The toxic effect of ionic silver on the lower organisms
by Jean McElroy

A THESIS Submitted to the Graduate Committee in partial fulfillment of the requirements for the Degree of Master of Science in Botany and Bacteriology
Montana State University

Abstract:
Ionic silver sterilization has gained widespread attention in recent years as a means of solving water-purification problems. Even further, its usage has extended into the fields of food preservation, food manufacture and medicine until a critical study of the process presents itself as being valuable at the present time.

The objective of this study is twofold; to note the effect of ionic silver sterilization on different organisms under varying dondi-tions, and, to examine the practicability of the process from the standpoint of Industrial Microbiology and Medical Bacteriology.

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Approved:

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In Charge of Major Work

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Chairman Graduate Committee

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THE TOXIC EFFECT OF IONIC SILVER ON THE LOWER ORGANISMS

Introduction

Ionic silver sterilization has gained widespread attention in recent years as a means of solving water-purification problems. Even further, its usage has extended into the fields of food preservation, food manufacture and medicine until a critical study of the process presents itself as being valuable at the present time.

The objective of this study is twofold; to note the effect of ionic silver sterilization on different organisms under varying dondi-tions, and, to examine the practicability of the process from the stand-point of Industrial Microbiology and Medical Bacteriology.

Historical

Miller[1/](22) was the first to notice the effect in antiseptic work of finely divided metals such as gold, silver and platinum and their germicidal action when made into false teeth. It was not until 1893, however, that the phenomenan of oligodynamic metal activity was demonstra-ted by the Swiss botanist V. Naegoli[1/](23). By oligodynamic phenomena, Naegali meant those produced by exceedingly small quantities of metallic substances in solution. Oligodynamic poisoning manifests itself in the

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1/As reported by Kissau, Luther, Beitrag Zur Sterilizierung des Trinkwasser durch das Katadyn-Verfahren. Tierarztl. Rundschau. 36 : 609-612; 37 : 629-633. Sept. 1933

living cell in a different way from true chemical poisoning. In the former case, the cell does not at once lose its rigidity. Naegali found that water in contact with metals such as copper, silver or aluminum took on bactericidal properties, and that this "activated" water caused the death of Algae.

Thiele and Wolff[1] (34) first noted the oligodynamic effect on bacteria, especially the pathogenic members. Messerschmidt[1] (21) reported a similar effect in gunshot wounds; even when the bullet was presumably germ-laden, the wound healed cleanly. He ascribed this to the oligodynamic effect of the metal bullets.

Gottschalk[1] (13) tried out this effect using twenty-four different purified five-mark pieces on twenty-five different strains of Escherichia coli (Migula) Castellani and Chalmers, thus demonstrating again the oligodynamic ability of certain metals.

Konrich[1] (16) cites figures for the relative spped and quantitative bactericidal results he obtained by using this method on Es. coli. Liter flasks were filled with a solution of Katadyn water (see page 7) containing 25,000 organisms per cc., with amounts of Katadyn sand ranging from 100 to 10 grams in each. In those containing 100 grams sterility was attained in 2 to 3 hours; with 10 grams it took 4 hours. In another test he killed 110,000 of these organisms per cc., in a flask

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[1] As reported by Kissau, Luther, Beitmag Zur Sterilizierung des Trinkwasser durch das Katadyn-Verfahren. Tierarztl. Rundschau. 36 : 609-612; 37 : 629-633. Sept. 1933.

containing 100 grams of the sand, in 4 to 5 hours. The size of the particles and consequently the total exposed surface was not indicated. Wollman and Wollman (37) found that the amount of surface exposed was not a limiting factor in oligodynamic action.

Konrich tested the power of activated water removed from the activating substance by leaving a liter of water 24 hours in contact with the sand (100 grams of sand containing 10 per cent of silver) then pouring it off and testing it on Es. coli. In 3 or 4 hours the water killed 88,000 organisms per cc. This seems to show that the quantity of Katadyn silver employed is relatively unimportant so far as either time or quantitative results is concerned.

Degkwitz[1] (6) however, thinks that for the best effect, in sterilization, there is a definite optimum relation between volume of water and surface of the oligodynamic agent. His results, both in the time factor and that of quantity of silver liberated, are much more favorable than Konrich's. By activating 500 cc. of water through 12 hours' contact with 50 grams of silver-containing sand, he killed one million Es. coli organisms in 2 hours, 5 million in 5 hours, with absolute certainty. A reduction of 10 per cent in the silver had no appreciable effect on the sterilizing power or the number of bacteria killed, but if the number treated is rather large and 10 per cent less silver used a correspondingly longer time is needed to kill them.

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The foregoing experiments being concerned only with Es. Coli one might think that they alone had been the subjects of investigation. But Krause (17 and 18), Degwitz (6) and Konrich (16) in test on other bacteria, have had results parallel in all respects to those given above. Since Es. coli is regarded to some extent as a "standard" organism, being preferred to other bacteria because it is easy to detect and recognize, grows well on artificial media and is not pathogenic, as well as having a fairly high resistance to ordinary methods of disinfection, these tests may be assumed to be fairly usable.

Other names associated with these earlier studies and who contributed much to our understanding of the work include Neisser and Eichbaum (24), Kronig and Paul, Elsenberg, and Delepine and Greenwood. In his publication on "The Oligodynamic Metal Strength in Theory and Practice", Neisser, as reported by Viesohn (35), cites the following principle: "oligodynamic action is due to the strength of the smallest particle of electro-positive charged metal ion." Krnnig and Paul, according to Suckling (33) showed that the toxicity to bacteria was not dependent upon the molecular concentration of the salt, but upon the concentration of free metallic ions in the solution. Elsenberg, as reported by Suckling (33) working with the salts, arranged the metals in order of their toxicity and headed the list with silver.

Although many investigators have occupied themselves with the study of this phenomenon, it was Krause (17 and 18) of Munich, as reported by Kissau (15), Loewe (19), Suckling (33) and Viesohn (35) who

first succeeded with a practical application for the treatment of liquids. In 1929, he described a new form of silver which he called "Catadyn Silver". With regard to this preparation, Dr. Krause stated: "its bactericidal efficiency is many times greater than with silver in any of the forms hitherto employed."

Krause's Katadyn work was built up on the basis of V. Naegoli's discovery. He rechecked the toxicity of certain metals on bacteria and found that silver exhibited the property to the highest degree. The first application was in the form of silver coated surfaces (porcelain or quartz). By sufficient exposure the water became germicidal and the organisms in it were killed. The water sometimes retained this property for as long as three weeks. The next application was in the form of silver-covered sand, known as the "static catadyn sand process". This was successful, but the more recent discovery of the "Electro-Katadyn Process" represents the latest stage of development and serves for the treatment of larger amounts of liquids. In this process the discharge of silver ions is brought about by a weak electric current and according to Faraday's[1] Law 4.023 milligrams of silver would be brought into solution by one milliampere hour, but in actual practice only about 50 per cent of this amount is dissolved when water is used as a electrolyte.

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[1] Faraday's Law states that 96,500 coulombs will decompose one gram-equivalent weight of a substance, which in the case of silver is 107.88 grams. A coulomb is the amount of electricity conveyed by 1 ampere per second, and since the basis of this work is milli-amperes per hour, 4.023 milligrams of silver would be brought into solution.

Reports describing the successful applications of the Katadyn Process to water sterilization (drinking water and swimming pools in particular), food preservation, sterilization of beer, aging of wines, destruction of algae and vinegar eels, washing of milk utensils, sterilization of surgical instruments, ice manufacture, margarine manufacture, fruit fermentations and preservation of cut flowers have appeared in great profusion.

The question has been raised as to the poisonous effect of ionic silver on the human body. If this treatment were used on water employed for drinking purposes, the question of whether silver in this form were accumulative would be of great significance. The following quotation was taken from an article by E. V. Suckling (33) and continues to say: "silver is one of the least poisonous of the metals, and the amount that water can take up after prolonged contact with Katadyn silver is exceedingly minute. If a person drank $\frac{1}{2}$ gallon per day, containing 0.06 p.p.m. of silver his daily dose would be less than 0.00014 grn."

In continuation, Suckling adds that silver in the body is at once converted into insoluble salts and as the chloride and albuminate and by far the greater part remains unabsorbed, being expelled with the feces. He thought it highly unlikely that any absorption at all will occur by the consumption of the Katadynized water. Silver is excreted in the feces and none in the urine so there is no danger of damage to the kidneys.

Gibbard (12) questioned the physiological action of silver as causing argyria, a wellknown but not very common manifestation of silver poisoning. Another important consideration is that of the effect of the ionic silver upon enzymes, especially those of the digestive tract. Waksman and Davison (36) have discussed the action of salts of the heavy metals upon enzymes and it would appear advisable to conduct a specific study to determine the effect of silver upon these digestive enzymes.

