* Counts made using 5 tube broth series. Other counts are direct plate counts.

[REDACTED]

imparted to the solution upon passage through the Asboloxane S-powder. When large volumes of suspension were passed through a single cake, examination of successive lots of the filtrate showed larger numbers of organisms appearing towards the end of the filtration, indicating the chemical action of the powder became spent as more and more liquid passed through it. One point of distinct practical importance was brought out in the trials conducted in the cold (2-3°C) and warm (37°C) rooms. The temperature coefficient seems to be quite small, as the performance at the lower temperature compares very favorably with the sterilization obtained at 22 and at 37 degrees.

There appeared, however, definite limitations in the use of the simple Buechner sintered glass funnels and vacuum flasks for use in laboratory trials. Particularly, it became evident that it would be advisable to have an apparatus where successive samples could be taken of the filtrate for bacteriological and chemical examination without interrupting the course of the filtration, and to have the filter cake more protected, since it often became disturbed as more suspension was added to the sintered glass funnels, or as the funnels were removed to other sterile suction flasks, when one became filled. Accordingly, the laboratory apparatus described in the next section was designed, and subsequent laboratory evaluations of the Asboloxane S-filter powder were conducted in it.

c. Laboratory Pressure Filter Apparatus

A pressure-filtration system was set up to simulate the action of the S-filter on a laboratory scale. A closed reservoir was used to contain the water to be filtered. Compressed air forced the suspension out through an opening at the bottom of this reservoir and through a Seitz filter unit, from which the valve and diaphragm were removed. The effluent from the Seitz filter led

[REDACTED]

to a calibrated glass chamber in which measured portions of the filtrate could be held and removed for chemical and bacteriological determinations.

One gram of the Asboloxane S-filter powder was weighed out, and filter cakes were made by filtering a water suspension on a Euechner funnel. The cakes and filter papers were placed upon the metal screen of the Seitz filter, which was then clamped tight. The water used in these pressure filtrations was autoclaved distilled water, unless otherwise noted, to which might be added any type of bacterial or chemical agent, depending upon the purpose of the test. The time which it took for each successive 200 ml. portion of water to pass through the filter cake was noted. When the run was over, the thickness of the filter cake was determined by measurements with a Randall and Stickney thickness gauge.

Chemical tests performed on the filtrate included Winkler's determination of dissolved oxygen, and later, the cupric dithizonate method for silver. A discussion of these factors will be presented later.

Bacteriological assays were done on every 200 ml. portion of filtrate. Triplicate plates were poured of "direct" and "1:100" samples of filtrate, using Nutrient agar or Friedlein's synthetic agar or both (1). Plates were incubated at 37°C for 48 hours.

In some cases the suspensions being filtered were made by adding the broth in which they were grown directly to the water. In most cases, however, where comparative data were desired, bacterial suspensions were centrifuged, the precipitated organisms were taken up in distilled water, centrifuged again, and re-suspended before being added to the test water.

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(1) Plating was begun within one minute of collection of the sample to avoid the self-sterilizing action or antibiotic potential of the filtrates themselves.

[REDACTED]

In all tests, counts were made of the bacteria in the test water both before and after a run, to determine original concentration and also the normal die-off, due to standing in the reservoir during the time portions were being treated.

Typical Data: In Table XIII is a standard laboratory sheet, such as is obtained from a single filtration experiment of this type. From this table we can see the method of deriving the time of contact of any particle with the actual filter cake. The time for 200 ml. to pass through the filter cake is recorded, and transformed to rate of flow in ml/sec. This rate is divided by the area of the filter cake, to give the velocity with which a mass of water penetrates the cake. From this figure, we may obtain the time of contact of any particle with the cake ( $T_c$ ) by dividing it into the thickness of the cake. It will be noted that this time of contact increases as the filtration proceeds under any given pressure of air in the reservoir. This may be accounted for by mechanical compression of the cake during filtration, or by the stoppage of pores due to accumulation of microscopic debris. Correlation of this time of contact with chemical or bacteriological assay of the filtrates has not as yet been fruitful, but we have continued to gather these data against the time when the latter determinations become more standardized.

In this table we can also note the typical characteristics of Asboloxane filtration. The first filtrates are essentially sterile, and then a point arrives when small numbers of organisms begin to come through the filter. In no case does the number of organisms ever become exceedingly great, due, probably, to mechanical, rather than chemical effects from this point forward. The volume of filtrate which contained no more than one or two viable bacteria per ml. has been reported as "volume sterilized," and is used as the basis of comparison.





TABLE XIV

ASBLOXANE S-FILTER POWDER IN LABORATORY PRESSURE

FILTER APPARATUS

Comparison of Filter Effectiveness with Cake Thickness

Run No.	Grams S-Powder	Cake thick- ness - cm.	Average Tc - sec.	Volume of E. coli Filtered	Suspension Sterilized
28	0.25	.021	.25	3000	10
27	0.50	.027	.29	2600	50
29	1.0	.070	.64	3000	2000
31	1.0	.072	.69	3000	1600
20	2.0	.114	2.49	2600	2600
21	2.0	.173	3.73	2400	2400

TABLE XV

## ASBOLOXANE S-FILTER POWDER IN LABORATORY PRESSURE

## FILTER A PARATUS

Sterilizing Action Against Various Types of Organisms

Contaminant	Test No.	Orig. Conc. org./ml.	Vol.* Sterile Effluent
<i>E. coli</i>	37	400,000	2800
<i>S. aureus</i>	38	250,000	2400
<i>S. aureus</i>	70	2,800,000	2800
<i>B. globigii</i> (veg.)	39	50,000	2800
<i>B. globigii</i> (spore)	40	45,000	2800
<i>B. globigii</i> (spore)	47	200,000	2800
<i>B. globigii</i> (spore)	43	13,500,000	0
<i>M. phlei</i>	42	450,000	2800
<i>M. phlei</i>	44	1,250,000	2800
<i>K. pneumoniae</i>	45	570,000	2800
<i>E. typhosa</i>	68	118,000	1400
<i>E. typhosa</i>	78	450,000	2800
<i>E. typhosa</i>	79	3,000,000	2300
<i>S. marcescens</i>	73	650,000	2000
<i>S. marcescens</i>	71	2,500,000	1600
<i>G. tetragena</i>	72	86,000	2800
<i>E. coli</i> T <sub>2</sub> Phage	76	4,000,000	0

\* 2800 ml. was the total amount filtered in all cases.