As to the exact mechanism by which the destruction of bacteria may be brought about by the ionic action of silver several theories have been advanced, such as:

1. The emission of lethal rays, or radio-activity.

2. Combination of the ion with the proteins of the bacteria.

3. The ions cause some change in the medium containing the bacteria and this then affects the bacteria.

4. The ions bear electrical charges to which the effect is due.

5. Selective adsopption.

The following discussion on the mechanism whereby ionic silver brings death to the lower organisms was taken from a paper by Kissau (15). Von Behring (2) thought it a real chemical "identification" due to tiny particles of the metal being dissolved by the water. Saxl (31) has stubbornly contended it to be a purely physical effect, made effective at a distance by a ray-like action. But Doerr (8) learned that on

the surface of silver, by the action of atmospheric oxygen or of hydrogen ions, compounds soluble in water are formed, which diffuse outward and have a harmful effect on cell structures. He also found that on heating silver loses its capacity for oligodynamic effect. Herzburg (14) does not regard the effect of the metal ions, but rather their function as a carrier of oxygen, as the real effective agent. But this viewpoint was still more confused by the discovery that the oligodynamic power can also be transferred to glass vessels, which exert this power and even in turn convey it to the water contained in them, activating it. Freundlich and Sollner (10) think this is because the silver ions are at first adsorbed by the glass, later penetrating deeper into it, ions being continually taken up by the outer layer. Thus independent silver ions may later be released into solutions or diffused through liquids, or even removed from the glass surfaces by organisms with strong attraction for silver ions. They thus regard the oligodynamic effect of metals as purely a chemical one, due to the "dissolving" of metallic silver into liquids such as distilled water. It is adsorbed by the organisms, then penetrates the cell walls and effects its damage by some still indefinite chemical process. They think this is proved by the effect of silver on algae, as shown by treatment with carefully limited quantities. They ascribe the "dissolving" of silver to oxygen, carbonic acid, or other impurities in the water.

Burgi and Laubenheimer (3) on the basis of present knowledge, consider the injury more likely to be due to the effect of small amounts of

metals in solution, but make no effort to explain its real physical or chemical nature. Bechhold (1) thought that finely divided silver precipitated on porous bodies might have a higher oligodynamic value because of its greater silver surface, and therefore used carbonized silver.

Gibbard (11) attributed the inhibitory or oligodynamic action of metallic silvers to traces of salts, oxides or sulphides on its surface.

Rideal and Rideal (30) suggest that the germicidal effect of ionic disinfectants is due to the absorption of cations with the simultaneous absorption of anions.

Eichholtz (9) offers evidence in support of selective adsorption as being the lethal agent with his work with algae in which he demonstrated that the actual concentration of metal associated with the algae was several parts per 10,000. Gibbard (11) too offers evidence in support of selective adsorption. He reinoculated bouillon tubes in which no growth had appeared and growth followed, showing that none of the inhibiting material had been carried over.

Krause (17 and 18), as reported by Kissau (15), ascribes to silver a higher ologodynamic effect than other metals. He calls the process "Katadyn" since the oligodynamic nature of it is connected with a catalytic one. He has no belief in a purely chemical explanation of it. It is generally explained as a combined chemical and physical effect, perhaps chemically poisonous insofar as atmospheric oxygen brings about the creation of metal compounds in very small quanties, which dissolve

ionically in water. Then, too, the metal itself diffuses its ions through the water, which attach themselves to bacteria as electrically charged particle and thus kill them by an electrical reaction. But most of our present explanations are guesswork and as yet we can not ascribe the extermination of lower animals and plants to any specific physical or chemical process.

The acceptance of the process has not been entirely without opposition, but rather by some investigators with a degree of skepticism and doubt. According to Kissau (15), Clark and Gage (5) regard the effect as uncertain, and Stick (32) rejects it, but on no very definite grounds.

Kissau (15) found that sterilization by this process proved ineffective on the bacteria whose German names are Rauschbrand, Pararauschbrand, Frankel's gas bacillus and the Tetanus bacillus. A diminution took place but the treatment was not sufficient to kill them even after twenty-four hours.

It is evident that the destructive mechanism has not been satisfactorily explained and offers an excellent field for further study.

Materials and Methods

The bacterial organisms studied in this paper include:

Escherichia coli (Migula) Castellani and Chalmers

Bacillus megatherium De Bary

Eberthella typhosa (Zopf) Weldin

Mycobacterium Butyricum (Petrie) Bergi, et al.

Serratia marcescens Bizio

Phytomonas tumefaciens (Smith and Townsend) Bergey, et al

In addition to the studies to determine the effect of ionic silver upon different bacteria, a group of experiments were set up to determine the effect of ionic silver upon other kinds of cells and materials. In this group were included the following:

Milk

Saccharomyces cereviseae

Cut flowers

Elodea candensis

Spirogyra sp.

Apple juice

Eggs

Spores of Mucor sp. and Penicillium sp.

The application of ionic silver was effected by means of a laboratory unit, model TA-1, and a Katadyn Pocket Sterilizer furnished for this purpose by the Katadyn Process Corporation, New York. The laboratory unit consisted of two parts, the activator and the switchboard. The switchboard contains several dry cells connected in parallel fashion. On the switchboard a dial milliammeter with a five milliampere reading scale is given and a rheostat is mounted below, permitting the variation of voltage. To the right of the switchboard a polarization reverse switch is mounted. The katadyn Pocket sterilizer consists of standard dry cells, contained in a metal cylinder to which two silver electrodes can be attached.

In the following tables, the column headed "Gammas of silver per liter" refers to the amount of silver which goes into solution but not that which is present as the destructive agent. Silver goes into solution at the anode and a part is plated out at the cathode with a portion remaining in the solution to effect the resulting sterilization. Several references to the "amount of silver" or "Gammas per liter" appear in the literature; statements which are obviously in error if interpreted to mean the gammas of silver present in the ionic state and effective as bringing about the destruction of bacteria. In support of this statement, a current was passed between the two electrodes with the effect that the loss in weight of the one electrode was, in part, accounted for by the gain in weight of the other electrode.

In general, the technique employed was relatively simple, consisting of a period of treatment with ionic silver followed by a plating of the organism from the water or other liquid under observation. Whenever it was necessary to do so, this method was modified to fit the need of the material which was being studied.

During the season of the year that this work was being conducted, an analysis of the water showed it to be high in calcium bicarbonate and not to contain much of anything else.

The Katadyn Pocket Sterilizer was shown to have a current strength of five milliamperes. The cells in this unit should be changed frequently as they become ineffective after about six months.

Series 1. Toxicity of Ionic Silver to Es.coli, using the Katadyn Pocket Sterilizer.

Age of Es.coli culture: 24 hours.

Temperature of water: 25°C.

pH of water: 7.6

Plating medium: Standard beef extract agar.

Incubation temperature: 28°C.

Length of incubation period: 48 hours.

The content of the beakers was plated immediately following the treatment.

Table 1.

Number of beaker	Contents of beaker	Amount plated	Gammas of silver per liter	Colonies per plate	Killed
		cc.			pct.
	Trial 1				
1	tap water, untreated	1.0	0	63,000	--
2	tap water, treated 15 seconds	1.0	166	5,000	92
3	tap water, treated 30 seconds	1.0	332	2,500	96
	Trial 2				
1	tap water, untreated	0.001	0	126,000,000	--
2	tap water, treated 15 seconds	0.001	166	6,000,000	95

Series 2. Toxicity of Ionic Silver to Es. coli, Using the Laboratory Unit, Model TA-1.

Material tested: tap water inoculated with Es. coli.

Age of Es. coli culture: 24 hours.

Temperature of water: 33° C.

pH of water: 7.6 (determined at 22.5° C.)

Plating medium: Standard beef extract agar.

Incubating temperature: 28° C.

Length of incubation period: 48 hours.

The content of the beakers was plated immediately following the treatment.

Table 2.

Number of beaker	Contents of beaker	Amount plated	Gammas of silver per liter	Colonies per plate	Killed
		cc.			pct.
	Trial 1				
1	tap water, untreated	0.1	0	6,900	--
2	tap water, treated 15 seconds	0.1	41.7	1,200	82
3	tap water, treated 30 seconds	0.1	66.0	1,000	85
	Trial 2				
1	tap water, untreated	0.01	0	350,000	--
2	tap water, treated 15 seconds	0.01	133	160,000	54
3	tap water, treated 30 seconds	0.01	266	10,000	97
4	tap water, treated 15 minutes	0.01	200	0	100

Series 3. Effect of Holding Time on Efficiency of Katadyn Pocket Sterilizer.

Material tested: tap water inoculated with Es. coli.

Age of Es. coli culture: 24 hours.

Temperature of water: 22° C.

pH of water: 7.6

Plating medium: Standard beef extract agar.

Incubation temperature: 35° C.

Length of incubation: 48 hours.

Table 3

Number of beaker	Contents of beaker	Gammas of silver per liter	Time of holding before plating	Colonies per plate	Killed
			min.		pct.
1	tap water, untreated	0	0	160	--
2	tap water, treated 20 seconds	280	15	0	100
3	tap water, treated 20 seconds	280	30	0	100
4	tap water, treated 20 seconds	280	45	0	100
5	tap water, treated 20 seconds	280	60	0	100

Series 4. Efficiency of Ionic Silver Sterilization Using Distilled Water.