[REDACTED]

In evaluating these data it is important to remember that we are dealing with a system which stands to the S-filter in the ratio 1:40. Therefore, each 200 ml. of filtrate collected in this test is equivalent to eight liters of filtrate from the S-filter. The rated safe capacity of the pump is 50 or 60 liters, so that 1250 or 1500 ml. of sterile effluent from the laboratory model may be considered a sterile run in the pump. On the other hand, these tests were carefully conducted in the absence of organic matter, so they represent conditions which are rather ideal.

Storage of Asboloxane Powder: Stoppered and open vials were filled with one gram samples of Asboloxane S-filter powder and exposed to different conditions of temperature by being stored as follows: in a dry ice chest ( $-60^{\circ}\text{C}$ ); in a cold room ( $5^{\circ}$ ); in a laboratory ( $25^{\circ}$ ); in an incubator ( $37^{\circ}\text{C}$ ); and in a water bath ( $45^{\circ}$ ). Water containing washed E. coli was filtered through one gram cakes of these powders after six months of storage at the various conditions. Results are reported in Table XVI.

Effect of Various Materials added to the Suspension: In an early attempt to rule out silver ion as the antibacterial agent, a solution of sodium chloride (1.25 per cent) was used as suspending medium for E. coli. Three liters of this solution were filtered through one gram of Asboloxane - all the filtrates were sterile. Later work, of course, indicated that the silver present is much below the solubility product of silver chloride, and so this experiment did not at all rule out the possibility that ionic silver was the active agent.

In another test, cysteine hydrochloride was added to distilled water in a concentration sufficient to give a titer of zero when the solution was assayed for dissolved oxygen by Winkler's alkaline manganous hydroxide method. It was of interest to see whether the strong reducing properties of such a solution

TABLE XVI

ASBOLOXANE S-FILTER POWDER IN LABORATORY PRESSURE  
 FILTER APPARATUS

Surveillance of Stored Powders After Six Months Storage

Vial	Temperature °C	Original concentration, E. coli/ml water	Volume* Sterile Effluent
Stoppered	-60	470,000	1700
Open		500,000	800
Stoppered	5	1,600,000	1800
Open		6,000	2800
Open		600,000	2800
Stoppered	25	1,000,000	2800
Open		500,000	2800
Stoppered	37	250,000	2000
Open		460,000	2300
Stoppered	45	560,000	2800
Open		550,000	2800

\* 2800 ml was the total volume filtered in all cases.

NOTE: It is possible that samples stored in the dry ice chest were affected by the large amount of CO<sub>2</sub> in the atmosphere. The effect of very low temperatures upon storage should certainly be studied further.

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[REDACTED]

interfered with the death of *E. coli* by filtration through Asboloxane. In this test a few contaminants appeared, but essentially all of three liters of *E. coli* suspension were sterilized by passage through one gram of Asboloxane. It is interesting that in the test the value of  $\Delta O_2$  for the first filtrate was almost that of a normal water filtration, but this value dropped exceedingly rapidly, until there was very little determinable oxygen after one liter had passed the filter. The reducing conditions of the water did not apparently affect the effectiveness of the sterilization.

A detrimental effect on sterilizing capacity is noted, however, when nutrient broth is added to the water. Normally Friedlein's synthetic medium was used to grow the *E. coli*, and 20 ml. of this culture medium was added to 3500 ml. of water. However, when nutrient broth was added, far less water was sterilized. To show this point, and to rule out the possibility that bacteria grown in different media might have different resistances, or that the products of their growth were interfering with the action of Asboloxane, the tests described in Table XVII were performed. Two grams of filter powder were used in each test.

It appears obvious that it is the nutrient broth, and not factors of resistance or of metabolic end-products, which gives rise to the observed differences in effectiveness of the Asboloxane filtration process.

The same antagonism of nutrient broth for some bactericidal factor contained in the filtrate is evident when dilutions are made in nutrient and in Friedlein's agar. The Friedlein's plates are likely to be all sterile, while nutrient agar plates may show growth, due to the reversal of bacteriostasis by the components of the nutrient broth. It is thus important that test methods be standardized as to media used, and as to time of incubation, if comparable results are to be obtained.

TABLE XVII

ASBOLOXANE S-FILTER POWDER IN LABORATORY PRESSURE

FILTER APPARATUS

Effect of Nutrient Broth on Asboloxane Filtrations

Run No.	E. coli grown in	Treatment	Additional Material added to water	Original conc. E. coli/ml	Volume of water Filtered ml.	Sterilized ml.
19	20 ml. N <sup>*</sup>	Added to 3500 ml. water	None	600,000	2400	800
21	20 ml. F <sup>**</sup>	"	None	215,000	2400	2400
23	20 ml. F	"	20 ml. N (sterile)	270,000	3200	800
24	20 ml. N	Centrifuged, twice washed, added to 3500 ml. water	None	285,000	2000	1800

\* N = Nutrient broth    F = Friedlein's synthetic medium

Antibiotic Potential: Mention has been made in previous sections that the Asboloxane filtrates appeared to be bacteriostatic. Dr. Goetz had reported this interesting phenomenon and called it "Antibiotic Potential." By passing various suspensions through Asboloxane S-filter powder cakes in the laboratory pressure filter apparatus, and re-inoculating portions of this filtrate with different amounts and types of bacteria, the existence of this antibiotic potential has definitely been established. *E. coli*, *E. typhosa*, *S. aureus*, *S. marcescens* and *G. tetragena* have all been observed to die off more rapidly in Asboloxane filtrates than they do in the same water before filtration. This killing action is not too rapid, and when relatively heavy inocula are added to the filtrate and aliquots examined for viable bacteria at periodic intervals up to 3 hours, the killing is often not yet complete. Comparison of the effectiveness of this antibiotic potential can be made however by comparing under different circumstances the velocity constant or death rate,  $k$ , which is often used for comparing various bactericidal effects. This constant,  $k$ , comes from the expression for a first-order reaction,

$$\log \frac{N_0}{N} = kt,$$

where  $N_0$  is the original bacterial concentration,  $N$  is the concentration after any contact time,  $t$ , and  $k$  is the velocity constant or death rate. The bacteria die off in a logarithmic order in the Asboloxane filtrates as in so many cases and thus obey the above expression. When the logarithm of the concentration ( $\log N$ ) is plotted against  $t$ , a straight line can be drawn through the points and the velocity constant  $k$  can be calculated by determining the slope of that line. It is customarily said that  $k$  is a numerical constant having no exact meaning, but that it becomes larger in value as the death rate becomes faster. Actually  $k$  has the dimensional units of time<sup>-1</sup>, and it is numerically the reciprocal of the time required to kill 90 per cent of the organisms in any given

suspension, since in this special case  $\text{Log } \frac{N_0}{N}$  becomes 1 and  $k = \frac{1}{T}$  in the equation given above.

A typical example of the fall in concentration of *E. coli* when added in two different amounts to an Asboloxane filtrate is given in Table XVIII. The bacteriostatic action is hard to compare in these two cases because of the difference in original concentration. The death rates however (.078 as against .096) tell us that the bacteria are dying off faster in the lower inocula where 90 per cent of the organisms die in just over 10 minutes on an average as against just under 13 minutes.

Using these death rate figures, it was possible to compare the effect of various factors such as organic or other material present in the water before it was filtered, and of the number of contaminating organisms added to the filtrate as is done in Table XIX.

Our data, to date, made by comparing these death rates, have indicated that the antibiotic potential is greatest in the first portions of suspensions passed through Asboloxane S-filter powder than in later portions, although it is present even towards the end of the filtration. Also as is evident in Table XIX referred to above, the death rate falls off as the number of organisms added to the filtrate is increased.

The literature is filled with similar observations, and silver, as a sterilizing agent, shows this relationship to a marked degree (1). This factor must be borne in mind in comparing the results of different investigations.

Another conclusion which is readily apparent from these data is that pure water, passing through the Asboloxane filter cake, acquires considerabl

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(1) "Abstracts of Articles on Oligodynamic Sterilization" - The Engineer Board, Project WS 768, 1 May 1947

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TABLE XVIII

DEATH RATE OF E. COLI IN FILTRATE FROM  
ASBOLOXANE S-FILTER POWDER

Time of Contact	Bacteria/ml.	
	0.5 ml. E. coli inocula	0.005 ml. E. coli inocula
0 min.	5,220,000	160,000
15 min.	700,000	4,400
30 min.	7,800	246
60 min.	800	6
120 min.	35	0
k (death rate)	.078	.096

TABLE XIX

DEATH RATE OF E. COLI IN VARIOUS  
ASBOLOXANE FILTRATES

<u>Filtrate from water originally containing</u>	<u>Organisms added per ml. filtrate</u>	<u>k*</u>
3 ml. nutrient broth - E. coli	$1.5 \times 10^6$	.030
	$1.8 \times 10^5$	.036
	$2.1 \times 10^4$	.048
E. coli only	$2.1 \times 10^6$	.042
	$3.5 \times 10^5$	.087
	$5.7 \times 10^4$	.120
Nothing	$3.6 \times 10^6$	.093
	$3.4 \times 10^5$	.126
	$2.4 \times 10^4$	.138

\* k = death rate of added organisms. The greater the number, the faster is the rate of death of the added bacteria.

[REDACTED]

more antibiotic potential than does water which originally contained washed E. coli organisms. The difference is still greater when the water originally contained broth as well as well as E. coli. These results are not in accordance with the findings of Dr. Goetz, who reported that the antibiotic potential of Asboloxane filtrates was greater if viable organisms had been present in the water before it was filtered. Our findings, confirmed in many experiments utilizing both E. coli and S. aureus to re-inoculate the filtrate, show that any organic matter present in the water as it is being filtered, whether viable or not, results in less antibiotic potential in the filtrate. Although many attempts have been made to elucidate the exact nature of this antibiotic potential, it is still an unexplained factor.

Its importance, however, may be considerable, for water sterilized with the Asboloxane S-filter pump will retain self-sterilizing properties for a considerable length of time. Thus the usual precautions for aseptic handling of drinking supplies may possibly be dispensed with to a certain extent.

d. Various Chemical and Biological Properties of the Filtrates from Asboloxane S-Filter Powder

In this section are reported various tests conducted at Camp Detrick on Asboloxane S-filter powder and the filtrates obtained from it. These tests are largely exploratory or fact-finding in nature, and describe some of the changes which occur both in the S-filter powder itself, the liquids which are passed through it, and the organisms which have been exposed to Asboloxane action. Certainly no definitive explanation can be given at this time as to how or why microorganisms are killed by Silver Asboloxanes, or by any other silver complexes. The tests do give, however, a little insight into a few of the chemical and biological forces at action in Asboloxane sterilization.

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Silver Relationships: Chemical analysis for silver was made on Asboloxane S-filter powder. Also, spectrographic analyses were made of the powder and of the dried residue from an Asboloxane filtrate. The data so obtained are in good agreement with the data for the composition of the powder, furnished later by Dr. Goetz, save for the fact that no zinc was reported in the spectrographic data. The figures are given together in Table XX.

Routine tests for silver were performed on many of the filtrates in the laboratory pressure filtration tests with the Asboloxane S-filter powder. The method used was the cupric dithizonate method (1) which detects as little as 5./liter. Typical data obtained are presented in Table XXI. Silver does appear in the filtrates, but in quantities far below the solubility of silver chloride. Spectrographic data also have shown that, when Asboloxane S-filter powder is deposited on hardened agar plates, small amounts of silver diffuse out into the agar.

It appears that silver is still present towards the end of the filtrations, where viable organisms often begin to show up in the filtrates. Moreover, ionic silver at a concentration several times as great as the silver concentrations usually found in the filtrate, is but slowly toxic to E. coli as measured in experiments conducted in this laboratory and shown in Table XXII. For these reasons, attempts thus far to correlate the data on silver in the filtrates with killing action of Silver Asboloxane have been unsuccessful.