The conditions under which this experiment was conducted agreed with those used in Series 3, except for the hydrogen ion concentration of the distilled water, this being 6.2

Table 4.

Number of beaker	Contents of beaker	Amount plated	Gammas of silver per liter	Colonies per plate	Killed
		cc.			pct.
	Trial 1				
1	distilled water, untreated	1.0	0	970,000	--
2	distilled water, treated 15 seconds	1.0	166	290,000	70
3	distilled water, treated 30 seconds	1.0	332	150,000	84
	Trial 2				
1	distilled water, untreated	0.001	0	1,400,000	--
2	distilled water, treated 15 seconds	0.001	166	1,300,000	7.14
3	distilled water, treated 30 seconds	0.001	332	1,120,000	20

Series 5. Influence of Temperature upon the Effectiveness of Ionic Silver Treatment.

This series was carried out to determine whether the temperature of the bacterial suspension or other liquid under treatment really effected the efficiency of the treatment, or if the latter were independent of external conditions.

The content of the beakers was plated immediately following the treatment.

Material tested: tap water inoculated with Es. coli.

Age of Es. coli culture: 24 hours.

Temperature of water: A. 11° C.; B. 22.5° C., C. 35° C.

pH of water: 7.6 (determined at 22.5° C.)

Plating medium: Standard beef extract agar.

Incubation temperature: 35° C.

Length of incubation period: 48 hours.

Table 5

A.

Number of beaker	Contents of beaker	Amount plated	Gammas of silver per liter	Colonies per plate	Killed
		cc.			pct.
	Trial 1				
1	tap water, untreated	1.0	0	33	--
2	tap water, treated 15 seconds	1.0	20	23	30
3	tap water, treated 30 seconds	1.0	41	10	69
4	tap water, treated 60 seconds	1.0	83	10	69
5	tap water, treated 120 seconds	1.0	166	3	90
	Trial 2				
1	tap water, untreated	1.0	0	90	--
2	tap water, treated 15 seconds	1.0	32	33	63
3	tap water, trested 30 seconds	1.0	64	20	77
	Trial 3				
1	tap water, untreated	0.001	0	33,000	--
2	tap water, treated 15 seconds	0.001	29	30,000	9
3	tap water, treated 30 seconds	0.001	58	14,000	57

Table 6.

B.

Number of beaker	Contents of beaker	Amount plated	Gammas of silver per liter	Colonies per plate	Killed
		dc.			pct.
	Trial 1				
1	tap water, untreated	1.0	0	27	--
2	tap water, treated 15 seconds	1.0	29	13	51
3	tap water, treated 30 seconds	1.0	58	10	62
4	tap water, treated 60 seconds	1.0	116	3	88
5	tap water, treated 120 seconds	1.0	233	0	100
	Trial 2				
1	tap water, untreated	1.0	0	53	--
2	tap water, treated 15 seconds	1.0	29	--	--
3	tap water, treated 30 seconds	1.0	58	23	56
4	tap water, treated 60 seconds	1.0	116	16	69
5	tap water, treated 120 seconds	1.0	233	13	75
	Trial 3				
1	tap water, untreated	0.001	0	230,000	--
2	tap water, treated 15 seconds	0.001	29	160,000	30
3	tap water, treated 30 seconds	0.001	58	150,000	34
4	tap water, treated 60 seconds	0.001	116	130,000	43
5	tap water, treated 120 seconds	0.001	233	90,000	60

Table 7.

C.

Number of beaker	Contents of beaker	Amount plated	Gammas of silver per liter	Colonies per plate	Killed
		cc.			pct.
	Trial 1				
1	tap water, untreated	1.0	0	30	--
2	tap water, treated 15 seconds	1.0	29	16	46
3	tap water, treated 30 seconds	1.0	58	13	56
4	tap water, treated 60 seconds	1.0	116	6	80
5	tap water, treated 120 seconds	1.0	233	3	90
	Trial 2				
1	tap water, untreated	1.0	0	113	--
2	tap water, treated 15 seconds	1.0	29	33	70
3	tap water, treated 30 seconds	1.0	58	13	88
4	tap water, treated 60 seconds	1.0	116	3	97
5	tap water, treated 120 seconds	1.0	233	3	97
	Trial 3				
1	tap water, untreated	0.001	0	140,000	--
2	tap water, treated 15 seconds	0.001	29	80,000	42
3	tap water, treated 30 seconds	0.001	58	40,000	71
4	tap water, treated 60 seconds	0.001	116	30,000	78
5	tap water, treated 120 seconds	0.001	233	6,000	95

Series 6. Efforts to Obtain a Resistant Strain of Es. coli.

This study was conducted to determine if it were possible for Es. coli to develop a tolerance to ionic silver.

Material tested: tap water inoculated with Es. coli.

Age of Escherichia coli cultures: 24 hours.

Temperature of water: 22° C.

pH of water: 7.6

Plating medium: Standard beef extract agar.

Incubation temperature: 35° C.

Length of incubation period: 48 hours.

Table 8.

A.

Number of beaker	Contents of beaker	Amount plated	Gammas of silver per liter	Colonies per plate	Killed
		cc.			pct.
	Trial 1				
1	tap water, untreated	0.01	0	53,000	--
2	tap water, treated 60 seconds (held for 5 minutes before plating)	0.01	166	1,800	96
	Trial 2 (Using a 24 hours broth culture taken from plate 2, trial 1)				
1	tap water, untreated	0.01	0	6,000	--
2	tap water, treated 30 seconds (held for 2.5 minutes before plating)	0.01	83	50	99

In trial 3, using a survivor of trial 2, there was a 100 per cent fatality.

B. Part A was repeated, with the following results.

Table 9.

Number of beaker	Contents of beaker	Gammas of silver per liter	Colonies per plate	Killed pct.
	Trial 1			
1	tap water, untreated	91,000	91,200	--
2	tap water, treated 30 seconds (held 2.5 minutes before plating)	83	32,000	50
	Trial 2 (Using a survivor of Trial 1.)			
1	tap water, untreated	0	182,000	--
2	tap water, treated 30 seconds	83	84,000	53.8

In trial 3, using a survivor of Trial 2, there was a 100 per cent fatality.

Series 7. Influence of Foreign Material upon the Effectiveness of Ionic Silver Treatment.

This study was designed to determine whether the organic materials present in milk would tend to cut down the efficiency of ionic silver treatment to a greater extent than a similar study on water.

Materials tested: Milk and water, inoculated with Eb. typhosa.

Age of Eb. typhosa culture: 24 hours.

pH: milk 6.7, water 7.6.

Plating medium: Standard beef extract agar.

Incubation temperature: 35° C.

Length of incubation period: 48 hours.

The content of the beakers was plated immediately following the treatment.

Table 9

Number of beaker	Contents of beaker	Amount plated	Gammas of silver per liter	Colonies per plate	Killed
		cc.			pct.
	Trial 1 (a)				
1	milk, untreated	0.01	0	3,000	--
2	milk, treated 30 seconds	0.01	83	2,100	30
3	milk, treated 60 seconds	0.01	166	2,000	33
4	milk, treated 120 seconds	0.01	332	1,700	43
	Trial 1 (b)				
1A	tap water, untreated	0.01	0	9,500	--
2A	tap water, treated 30 seconds	0.01	83	2,750	71
3A	tap water, treated 60 seconds	0.01	166	1,250	86
4A	tap water, treated 120 seconds	0.01	332	250	97
	Trial 2 (a)				
1	milk, untreated	0.01	0	12,300	--
2	milk, treated 60 seconds	0.01	166	8,300	32
3	milk, treated 120 seconds	0.01	332	6,300	48
	Trial 2 (b)				
1	tap water, untreated	0.01	0	9,000	--
2	tap water, treated 30 seconds	0.01	83	0	100
3	tap water, treated 60 seconds	0.01	166	0	100
4	tap water, treated 120 seconds	0.01	332	0	100

Series 8. Influence of Oxygen upon the Effectiveness of Ionic Silver Treatment.

The results of this experiment were quite contradictory as shown in the following tables.

Material tested: (a) tap water inoculated with Eb. typhosa.
(b) tap water boiled to removed oxygen, inoculated with Eb. typhosa.

Age of Eb. typhosa culture: 24 hours.

Temperature of water: 25° C.

pH of water: 7.6

Plating medium: Standard beet extract agar.

Incubation temperature: 35° C.

Length of incubation period: 48 hours.

All treated liquids in this experiment were held for five minutes before plating.

Table 10.