It is interesting to note in this connection that very little silver (5)/liter or less) appears in the water in canteen trials where the special Asboloxane Canteen charge is added to the water, and is in contact with it for 15 minutes before filtering.

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(1) E. B. Sandell, "Colorimetric Determination of Traces of Metals" - Interscience Publishers Inc., New York, N.Y. (1944) p. 399.

[REDACTED]

TABLE XX

COMPOSITION OF ASBOLOXANE S-FILTER POWDER

Chemical Test for Silver

Asboloxane S-Filter Powder                      Approx. 0.5% silver

Spectrographic Data (Combined for both Asboloxane S-Filter Powder and residue from Filtrate)

Major Elements	Ca, Si, Mg
Minor and Trace Elements	Al, Fe, Na, Ag, Cu*, Ti**

\* Reported only for Filtrate Residue

\*\* Reported only for Asboloxane S-powder

Composition of Asboloxane S-filter Powder as Furnished by Dr. Goetz

<u>Ingredient</u>	<u>% by Dry Weight</u>
Diatomaceous Earth	78.4
CaO <sub>2</sub> · nH <sub>2</sub> O + Ca(OH) <sub>2</sub>	13.2
ZnO (U.S.P.)	1.9
Colloidal Carbon (Black)	2.9
Ag (as Ag <sub>2</sub> O) (U.S.P.)	0.5
Asboloxane	5.3
Polyvinyl Compds. (dry)	2.7
Detergent (wet)	0.4
Total	100.0 %

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TABLE XXI

ASBOLOXANE S-FILTER POWDER IN LABORATORY

PRESSURE FILTER APPARATUS

Amount of Silver Appearing in Filtrate

(Filtrates digested with H<sub>2</sub>SO<sub>4</sub>)

Volume passed through Filter Cake (ml)	Liquid Being Filtered					
	Distilled Water	Tap Water (Average of 2)	Distilled water washed coli (Average of 2)	Tap water washed coli	Distilled water + nutrient broth	Tap water + nutrient broth
	Silver in Filtrate in $\mu$ /Liter					
200						
400	5	50	10	25	100	150
600		65				
800						
1000	5	60	17	80	70	90
1200			17			
1400	30			75	45	45
1600						
1800	10	50	17	65	35	25
2000						
2200	20	45	15	60	20	30
2400						
2600	20		12	50	10	20
2800		30				
Control	0	3	5	0	0	3

NOTE: For Comparison -  
 Solubility AgCl in Water - 1,500  $\mu$ /L or 1,130  $\mu$ /L of Silver  
 Solubility AgBr in Water - 130  $\mu$ /L or 75  $\mu$ /L of Silver

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TABLE XXII

BACTERICIDAL ACTION OF  $\text{AgNO}_3$  AGAINST *E. COLI*

(Concentration of Silver 104  $\gamma$ /Liter)

Contact Times	Organisms/ml at Various pH Values		
	pH 3.42	pH 6.35	pH 9.85
0 min.	190,000	30,500	14,100
5 min.	10,700	10,800	375
10 min.	4,800	3,100	101
15 min.	1,850	920	35
30 min.	260	172	2
60 min.	4	28	0
120 min.	0	2	0
120 control*	2,900	25,600	11,100

\* In contact with Buffer only. No  $\text{AgNO}_3$ .

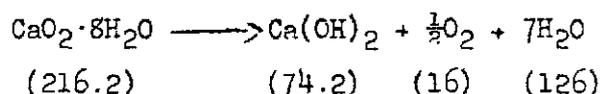
Experimental Conditions:

1 ml of 0.00005 N  $\text{AgNO}_3$   
1 ml of *E. coli* Suspension  
Added to 50 ml Various Buffers.  
Aliquots removed for viable count at indicated times.  
Tests run at room temperature.

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Oxygen Relationships: The Asboloxane S filter powder contains both a silver catalyst and an insoluble peroxide (CaO<sub>2</sub>). In the course of filtration the peroxide is consumed, and the filter powder loses effectiveness. It is therefore interesting to try to trace what is happening to the peroxide originally present in the powder when it comes in contact with water.

One possibility is that the silver could catalyze the decomposition of the calcium peroxide to oxygen and calcium hydroxide according to the following equation:



Actually some gas is given off when the powder becomes wet. Experiments were conducted in the Warburg respirometer to collect and analyze this gas. As the data in Table XXIII indicate, the gas is oxygen since it is adsorbed by pyrogallol and not by alkali. In 30 minutes shaking with water, 0.32 milliliters or 0.42 milligrams of oxygen are given off by one gram of Asboloxane S-filter powder.

Some simple calculations can show that this amount of gaseous oxygen is not enough to account for all of the peroxide originally present in the filter powder or extracted by the water. For example the equivalent weight of calcium peroxide, according to the equation above, is  $0.42 \times 216/16 = 5.68$  mg. of calcium peroxide per gram of Asboloxane. Thus the oxygen liberated accounts for only about 0.6 per cent CaO<sub>2</sub> in the original Asboloxane composition.

In another experiment a sample of Asboloxane S-filter powder was carefully weighed and then extracted with 100 ml. of water. The extracted powder was dried to a constant weight in a vacuum desiccator for two days, and the filtrate was dried frozen under high vacuum in a lyophilizer. The drying

[REDACTED]

TABLE XXIII

EVOLUTION OF GAS BY WETTED  
ASBCLOXANE S-FILTER POWDER

(Milliliters of Gas per gram of Powder after 30 minutes)

Collected in the presence of:	<u>Water</u>	<u>20% KOH</u>	<u>Pyrogallol</u>
	.25	.42	0
	.43	.27	0
	.37	.22	0
	.29		
	—	—	—
Average	.34	.30	0

[REDACTED]

[REDACTED]

processes were such that no decomposition due to heat should take place. The net loss in weight was 0.017 grams per gram of Asboloxane S-filter powder. If one assumes that this non-recoverable weight is due to oxygen and water, formed according to the equation above, then  $0.017 \times 216/142$  or .026 grams of  $\text{CaO}_2$  must be decomposing per gram of filter powder. This figure is about five times as high as that obtained in the Warburg experiment.