Number of beaker	Contents of beaker	Amount plated	Gammas of silver per liter	Colonies per plate	Killed
		cc.			pct.
	Trial 1 (a)				
1	tap water, untreated	0.01	0	5,000	--
2	tap water, treated 30 seconds	0.01	83	200	96
3	tap water, treated 120 seconds	0.01	332	0	100
	Trial 1 (b)				
1A	tap water, untreated	0.01	0	14,750	--
2A	tap water, treated 30 seconds	0.01	83	0	100
3A	tap water, treated 60 seconds	0.01	166	0	100
4A	tap water, treated 120 seconds	0.01	332	0	100

Table 10 (continued)

Number of beaker	Contents of beaker	Amount plated	Gammas of silver per liter	Colonies per plate	Killed
		cc.			pct.
	Trial 2 (a)				
1	tap water, untreated	0.01	0	1,000	--
2	tap water, treated 30 seconds	0.01	83	200	80
3	tap water, treated 60 seconds	0.01	166	200	80
4	tap water, treated 120 seconds	0.01	332	0	100
	Trial 2 (b)				
1	tap water, untreated	0.01	0	17,000	--
2	tap water, treated 30 seconds	0.01	83	0	100
3	tap water, treated 60 seconds	0.01	166	0	100
4	tap water, treated 120 seconds	0.01	332	0	100
	Trial 3 (a)				
1	tap water, untreated	0.01	0	9,000	--
2	tap water, treated 30 seconds	0.01	83	0	100
3	tap water, treated 60 seconds	0.01	166	0	100
4	tap water, treated 120 seconds	0.01	332	0	100
	Trial 3 (b)				
1	tap water, untreated	0.01	0	13,000	--
2	tap water, treated 30 seconds	0.01	83	0	100
3	tap water, treated 60 seconds	0.01	166	0	100
4	tap water, treated 120 seconds	0.01	332	0	100
	Trial 4 (a)				
1	tap water, untreated	1.0	0	13,670	--
2	tap water, treated 30 seconds	1.0	83	435	96
3	tap water, treated 60 seconds	1.0	166	22	99.83
4	tap water, treated 120 seconds	1.0	332	16	99.88
	Trial 4 (b)				
1	tap water, untreated	1.0	0	17,220	--
2	tap water, treated 30 seconds	1.0	83	7,650	55
3	tap water, treated 60 seconds	1.0	166	860	95
4	tap water, treated 120 seconds	1.0	332	110	99.3

Series 9. Influence of Light and Darkness upon the Effectiveness of Ionic Silver Treatment.

Material tested: Tap water inoculated with Eb. typhosa.

Age of Eb. typhosa cultures: 24 hours

Temperature of water: 22° C.

pH of water: 7.6

Plating medium: Standard beef extract agar.

Incubation temperature: 35° C.

Length of incubation period: 48 hours.

The content of the beakers was held for five minutes before plating. Part (a) was conducted in daylight and part (b) in darkness.

Table 11.

Number of beaker	Contents of beaker	Amount plated	Gammas of silver per liter	Colonies per plate	Killed
		cc.			pct.
	Trial 1 (a)				
1	tap water, untreated	0.01	0	5,000	--
2	tap water, treated 30 seconds	0.01	83	200	96
3	tap water, treated 120 seconds	0.01	332	0	100
	Trial 1 (b)				
1	tap water, untreated	0.01	0	19,000	--
2	tap water, treated 30 seconds	0.01	83	10	99
3	tap water, treated 60 seconds	0.01	166	0	100
4	tap water, treated 120 seconds	0.01	332	0	100
	Trial 2 (a)				
1	tap water, untreated	0.01	0	1,000	--
2	tap water, treated 30 seconds	0.01	83	200	80
3	tap water, treated 60 seconds	0.01	166	200	80
4	tap water, treated 120 seconds	0.01	332	0	100
	Trial 2 (b)				
1	tap water, untreated	0.01	0	30,000	--
2	tap water, treated 30 seconds	0.01	83	0	100
3	tap water, treated 60 seconds	0.01	166	0	100
4	tap water, treated 120 seconds	0.01	332	0	100

Series 10. Toxicity of Ionic Silver to Bacillus megatherium.

Material tested: tap water inoculated with B. megatherium.

Age of B. megatherium: (a) 24 hours; (b) 48 hours.

Temperature of water: 21° C.

pH of water: 7.6 (determined at 22.5° C.)

Plating medium: Standard beef extract agar.

Incubation temperature: 35° C.

Length of incubation period: 48 hours.

The content of the beaker was poured immediately following the treatment.

Table 12.

Number of beaker	Contents of beaker	Amount plated	Gammas of silver per liter	Colonies per plate	Killed
		cc.			pct.
	(a)				
1	tap water, untreated	1.0	0	84	--
2	tap water, treated 15 seconds	1.0	83	25	70
3	tap water, treated 30 seconds	1.0	166	15	82
	(b)				
1	tap water, untreated	0.01	0	59,000	--
2	tap water, traated 15 seconds	0.01	50	30,000	49
3	tap water, treated 30 seconds	0.01	150	27,000	54

Series 11. Effect of Holding upon the Toxicity of Ionic Silver to Bacillus megatherium.

Beakers 2 and 3, part (a) of series 10 were allowed to stand for one hour before plating.

Table 13.

Number of beaker	Contents of beaker	Gammas of silver per liter	Colonies per plate	Killed
				pct.
1	tap water, untreated	0	84	--
2	tap water, treated 15 seconds	83	15	82
3	tap water, treated 30 seconds	166	6	92

Series 12. Toxicity of Ionic Silver to Bacillus Megatherium.

This experiment was set up under the same conditions as series 10, differing only in that a week old culture of B. megatherium was used, and which therefore contained many young spores.

Table 14.

Number of beaker	Contents of beaker	Amount plated	Gammas of silver per liter	Colonies per plate	Killed
		cc.			pct.
	Trial 1				
1	tap water, untreated		0	2,100	--
2	tap water, treated 15 seconds		20	1,700	19
3	tap water, treated 30 seconds		41	1,360	35
	Trial 2				
1	tap water, untreated	0.01	0	18,000	--
2	tap water, treated 15 seconds	0.01	20	16,600	7.7
3	tap water, treated 30 seconds	0.01	41	15,600	13

Series 13. Effect of Holding upon the Toxicity of Ionic Silver to Bacillus megatherium.

Beakers 2 and 3, trial 2, of series 12 were allowed to stand for one hour before plating.

Table 15

Number of beaker	Contents of beaker	Amount plated cc.	Gammas of silver per liter	Colonies per plate	Killed pct.
1	tap water, untreated, (beaker 1, trial 2, series 12)	0.01	0	18,000	--
2	tap water, treated 15 seconds	0.01	20	15,300	15
3	tap water, treated 30 seconds	0.01	41	14,300	20

Series 14. Effect of Temperature upon the Effectiveness of Ionic Silver Treatment to Bacillus megatherium.

Material tested: tap water inoculated with B. megatherium.

Age of culture: 24 hours.

Temperature of water: A. 11° C.; B. 22.5° C.; C. 35° C.

pH of water: 7.6 (determined at 22.5° C.)

Plating medium: Standard beef extract agar.

Incubation temperature: 35° C.

Length of incubation period: 48 hours.

The content of the beakers was plated immediately following the treatment.

Table 16

A.

Number of beaker	Contents of beaker	Amount plated	Gammas of silver per liter	Colonies per plate	Killed
		cc.			pct.
	Trial 1				
1	tap water, untreated	0.01	0	97,000	--
2	tap water, treated 15 seconds	0.01	29	14,000	86
3	tap water, treated 30 seconds	0.01	58	10,000	89
4	tap water, treated 60 seconds	0.01	116	9,000	90
5	tap water, treated 120 seconds	0.01	233	6,000	93
	Trial 2				
1	tap water, untreated	0.1	0	2,700	--
2	tap water, treated 15 seconds	0.1	29	800	70
3	tap water, treated 30 seconds	0.1	58	350	87
4	tap water, treated 60 seconds	0.1	116	150	94
5	tap water, treated 120 seconds	0.1	233	150	94

Table 17

B.

Number of beaker	Contents of beaker	Amount plated	Gammas of silver per liter	Colonies per plate	Killed
		cc.			pct.
1	tap water, untreated	0.01	0	14,600	--
2	tap water, treated 15 seconds	0.01	29	6,600	54
3	tap water, treated 30 seconds	0.01	58	4,000	72
4	tap water, treated 60 seconds	0.01	116	3,300	77
5	tap water, treated 120 seconds	0.01	233	3,300	77

Table 18

C.

Number of beaker	Contents of beaker	Amount plated	Gammas of silver per liter	Colonies per plate	Killed
		cc.			pct.
1	tap water, untreated	0.1	0	3,600	--
2	tap water, treated 15 seconds	0.1	29	425	88
3	tap water, treated 30 seconds	0.1	58	250	93
4	tap water, treated 60 seconds	0.1	116	250	93
5	tap water, treated 120 seconds	0.1	233	150	95

Series 15. Toxicity of Ionic Silver to Bacterial Spores.

Material tested: tap water inoculated with B. megatherium.

Age of B. megatherium: 4 weeks (spores well matured).

Temperature of water: A, 11° C.; B, 21° C.; C, 35° C.

pH of water: 7.6 (determined at 22.5° C.)

Plating medium: Standard beef extract agar.

Incubation temperature: 35° C.

Length of incubation period: 48 hours.

This study was conducted to determine how much protection the spore wall offered against ionic silver.

Table 19

A.

Number of beaker	Contents of beaker	Gammas of silver per liter	Colonies per plate	Killed
				pct.
1	tap water, untreated	0	19,300	--
2	tap water, treated 15 seconds	29	12,600	34
3	tap water, treated 30 seconds	58	12,000	38
4	tap water, treated 60 seconds	116	10,600	45
5	tap water, treated 120 seconds	233	9,300	51

Table 20

B.

Number of beaker	Contents of beaker	Gammas of silver per liter	Colonies per plate	Killed
				pct.
1	tap water, untreated	0	28,300	--
2	tap water, treated 15 seconds	29	8,300	70
3	tap water, treated 30 seconds	58	6,600	76
4	tap water, treated 60 seconds	116	6,300	77
5	tap water, treated 120 seconds	233	5,000	82
6	tap water, treated 120 seconds	233	4	99

The content of beakers 1-5 was plated five minutes following treatment, and the content of beaker 6 was held for one hour before plating.

Table 21

C.

Number of beaker	Contents of beaker	Amount plated	Gammas of silver per liter	Colonies per plate	Killed
		cc.			pct
1	tap water, untreated	0.01	0	2,600	--
2	tap water, treated 15 seconds	0.01	29	2,300	11
3	tap water, treated 30 seconds	0.01	58	1,300	50
4	tap water, treated 60 seconds	0.01	116	1,100	57
5	tap water, treated 120 seconds	0.01	233	660	74
6	tap water, treated 120 seconds	0.01	233	2	99

The content of beakers 1-5 was plated five minutes following treatment and the content of beaker 6 was held for one hour after plating.

This experiment was repeated three times in May 1937, with no reduction in the colony count. Just prior to the treatment, the electrodes were heated to "inactivate" them, then treated with dilute HCl to "activate" them again. The results suggest that this treatment may have damaged the silver electrodes such that the quantity of ionic silver going into solution was reduced.

Series 16. Toxicity of Ionic Silver to Capsule-Forming Organisms.

Material tested: tap water inoculated with Serratia Marcescens

Age of cultures: 24 hours.

Temperature of water: 22.5° C.

pH of water: 7.6

Plating medium: Standard beef extract agar.

Incubation temperature: 35° C. and room temperature.

Length of incubation period: 24 hours at 35° C and two days at room temperature.

This study was conducted to determine whether an organism which forms capsules would be more resistant to ionic silver than those which do not form capsules. (See plate III, page 63)

Table 22

Number of beaker	Contents of beaker	Amount plated	Gammas of silver per liter	Colonies per plate	Killed
		cc.			pct.
	Trial 1				
1	tap water, untreated	0.01	0	21,300	--
2	tap water, treated 30 seconds	0.01	83	0	100
3	tap water, treated 60 seconds	0.01	116	0	100
4	tap water, treated 120 seconds	0.01	332	0	100
	Trial 2				
1	tap water, untreated	0.01	0	25,000	--
2	tap water, treated 30 seconds	0.01	83	0	100
3	tap water, treated 60 seconds	0.01	116	0	100
4	tap water, treated 120 seconds	0.01	332	0	100
	Trial 3				
1	tap water, untreated		0	1,780	--
2	tap water, treated 20 seconds		55	0	100
3	tap water, treated 40 seconds		110	0	100
4	tap water, treated 60 seconds		166	0	100

Table 22 (continued)

Number of beaker	Contents of beaker	Gammas of silver per liter	Colonies per plate	Killed pct.
	Trial 4			
1	tap water, untreated	0	300	--
2	tap water, treated 20 seconds	55	0	100
3	tap water, treated 40 seconds	110	0	100
4	tap water, treated 60 seconds	166	0	100
	Trial 5			
1	tap water, untreated	0	100	--
2	tap water, treated 20 seconds	55	0	100
3	tap water, treated 40 seconds	110	0	100
4	tap water, treated 60 seconds	166	0	100

The treated solutions in trials 1 and 2 were held for five minutes before plating; in trials 3,4 and 5 they were held for two minutes before plating.

Series 17. Toxicity of Ionic Silver to Acid Fast Organisms.

Material tested: tap water inoculated with Myco. butyricum.

Age of Myco. butyricum culture: 1 week.

Temperature of water: 25° C.

pH of water: 7.6

Plating medium: Beef extract agar with 5 per cent glyderine.

Incubation temperature: Room temperature.

Length of incubation period: 1 week.

The content of the treated beakers was held for five minutes before plating.

This study was carried out to determine how much protection the waxy cell wall of Myco. butyricum offered against ionic silver.

Table 23

Number of beaker	Contents pf beaker	Gammas of silver per liter	Colonies per plate	Killed pct.
	Trial 1			
1	tap water, untreated	0	7,400	--
2	tap water, treated 30 seconds	83	340	95
3	tap water, treated 120 seconds	332	170	97
	Trial 2			
1	tap water, untreated	0	6,075	--
2	tap water, treated 30 seconds	83	570	91
3	tap water, treated 60 seconds	166	270	95
4	tap water, treated 120 seconds	332	13	99
	Trial 3			
1	tap water, untreated	0	9,990	--
2	tap water, treated 30 seconds	83	6,150	38
3	tap water, treated 60 seconds	166	630	93
4	tap water, treated 120 seconds	332	313	96

Three additional trials showed the following death rates for beakers 2, 3 and 4: (2) 30 seconds treatment = 90%, 86%, 90%.
(3) 60 seconds treatment = 99.5%, 99.3%, 99.02%.
(4) 120 seconds treatment = 99.7%, 99.8%, 99.8%.

Series 18. Toxicity of Ionic Silver to Saccharomyces cerevisease.

Material tested: tap water inoculated with yeast cells.

Source of Saccharomyces cerevisease: Fleischmann's yeast cake.

Temperature of water: 25° C.

pH of water: 7.6

Plating medium: Whey agar plus 1 cc of 1 per cent tartaric acid per plate.

Incubation temperature: Room temperature.

Length of incubation period: 48 hours.

The treated solutions in trial 1 and 2 were held for five minutes before plating.

Table 24

Number of beaker	Contents of beaker	Gammas of silver per liter	Colonies per plate	Killed
				pct.
	Trial 1			
1	tap water, untreated	0	500	--
2	tap water, treated 30 seconds	83	40	92
3	tap water, treated 60 seconds	166	17	96
	Trial 2			
1	tap water, untreated	0	1,365	--
2	tap water, treated 30 seconds	83	13	99.04
3	tap water, treated 60 seconds	166	10	99.2
	Trial 3			
1	tap water, untreated	0	2,090	--
2	tap water, treated 30 seconds	83	550	73
2A	tap water, treated 30 seconds	83	40	98
3	tap water, treated 60 seconds	166	10	99.5
3A	tap water, treated 60 seconds	166	6	99.7

The content of beakers 2 and 3 was plated immediately following treatment, 2A and 3A being held five minutes following treatment. One week after plating the plates were re-counted and there was no increase in the number of colonies (see plate 1, page 62).

Series 19. Toxicity of Ionic Silver to Mold Spores.

Material tested: tap water inoculated with mold spores,

A. Mucor sp. B. Penicillium sp.

Plating medium: Beef extract agar with 1 per cent dextrose.

Incubation temperature: Room temperature.

Length of incubation period: 72 hours.

Table 25

A.

Number of beaker	Contents of beaker	Holding time	Gammas of silver per liter	Colonies per plate	Killed
		min.			pct.
1	tap water, untreated		0	50	--
2	tap water, treated 10 minutes	30	666	23	54
3	tap water, treated 10 minutes	60	666	8	84
4	tap water, treated 10 minutes	120	666	3	94

Table 26

B.

Number of beaker	Contents of beaker	Holding time	Gammas of silver per liter	Colonies per plate	Killed
		min.			pct.
	Trial 1				
1	tap water, untreated		0	29	--
2	tap water, treated 2 minutes	0	66	16	44
3	tap water, treated 2 minutes	15	66	10	65
4	tap water, treated 2 minutes	30	66	10	65
5	tap water, treated 2 minutes	45	66	5	82
6	tap water, treated 2 minutes	60	66	5	82
7	tap water, treated 2 minutes	120	66	3	89
	Trial 2				
1	tap water, untreated		0	230	--
2	tap water, treated 5 minutes	30	33	190	17
3	tap water, treated 5 minutes	60	33	170	26
4	tap water, treated 5 minutes	120	33	13	94

Table 26 (continued)

Number of beaker	Contents of beaker	Holding time min.	Gammas of silver per liter	Colonies per plate	Killed pct.
	Trial 3				
1	tap water, untreated		0	63	--
2	tap water, treated 10 minutes	30	66	47	25
3	tap water, treated 10 minutes	60	66	10	84
4	tap water, treated 10 minutes	120	66	3	95

Series 20. Toxicity of Ionic Silver to Elodea candensis.

Three beakers, each containing 800 cc., of tap water, pH = 7.6, room temperature, were subjected to ionic silver treatment. Elodea candensis was placed in each beaker and observed. A few hours after treatment, a leaf was mounted on a microscopic slide and examined. All movement of the cytoplasm had ceased, but there was no other visible change in the cell.