If analyses are run on the Asboloxane filtrates, however, oxygen compounds are found in the filtrate. The method used in this laboratory to determine these compounds was that of Winkler (1) for total dissolved oxygen in water. Peroxides are also determined by this method, so that the values obtained for "Total Oxygen" in the filtrates represent the total of oxygen gas and soluble peroxy type compounds. When the control values, representing the amount of dissolved oxygen present in the water before filtration, are subtracted, one obtains values for  $\Delta \text{O}_2$ , or for oxygen and peroxy compounds added to the water in the course of filtration. The values for total oxygen and for  $\Delta \text{O}_2$  as obtained in this way when 2800 ml. of distilled or tap water are filtered through one gram of Asboloxane are given in Figures 8 and 9.

The values for  $\Delta \text{O}_2$  must largely represent peroxides of some sort, since the water usually is nearly saturated with oxygen before passage through the filter cake, and any oxygen liberated as such would mainly be lost to the atmosphere before the samples could be titrated. Dr. Goetz has reported to us that he has used acid permanganate solutions to titrate peroxides in Asboloxane filtrates. When this method is used, curves very similar but slightly lower than the  $\Delta \text{O}_2$  curves are obtained, as is also shown in Figures 8 and 9 where data are presented for both methods used on the same filtrate. For comparative purposes, all values in these figures are given as hydrogen peroxide, although it is doubtful that this is the actual compound formed.

(1) Method given in most analytical texts such as Treadwell-Hall "Analytical Chemistry." Manganous hydroxide is oxidized by oxygen to manganese dioxide, which is then determined iodometrically.

FIGURE No. 8

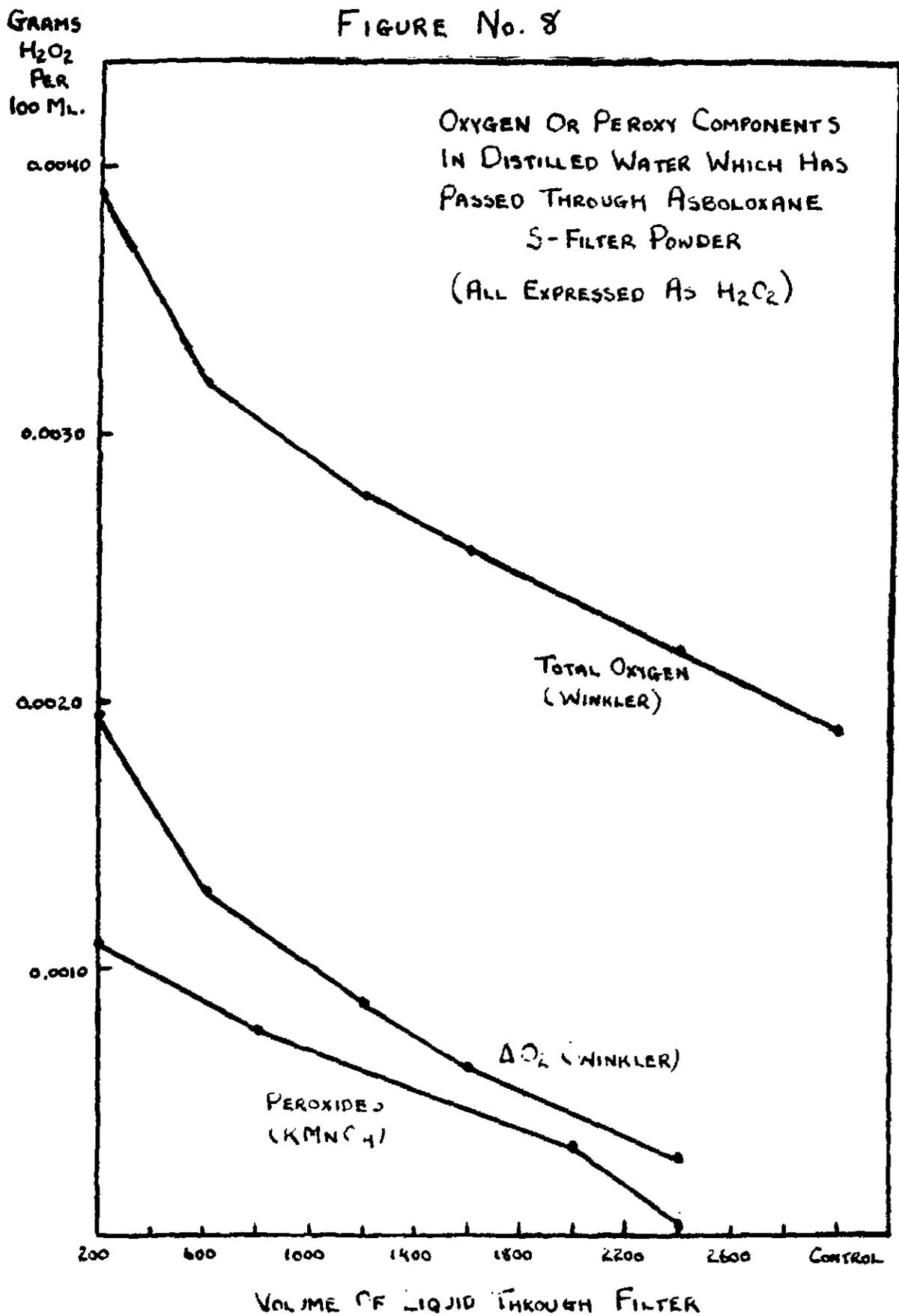
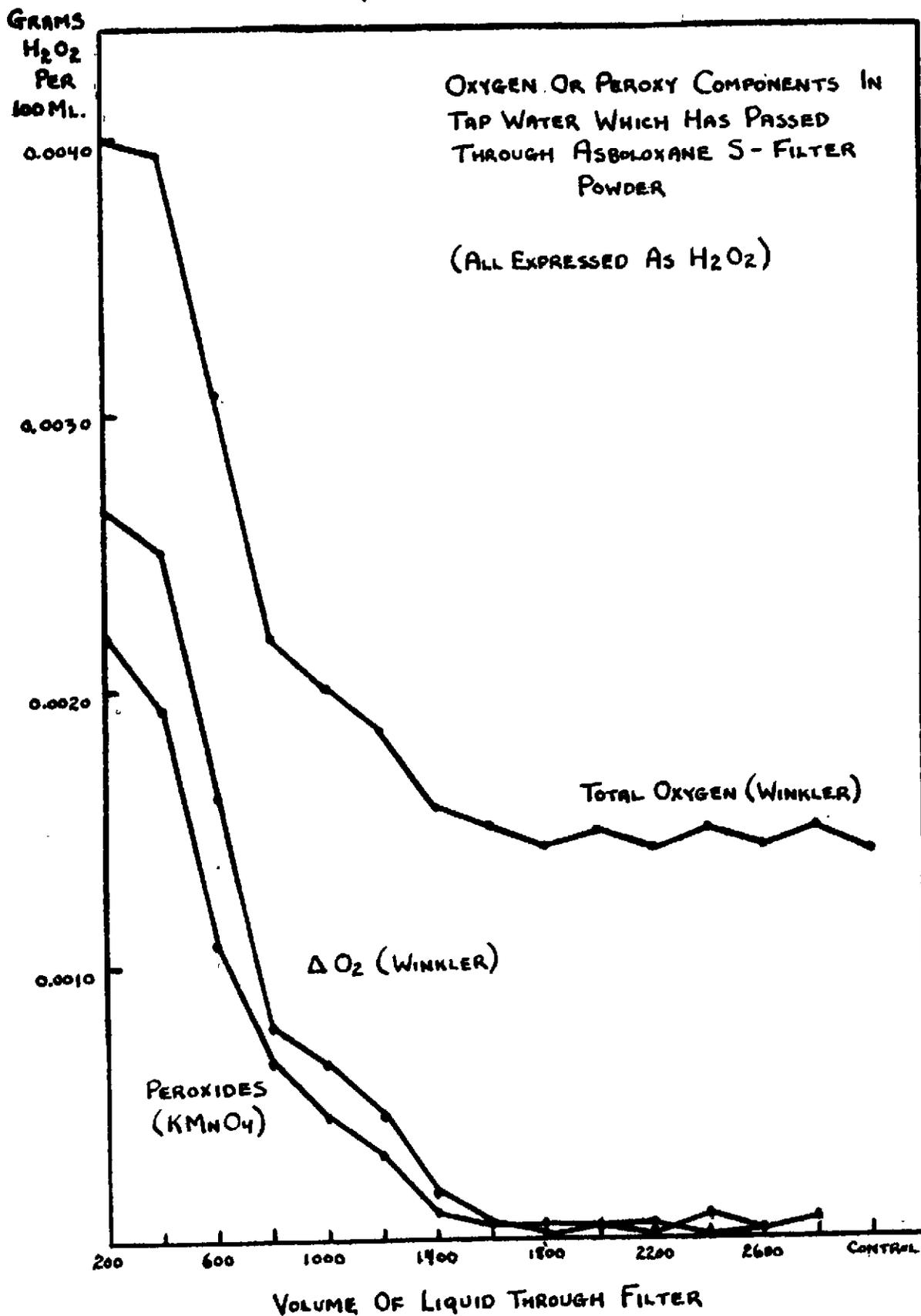