Table 27

Number of beaker	Contents of beaker	Gammas of silver per liter	Observation 3/2/36	3/3/36	3/5/36
1	tap water, untreated	0	unchanged	unchanged	unchanged
2	tap water, treated	291	dead	dead	dead
3	tap water, treated	875	dead	dead	dead

Series 21. Effect of Ionic Silver on the Keeping Quality of Cut Flowers.

This experiment was conducted at the Freshman Women's Dormitory, using the flowers from the dining room tables. There were ten bouquets

of mixed flowers in soft glass vases. These were divided into two groups, one containing treated tap water, and the other containing untreated tap water and serving as controls. The water was changed daily and the vases were kept in the refrigerator overnight. When the table flowers were changed, the old flowers were collected into two large bouquets and kept at room temperature for observation.

A. The treated water contained 1,666 gammas of silver per liter. The flowers were kept under observation for two weeks and no difference in the appearance of the two groups was observed. When transferred to the large containers and held at room temperature, there was still no difference in their keeping qualities, although the treated water was not as murky in appearance and did not have as disagreeable an odor as did the untreated water, due to the inhibition of bacteria and other micro-organisms present in the water.

This study was repeated, using water which contained 5,555 gammas of silver per liter, with the same results.

Series 22: Preservation of Cut Flowers.

The study was set up on October 28, 1935. Each beaker contained geranium flowers of approximately the same age and size. The beakers contained 250 cc., of tap water and were held at room temperature. Observations were made every day to determine the effectiveness of ionic silver on the keeping quality of cut flowers. The first six observations showed little change beyond the usual dropping of petals which accompanies the aging of cut flowers. The final observation made on November 8, 1935

indicated that the flowers in the beaker containing untreated distilled water and those in the beaker containing treated water (500-1,333 gammas of silver per liter) did not dry up as rapidly as those flowers in the remaining beakers. However, the difference in the appearance of the two groups was hardly marked enough to draw any definite conclusion as to the effect of ionic silver on the preservation of cut flowers.

Series 23. Ionic Silver as an Algicide.

Material tested: Spirogyra sp.

Containers: Soft glass Economy jars.

Table 28

Number of jar	Content of jar	Gammas of silver per liter
1	tap water, untreated	0
2	tap water, treated 5 minutes	666
3	tap water, treated 10 minutes	1,333
4	tap water, treated 2 minutes	266
5	tap water, Spirogyra, treated 5 minutes	666
6	tap water, Spirogyra, treated 10 minutes	1,333
7	tap water, Spirogyra, treated 2 minutes	266

The water in jars 2, 3 and 4 was treated, then the alga was added, whereas in jars 5, 6 and 7 the alga was put in the jars first and the treatment applied later.

The experiment was set up on June 28, 1936 at one o'clock. By five o'clock the same day the alga showed signs of dying, especially samples 2, 3, 5 and 6, with 4 and 7 less so.

June 29, 1936: The Spirogyra in jars 2, 3, 4, 5, 6 and 7 was dead - the water being discolored and having a disagreeable odor. The Spirogyra in jar 1 was unchanged.

June 30, 1936: Same as on June 29. The Spirogyra was removed from the jars, the water was strained free of visible dirt and fresh Spirogyra added.

July 9, 1936: The Spirogyra in jars 2, 3, 4, 5, 6 and 7 was dead and the water possessed a disagreeable odor. The Spirogyra in jar 1 was unchanged.

Series 24. Ionic Silver as a Food Preservative.

Materials tested: A. eggs; B. apple juice; C. milk.

A. (1) Date of preserving: July 9, 1936.

Date of examining: January 1, 1937.

Containers: Soft glass Economy jars.

Holding temperature: House cellar.

(On July 17, and 28 and again on August 12, jar number 3 was treated for periods of 5 minutes each, adding 1,666 gammas of silver each time.)

(2) Date of preserving: November 1, 1936.

Date of Examining: March 3, 1937.

Containers: Pyrex beakers (1,000 cc.).

Holding temperature: 5° C.

Plating medium: Standard beef extract agar.

Incubation temperature: Room temperature.

Length of Incubation period: 48 hours.

Each container held six fresh eggs.

Table 29

Number of beaker	Contents of beaker	Gammas of silver per liter	Colonies per plate	Remarks
		Trial 1		
1	water glass	0	--	well preserved, used for cooking purposes
2	tap water, treated	1,666	--	spoiled
3	tap water, treated	333	--	spoiled
		Trial 2		
1	water glass (1-25)	0	--	there was neither bacterial nor mold growth
2	tap water, treated	1,000	--	"
3	tap water, treated	2,000	--	"

B. Date of preserving: September 11, 1936.

Date of examining: February 23, 1937

Containers: Soft glass, screw top, Mason jars.

Holding temperature: house cellar.

Table 30

Number of beaker	Contents of beaker	Gammas of silver per liter	Colonies per plate	Remarks
1	apple juice, boiled	0	2,730	no mold appeared on plates.
2	apple juice, treated	1,330	0	mold on plates. taste: sour.

C. Material tested: Milk.

Temperature: 35° C., 20° C., 10° C.

pH: 6.7

Plating medium: Standard beef extract agar.

Incubation temperature: 35° C., 20°C., 10° C.

Length of incubation period: 48 hours.

Eighteen samples of raw milk and six samples of pasteurized milk, from the same lot, were obtained from the College Dairy and marked in the following manner:

This study was carried out to determine how favorably ionic silver compared with pasteurization in improving the keeping quality of milk.

Table 31

Number of beaker	Contents of beaker	Holding temperature	Gammas of silver per 1,000 cc.
1	raw milk	37° C.	0
1A	raw milk	37° C.	0
2	raw milk	20° C.	0
2A	raw milk	20° C.	0
3	raw milk	10° C.	0
3A	raw milk	10° C.	0
4	pasteurized milk	37° C.	0
4A	pasteurized milk	37° C.	0
5	pasteurized milk	20° C.	0
5A	pasteurized milk	20° C.	0
6	pasteurized milk	10° C.	0
6A	pasteurized milk	10° C.	0
7	raw milk	37° C.	618
7A	raw milk	37° C.	660
8	raw milk	20° C.	618
8A	raw milk	20° C.	640
9	raw milk	10° C.	640

Table 31 (continued)

Number of beaker	Contents of beaker	Holding temperature	Gammas of silver per 1,000 cc.
9A	raw milk	10° C.	640
10	raw milk	37° C.	1050
10A	raw milk	37° C.	830
11	raw milk	20° C.	860
11A	raw milk	20° C.	800
12	raw milk	10° C.	760
12A	raw milk	10° C.	760

Table 32

Number of beaker	Observation						
	4-4-36	4-6-36	4-7-36	4-8-36	4-9-36	4-10-36 colonies per plate	4-13-36
1	unchanged	sour, white mold on surface, disagreeable flavor	sour cheesy odor and flavor	unchanged from 4-7-36	unchanged from 4-7-36		
1A	unchanged	sour, white mold on surface, disagreeable flavor	extrusion of whey yellow in color	unchanged from 4-7-36	unchanged from 4-7-36		
2	unchanged	sour, white mold on surface, disagreeable flavor	unchanged from 4-6-36	unchanged from 4-7-36	unchanged from 4-7-36	too many to count	
2A	unchanged	sour, white mold on surface, disagreeable flavor	unchanged from 4-6-36	unchanged from 4-7-36	unchanged from 4-7-36	too many to count	
3	unchanged	unchanged	unchanged	unchanged	unchanged	29,700	disagreeable flavor
3A	unchanged	unchanged	unchanged	unchanged	unchanged	32,700	disagreeable flavor
4	unchanged	sour	sour, mold on surface	unchanged from 4-7-36	unchanged from 4-7-36		
4A	unchanged	sour	sour, cheesy odor	unchanged from 4-7-36	unchanged from 4-7-36		
5	unchanged	solidified, sour	extrusion of whey	unchanged from 4-7-36	unchanged from 4-7-36		
5A	unchanged	solidified	extrusion of whey	unchanged from 4-7-36	unchanged from 4-7-36		
6	unchanged	unchanged	unchanged	unchanged	strong odor	2,000	unchanged from 4-9-36

Table 32 (continued)

Number of beaker	Observation						
	4-4-36	4-6-36	4-7-36	4-8-36	4-9-36	4-10-36 colonies per plate	4-13-36
6A	unchanged	unchanged	unchanged	unchanged	strong odor	30,000	unchanged from 4-9-36
7	strong o odor	sour, extrusion of whey, cheesy odor	unchanged from 4-6-36	unchanged from 4-6-36	unchanged from 4-6-37		
7A	strong odor	sour, extrusion of whey, cheesy odor	unchanged from 4-6-36	unchanged from 4-6-36	unchanged from 4-6-36		
8	unchanged	sour, green mold on surface, disagreeable flavor	extrusion of whey	unchanged from 4-7-36	unchanged from 4-7-36		
8A	unchanged	sour, green mold on surface, disagreeable flavor	extrusion of whey	unchanged from 4-7-36	unchanged from 4-7-36		
9	unchanged	unchanged	unchanged	unchanged	unchanged	14,000	disagreeable flavor
9A	unchanged	unchanged	unchanged	unchanged	unchanged	16,000	disaggreeable flavor
10	strong odor	sour, extrusion of whey	unchanged from 4-6-36	unchanged from 4-6-36	unchanged from 4-6-36		
10A	strong odor	sour, extrusion of whey	unchanged from 4-6-36	unchanged from 4-6-36	unchanged from 4-6-36		
11	unchanged	sour, mold on surface, solidified	extrusion of whey	unchanged from 4-7-36	unchanged from 4-7-36	too many to count	
11A	unchanged	sour, mold on surface solidified	extrusion of whey	unchanged from 4-7-36	unchanged from 4-7-36	too many to count	
12	unchanged	unchanged	unchanged	unchanged	unchanged	10,500	disagreeable flavor, sour
12A	unchanged	unchanged	unchanged	unchanged	unchanged	40,000	disagreeable flavor, sour

Series 25. Reduction of the Virulence of Pathogenic Bacteria by Ionic Silver.