FIGURE No. 9



[REDACTED]

From these graphs, by summing up the area under the  $\text{AO}_2$  curves, one can calculate the amount of peroxy type compound added to the water during filtration. This amounts to 0.0248 grams of  $\text{H}_2\text{O}_2$ , or its equivalent in oxygen or other peroxides, for the distilled water curve, and to 0.0210 grams of  $\text{H}_2\text{O}_2$  in the figure for the tap water filtration. The former figure is equivalent to that which would be formed by the decomposition of 0.170 grams of calcium peroxide per gram of filter powder. The latter is equivalent to 0.134 grams of the calcium peroxide per gram of filter powder.

When these figures are compared with those appearing in Table XX for the per cent composition of Asboloxane S-filter powder (13.2 per cent calcium peroxide as stated by the manufacturer), it is apparent that all of the calcium peroxide must be decomposed during the course of filtration. The values for  $\text{AO}_2$  obtained by the Winkler method indicate a little more calcium peroxide than that stated. The values for peroxides as determined by the permanganate method would indicate a little less calcium peroxide in the powder than this stated amount. Almost all of this calcium peroxide (about 13 per cent) which is decomposing appears as peroxy compounds in the filtrate, since that which decomposes to form gaseous oxygen is very little (about 0.6 per cent in the Warburg test), and that which goes to form compounds which are lost when the products are dried in the absence of heat is not very great (about 2.6 per cent in the loss of weight test).

The low figure in the loss of weight test indicates that the soluble peroxy compound in the filtrate is not hydrogen peroxide. There are other good reasons for believing that it is some other compound or compounds. The common qualitative tests for hydrogen peroxide are negative for the Asboloxane filtrates, (Table XXIV). Moreover, when hydrogen peroxide and sodium carbonate are added to distilled water in amounts large enough to produce a total oxygen value just

TABLE XXIV

TESTS FOR HYDROGEN PEROXIDE IN FILTRATES  
FROM ASBOLOXANE S-FILTER POWDER

Reagent	Sensitivity Mg/Liter	Result, if Peroxide present	Observed
$\text{Co}(\text{NO}_3)_2$ (alkaline)	-	Black ppt.	Nothing
$\text{FeCl}_3 + \text{K}_2\text{Fe}(\text{CN})_6$	0.02	Green + blue ppt.	Nothing
$\text{K}_2\text{CrO}_4 + \text{ether}$ (acid)	0.1	Blue ether layer	Nothing
$\text{KI} + \text{starch}$ (acid)	0.05	Blue color immediately	Blue, slight, slow

[REDACTED]

under that obtained for the Asboloxane filtrates, the killing action is considerably greater than that of the filtrates, as shown in Table XXV. Expressed in another way, the antibiotic potential of the Asboloxane filtrates is less than that of hydrogen peroxide solutions of equivalent or slightly lower strength. The exact composition of the peroxy substance or substances imparted to the filtrate by the Asboloxane S-filter powder is not evident from the work done in this laboratory to date.

The earlier work done here indicated that there may be some correlation between the amount of these compounds appearing in the filtrate, and the sterilizing action obtained. In Table XXVI appear data obtained from earlier runs giving the  $\Delta O_2$  values found in the last portion of the filtrate which came through sterile. In the earlier portions of these filtrates the  $\Delta O_2$  values were higher, and the filtrates all sterile. In the later portions, viable organisms began to appear in the filtrates and the  $\Delta O_2$  values were lower. It was hoped that it might be possible to use these data to adapt a chemical test for evaluating the effectiveness of Asboloxane filtrations by simply checking the filtrate to see if the  $\Delta O_2$ , or peroxy titer, was above a certain level. This value differs, however, according to the amount of organic material present, as is shown in Table XXVI referred to above. Moreover, it is possible to achieve sterility even when no  $\Delta O_2$  is evident in the filtrate as in the run described on page 55 in which cysteine hydrochloride was present. For naturally occurring waters it still may be possible to set up such a control test for sterility.

Effect on Bacterial Enzyme Systems: The effect of the Silver Asboloxanes on various bacterial enzyme systems has been studied, since it is by attack on these sensitive components of the cell that most disinfectants work.

TABLE XXV

[REDACTED]

COMPARISON OF ANTIBIOTIC POTENTIAL OF ASBOLOXANE FILTRATE  
AND HYDROGEN PEROXIDE SOLUTION

(Asboloxane Filtrate = 1200 ml thru 1 gram Asboloxane S-Filter Powder)

(Peroxide Solution = 0.47 ml 3% H<sub>2</sub>O<sub>2</sub> + 5 g Na<sub>2</sub>CO<sub>3</sub> to 1 Liter water)

Heavy E. coli Suspension Added To	Organisms/ml After				
	1 min.	5 min.	10 min.	30 min.	60 min.
Distilled Water	4.5 M	4.4 M	3.9 M	4.8 M	4.4 M
Asboloxane Filtrate	4.3 M	0.8 M	3.6 M	1.2 M	58 T
Peroxide Solution	15	9	8	8	7

Total O <sub>2</sub> and peroxides (Expressed as H <sub>2</sub> O <sub>2</sub> )	Asboloxane Filtrate	0.0030 g/100 ml
	Peroxide Solution	0.0026 g/100 ml

Light E. coli Suspension Added to	Organisms/ml After			
	1 min.	5 min.	10 min.	30 min.
Distilled Water	12,300	10,700	8,850	6,750
Asboloxane Filtrate	7,500	7,300	5,650	3,350
Peroxide Solution	67	0	0	0

Total O <sub>2</sub> and peroxides (Expressed as H <sub>2</sub> O <sub>2</sub> )	Asboloxane Filtrate	0.0029 g/100 ml
	Peroxide Solution	0.0026 g/100 ml

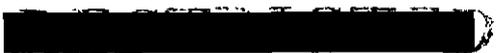
RESTRICTED TABLE XXVI

ASBOLOXANE S-FILTER IN LABORATORY

PRESSURE FILTER APPARATUS

Lowest Value of  $\Delta O_2$  Accompanying Sterile Filtrates  
of E. coli Suspensions

<u>Run No.</u>	<u>Friedlein Broth Suspension</u> <u><math>\Delta O_2</math> (minimal)</u> <u>(mg. <math>O_2</math>/100 ml.)</u>	<u>Run No.</u>	<u>Nutrient Broth Suspension</u> <u><math>\Delta O_2</math> (minimal)</u> <u>(mg. <math>O_2</math>/100 ml.)</u>
14	.26	15	.74
21	.37	17	.75
24	.30	19	.58
25	.29	20	.59
26	.41	23	.80
29	.24	27	.75
31	.32	28	.54
Average	.31 $\pm$ .04	Average	.68 $\pm$ .09



████████████████████

A technique of treating organisms to varying dosages of Asboloxane S-filter powder was evolved in which pour plates of hardened nutrient agar was prepared and the surface uniformly seeded with a suspension of the organism chosen for study. In the center of the plate about half a gram of Asboloxane S-filter powder was placed, and the plate incubated. The bacteria are thus subjected to successively diminishing dosages the farther away they are from the solid powder in the middle of the plate. Chemical tests have shown that silver, peroxides, and alkali all diffuse out from the S-filter powder into the nearby agar.

The organisms farthest away, near the edge of the Petri dish, grow normally. Usually those in the area next to Asboloxane S-filter powder fail to grow at all, and a clear zone of inhibition in which no colonies develop encircles the filter powder. These zones of no growth, and of normal growth, do not merge imperceptibly, as one might imagine. Instead, with *S. aureus* at least, the zone of inhibition stops abruptly and there is a narrow band just around it where the growth of the colonies is more vigorous than in the area of normal growth behind it. This is another example of contact with sub-lethal concentrations of a toxic substance causing a stimulation in growth, as has been noted in so many fields of biology. The tests summarized in Table XXVII were performed on organisms which survived a sub-lethal dosage of Asboloxane S-filter powder. These organisms were collected from the zone of enhanced growth or stimulation just next to the lethal zone on the plate. The activities of the enzyme systems in these particular organisms are compared with those of normal unexposed organisms.

At the present state of the investigation, no theory can be postulated as to the mechanism of the Asboloxane action based on these enzyme studies. It is evident, however, that with *S. aureus* at least, a wide range of enzyme systems are affected as the organism attempts to overcome the Asboloxane action.

TABLE XXVII

EFFECT OF SUB LETHAL DOSAGES OF ASBOLOXANE S-FILTER  
POWDER UPON ENZYMES OF STAPH. AUREUS

<u>Enzyme</u>	<u>How Measured</u>	<u>Activity in Asboloxane Treated Organisms</u>
Coagulase	Clotting of human blood plasma	Increased
Catalase	Ability to decom- pose H <sub>2</sub> O <sub>2</sub>	Increased*
Gelatin Liquefaction	Stab puncture in Gelatin tube	No effect
Hemolysin	Action against defi- brinated rabbit blood cells	Decreased*
Succinic Dehydrogenase	Ability to oxidize substrate - dye indicator	Decreased

\* Thioglycollic acid does not inhibit this action.