Test organism: Phytomonas tumefaciens.

Age of Phytomonas tumefaciens culture: 24 hours.

Two strains of the test organism Phytomonas tumefaciens were studied. These were designated as A and A6. The organisms were subjected to ionic silver treatment, plated on standard beef extract agar and from the colony surviving the longest period of treatment, a broth culture was prepared. From this and a control of untreated Phytomonas tumefaciens, tomato plants were inoculated and the subsequent gall formation was observed. Five series of tomato plants were inoculated in this manner and of the series one showed a slightly retarded gall formation in the case of the treated Phytomonas tumefaciens A6, a second series showed complete inhibition of gall formation and the remaining three showed no inhibitory action in gall formation by ionic silver (see plate 2, page 62).

Series 26. Influence of Ionic Silver on the Staining Reactions of Es. coli, B. megatherium, Alc. viscosus, and Saccharomyces cereviseae.

Twenty-four cultures of Es. coli, Alc. viscosus, and B. megatherium were stained by the Gram method before and immediately after being treated with ionic silver. The Gram reaction was in no way changed, nor was the appearance of the cell altered. Saccharomyces cereviseae was stained with methylene blue and no change in the staining properties of the cell could be detected.

Series 27. Effect of Ionic Silver upon the Biochemical Properties of Es. coli.

A. Tap water inoculated with a twenty-four hour culture of Es. coli was subjected to ionic silver treatment, (the bacterial suspension containing 250 gammas of silver per liter), was plated on standard beef extract agar, and the following tests were made on the survivors.

Table 33

Test		Reactions of control	Reactions of treated organisms
Indol		+	+
Nitrate reduction		+	+
H_2S production		-	-
Starch hydrolysis		-	-
Acetyl methyl carbinal prod.		-	-
Sugar fermentation:	sucrose	acid only	acid only
	lactrose	acid and gas	acid and gas
	dextrose	acid and gas	acid and gas

B. Part A was repeated, with one modification. Instead of plating the treated organisms, they were inoculated directly into the special media. The results were the same as in Part A.

Series 28. Length of Time that Treated Water Will Retain its Germicidal Power.

Test organism: Es. coli.

Age of Es. coli culture: 48 hours.

Plating medium: Standard beef extract agar.

Incubation temperature: 35° C.

Length of incubation period: 48 hours.

A liter of tap water was treatdd with ionic silver and stored in a pyrex flask at room temperature. The treated water contained 333 gammas of silver per liter. At weekly intervals, the water was inoculated with Es. coli, plates were poured and from the colony counts, the length of time that the water retained its germicidal power was determined.

Table 34

Number of beaker	Contents of beaker	Colonies per plate	Killed
			pct.
1	tap water, untreated	103,000	
2	tap water, treated, held 60 minutes before plating	100	99.9
	Second Week		
1	tap water, plated immediately	62,750	
2	tap water, held 60 minutes before plating	500	99.2
	Third Week		
1	tap water, plated immediately	900	
2	tap water, held 60 minutes before plating	250	70.2
	Fourth Week		
1	tap water, plated immediately	total count	
2	tap water, held 60 minutes before plating	was not cut down	

The experiment was repeated twice more with the same results.

General Discussion

It is evident that there is a real value to ionic silver as a sterilizing agent, but it is also evident that a much better understanding of certain of its properties is necessary before it can be substituted for those methods now in use.

In the hands of a trained technician and under laboratory conditions, the results obtained with the process are gratifying; but to insure results that are equally as successful for the layman will require more efficient control measures than are now available.

The quantity of ionic silver in solution at a given time is of great importance as may be noted in the study using Bacillus megatherium spores. Table 19, 20 and 21 (pages 32 and 33) show that the spores can be killed by the treatment. But later, after the electrodes had been subjected to a preliminary heating and acid treatment, the generation of the ionic silver with the same apparatus failed to reduce the number of spores. This suggests that the silver content of the bacterial suspension was cut down. In the absence of an easy and simple method for determining the amount of ionic silver actually present in a solution, serious consequences might arise. That this point is an important one is further evidenced in the studies devoted to the influence exerted by foreign material present in the liquids under treatment. Here then, a question arises. Under the same conditions of operation, will the results be uniform in waters from different localities and hence of different composition? It would appear necessary to make allowances for turbidity, mineral content, etc.

A comparison of the death rate of Eberthella typhosa in milk and in water (table 9, page 24) will also serve to illustrate the point in question. The conditions under which the two parts of the experiment were conducted were identical, yet the results were very different. There were two factors to which this difference could have been due, neither being apparent to one applying the treatment on the basis of milliampere reading and application period. These factors are the hydrogen-ion concentration and the milk components (protein, butterfat, sugars and salts).

When tap water containing calcium bicarbonate is treated with ionic silver for a rather long period of time, a cloudiness and grey sediment appears. On the other hand, when veal infusion broth is treated, almost immediately a cloudiness appears, which is at first white, becoming darkened later. Obviously to say that the two liquids contain the same amount of active silver, as calculated from the milliampere reading would be erroneous. Yet that is what may be done in actual practice.

Katadyn Pocket Sterilizer

The Katadyn pocket sterilizer, in a compact, fitted case, is especially convenient for campers using water from an unknown source. By placing a knife blade across the two electrodes it is possible to determine when the cell is drawing current, but there is no way of determining how much silver is going into solution. Keeping this fact in mind, it would seem advisable to over-treat the water rather than to run the risk of the water not being sufficiently sterilized.

This raises the question of the accumulative nature of ionic silver in the human body. Suckling (33) and Gibbard (12) disagree on this point and the supporting evidence is such as to justify further study.

Influence of Temperature

A study of tables 5, 6, 7, 16, 17, 18, 19, 20 and 21, (pages 19, 20, 21, 31, 32 and 33) indicates that the temperature of the liquid during treatment is a factor to be considered. The increase in efficiency at 22.5° C., over 10° C., is marked, whereas the increase at 35° C., over 22.5° C., is not as great.

Ionic Silver as a Food Preservative

Ionic silver does not promise as much success as a food preserving agent as it does as a water-purifying one. The study conducted with milk (table 32, pages 46 and 47) indicates that while the bacterial count was cut down to about the same level as in pasteurization, the mold growth was not inhibited, especially in those samples held at room temperature. At the lower temperature of 10° C., the keeping quality, odor and flavor of the treated milk compare very favorably with pasteurized milk. Demeter (7) reports that a preliminary heating to 63° C., will greatly enhance the effectiveness of ionic silver in milk.

As an egg preservative, ionic silver gave greater promise and further studies should be conducted. Table 29, page 43 gave the experimental results.

Apple juice treated with ionic silver retained its original color and the bacterial count was lower than in the sample sterilized by boiling. However, the mold growth was not inhibited.

The results obtained, using ionic silver as a food preservative justify further experimentation and under better controlled conditions, it can perhaps be used to good advantage in this field.

Bacterial Number in Suspension vs. Ionic Silver

A survey of the colony counts indicate that a definite ratio between the number of bacteria per cc., and the efficiency of the treatment does not exist. Table 2, 3, 4, 5, 6, 7, 12, 13 and 16, pages 16, 17, 18, 19, 20, 21, 28, 29, and 31, indicate that the number of bacteria in the initial inoculation and the silver content of the liquid do not offer an index to the death rate.

The success of ionic silver in the laboratory suggests that this form of sterilization may be profitably applied in industry. However, a recent publication by Mallman (20) reports ionic silver to be slower in germicidal action than chlorine when applied as a disinfectant for swimming pools under the conditions reported in his study.

Aeration

From repeated usage of sterilized tap water and tap water which was not sterilized aeration did not seem to be of great significance. In some of the earlier work, tap water which had been sterilized in the autoclave and cooled rapidly was used and later, when the same study was

repeated tap water (not sterilized in autoclave) was used with the same results.