[REDACTED]

Its ability to produce catalase, and hence destroy peroxides, is enhanced and so is its production of coagulase. On the other hand, the production of proteolytic enzymes which can liquefy gelatin are not affected. The succinic dehydrogenase, which is used by the organism for metabolism in the absence of oxygen, is produced in lessened amount; again indicating that the organism is adapting itself to an environment of increasing oxygen supply.

Sulfhydryl compounds, which are probably contained in many of the enzymes, often have an effect on the Asboloxane action. With *S. aureus* for example, no zone of inhibition appears about the Asboloxane powder in the above described technique, if mercaptoethanol or cysteine hydrochloride is present in the agar. The organisms grow up to the very edge of the Asboloxane deposit. Thioglycollic acid, however, does not have this power of overcoming the Asboloxane action against *S. aureus*.

When *E. coli* is seeded on nutrient agar plates, the zone of inhibition about the Asboloxane S-filter powder which one observes with *S. aureus* does not appear. On Friedlein's completely synthetic agar, however, the zone of total inhibition and zone of increased growth do appear. By adding the various components of nutrient agar to the Friedlein's agar, it can be shown that the beef extract, and, to a lesser degree, the yeast in the former enable *E. coli* to overcome the Asboloxane toxic action. The sulfhydryl compound, thioglycollic acid, likewise neutralizes this action. Cysteine hydrochloride and mercaptoethanol do not act as neutralizers, although they both contain the same reactive SH group.

These experiments with various neutralizing agents point out quite strongly the difference in enzyme systems of different bacterial species, and emphasize how difficult it is to base a theory as to mechanism of action of a disinfectant on tests with only one organism.

[REDACTED]

D. SUMMARY

The Silver Asboloxanes have been described. Two pieces of apparatus, a canteen with a special filter head, and the S-filter, designed as military items to be used in conjunction with the Silver Asboloxanes to sterilize water on a small scale, have been described. The experiments of Dr. Goetz in which these pieces of equipment were given field trials in Mexico, to determine their effectiveness in removing water-borne organisms from surface waters, are quoted in full.

Results of laboratory trials conducted at Camp Detrick on these items are given in some detail. These trials have in general centered around their performance in the presence of very high concentrations of organisms chosen to represent various typical groups of bacteria. Chemical tests and biological tests which shed some light on the mechanism of action of the Asboloxanes are reported in lesser detail.

Briefly, the findings from the Camp Detrick trials may be summarized as follows:

The use of the Asboloxane powders, in a canteen or in the S-filter can provide a quick, convenient, reliable method of obtaining small scale supplies of sterile drinking water. The canteen powder is intended for the treatment of individual supplies of water, while the pump may be used to deliver about 100 liters of sterile water per hour.

Experiments at Camp Detrick have indicated that both types of Asboloxane powder, when used in the prescribed method and apparatus, can sterilize water suspensions of bacteria of concentrations as high as 3 million viable organisms per ml. Organisms shown to be susceptible to the action of the Asboloxanes include *Escherichia coli*, *Staphylococcus aureus*, *Flavobacterium pneumoniae*, *Eberthella*

[REDACTED]

typhosa, Mycobacterium phlei, Serratia marcescens, Gaffkya tetragena, and Bacillus globigii. Tests against bacteriophage have indicated that these organisms are more resistant to the Asboloxanes.

Our most intensive work has been done on the S-filter and powder. This appears to be remarkably effective in removing high concentrations of bacteria from water. It appears that a considerable proportion of the bacteria are removed mechanically, but this filtration effect is not the significant part of the action of the S-filter. The Asboloxane powders actually kill bacteria (or render them incapable of growth) while the water containing them is in contact with the filter cake.

Water treated by the apparatus under consideration is clear and perfectly potable. It has, however, a rather high pH (9-10) and a considerable degree of temporary hardness ( $\text{Ca}^{++}$ ). The efficiency of the S-filter can be improved by using more powder, or by repeated treatment of the water, though these procedures would not seem to be necessary under ordinary circumstances, since viable organisms appear in the filtrates only when initial concentrations are about  $10^6$  organisms/ml. The filter powder is still effective after storage for six months in sealed containers at temperatures between  $0^\circ$  and  $45^\circ\text{C}$ .

The presence of nutrient broth and, presumably, of other protein materials has a detrimental effect on the sterilizing capacity of S-filter powder. This interfering action of nutrient broth extends also to a diminution of the self-sterilizing properties or antibiotic potential of Asboloxane filtrates.

This self-sterilizing capacity, which is apparently of long duration, may be of considerable practical importance in preventing the recontamination of treated water. Its intensity appears to depend upon the concentration of contaminating organisms added to the treated water, and upon the amount of organic matter originally present.

████████████████████

In an attempt to determine the active agent responsible for the bactericidal action of S-filter powder, a great number of filtrates obtained under various conditions were examined. Two observations as to the chemical composition of the filtrates have been made; namely, that silver and some form of active oxygen compound are present. The role of these materials has not yet been clearly delineated. Some of the data obtained in experiments done to determine the effect of ionic silver, and of hydrogen peroxide, are presented. It may be possible by using the oxygen-peroxy content of filtrates to devise a rapid spot-test system which will indicate whether such filtrates are likely to be bacteriologically safe to drink.

Some interesting results have been obtained from investigations of the enzyme systems of bacteria which have barely escaped bacteriostasis by Asboloxane S-filter powder on solid media. It appears that the ability of *S. aureus* to produce catalase (which destroys peroxides) is greatly enhanced while the succinic dehydrogenase system is decreased in amount. These results might be expected in such a case when organisms are growing on a substrate rather rich in oxygen. It is apparent that the enzyme systems are being affected by Asboloxane powders, but generalizations cannot be made on the basis of the limited number of tests we have performed.

[REDACTED]

[REDACTED]