Destructive Mechanism

The work of this paper favors the view that ionic silver brings about the death of the cell through selective adsorption. The fact that the bacterial cell is almost instantaneously killed suggests that death must be due to a process more rapid than a chemical combination of the ion with the proteins of the bacteria, particularly in the case of those organisms possessing a capsule or waxy cell wall, which would delay penetration. Likewise, the time factor would seem to be against the view that the ion causes some change in the medium containing the bacteria, this then affecting the cell.

The theories of radio-activity, of selective adsorption, and that the ions bear electrical charges to which the effect is due seem to overlap somewhat. That the action is, in part, electrical in nature is evidenced in Neisser and Eichbaum's (24) paper in which they attribute the oligodynamic action of the electro-positively charged metal ion; and from the statement by Rideal and McCombie (29) that most bacteria in water containing a trace of salt are electro-negatively charged. Peters (25 and 28) according to Clark (4), characterizes the cell as having a three dimensional mosaic extending throughout the cell, consisting of a net work of proteins. The surface proteins are connected to the directive proteins of the nucleus by threads of protaplasm. If such an organization does actually exist, the mosaic has been compared to the nervous system of

higher animals. This view makes it possible to understand how a stimulus applied to the surface of the cell could be transmitted to all parts of the cell. In further keeping with conception of cell organization, Rideal and McCombie (29) characterize the bacterial cell as a chromatin net work in an emulsoid protoplasm, the endoplasm contained in a semi-permeable membrane, the ectoplasm differing from the endoplasm only in its lower water content. If this view is accepted, the likelihood of selective adsorption as the destructive mechanism becomes more apparent. Further Rideal and McCombie (29) picture the surface membrane as possessing definite structure with the cell molecules definitely oriented. Present are both acid and basic groups ($-NH_2$ and $-COOH$) commonly referred to as acceptors which react with any molecule present which likewise possesses active groups. Peters (25 and 28) expands the idea when he pictures the surface proteins as the acceptors, the cytoplasmic proteins as the conductors and the accepting groups as the active groups of the proteins themselves, the prosthetic groups of enzymes associated with the proteins or of groups belonging to molecules in very intimate contact with the proteins.

The study of cell organization is an extremely interesting and important one as related to sterilization and disinfection in general, and should be investigated very thoroughly if we are to gain an insight to the destructive mechanism of cell destroying agents.

Summary

1. Ionic silver exerts a distinctly toxic effect on living cells of bacteria, yeasts, molds and higher plants.

2. Spore walls, waxy walls and capsules offer little protection to the protoplasm against ionic silver.

3. The spores of Mucor sp. and Penicillium sp. displayed a greater resistance to ionic silver than did any of the other microorganisms tested.

4. Ionic silver did not improve the keeping quality of cut flowers, under the conditions tested.

5. No visible change, other than a cessation of cytoplasmic movement, could be detected in the cell of Elodea candensis immediately following treatment with ionic silver.

6. Two trials out of five were successful in reducing the virulence of Phytomonas tumefaciens.

7. Those biochemical properties of Es. coli which were investigated remained unaltered after treatment of the organism with ionic silver.

8. The development of a strain of Es. coli, resistant to ionic silver, was not accomplished under the conditions employed.

9. The examination of numerous plates caused the writer to conclude that the action of ionic silver is germicidal rather than bacteriostatic.

10. The presence or absence of oxygen as tested does not alter the effectiveness of the ionic silver treatment.

11. Ionic silver is slightly more active in darkness than in light.

12. The temperature of the water or other liquid during treatment affects the degree of sterilization.

13. A simple analytical method of determining the silver content of any given solution would be a distinct advance in the studies using ionic silver as a sterilizing agent.

14. Such a method would perhaps offer a clue as to why the first results obtained from studies with Bacillus megatherium spores were not successfully repeated.

15. The use of ionic silver to sterilize swimming pools and supplies of drinking water would be promoted by such a control method.

16. As a food-preserving agent, ionic silver does not prove to be entirely satisfactory, although the results justify further experimentation.

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Description of Plates

Plate I.

Cultures of Saccharomyces cerevisceae 72 hours old, showing the effect of ionic silver treatment upon the cells. (see table 24, trial 3, page 37).

Figure 1. Agar plate from an untreated sample of tap water inoculated with yeast cells.

Figure 2. Agar plate from the same sample, which had been subjected to treatment for 30 seconds and plated immediately.

Figure 2A. From the same sample as Figure 2, but held for 5 minutes before plating.

Figure 3. Agar plate from the same sample as Figure 1 which had been subjected to treatment for 60 seconds and plated immediately.

Figure 3A. From the same sample as Figure 3, but held for 5 minutes before plating.

Plate II.

Stem of tomato plant showing reduction of virulence in Phytomonas tumefaciens A-6 as a result of ionic silver treatment.

Figure 1. Lower scar indicates site of inoculation of the control (untreated Phytomonas tumefaciens A-6).

Upper scar indicates site of inoculation of the treated Phytomonas tumefaciens A-6 showing complete absence of gall formation.

Figure 2. Lower scar indicates site of inoculation of the control (untreated Phytomonas tumefaciens A-6).

Upper scar indicates site of inoculation of the treated Phytomonas tumefaciens A-6, showing an inhibition in the gall formation.

Plate III.

These plates were incubated for 24 hours at 35° C., and 48 hours at room temperature.

Figure 1. Tap water, inoculated with Serratia Marcescens, untreated.

Figure 2. Tap water, inoculated with Serratia marcescens, treated for 20 seconds and held for 2 minutes before plating.

Figure 3. Tap water, inoculated with Serratia marcescens, treated for 40 seconds and held for two minutes before plating.

Figure 4. Tap water, inoculated with Serratia marcescens, treated for 60 seconds and held for two minutes before plating.

Plate I

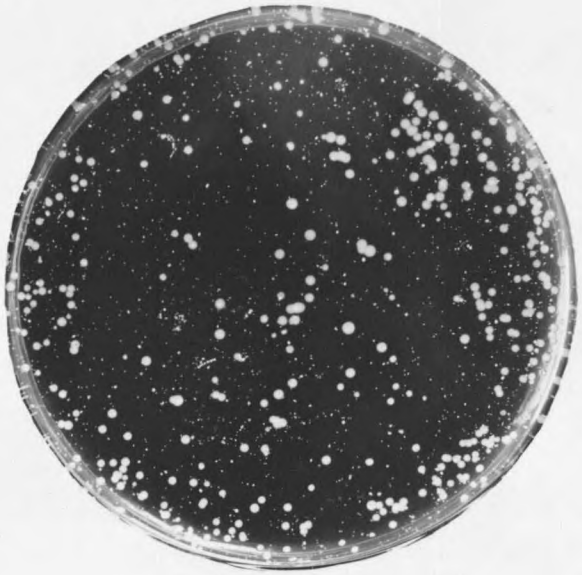

Fig. 1

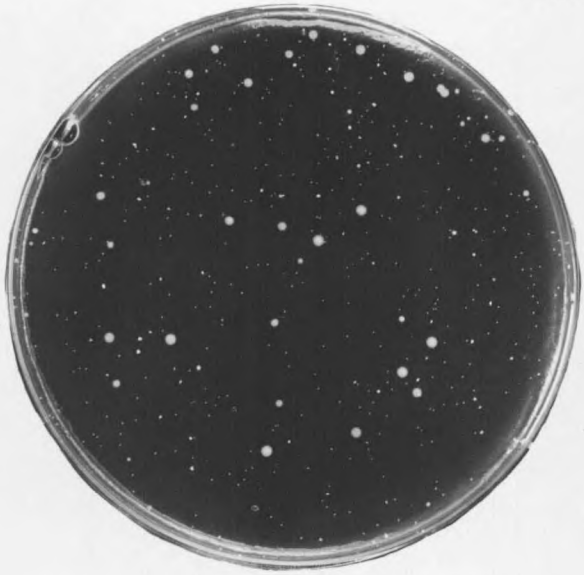

Fig. 2

Fig. 2A

Fig. 3

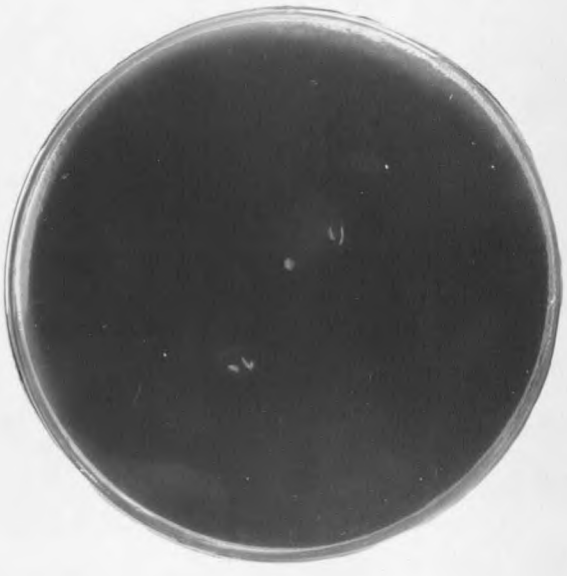

Fig. 3A

Plate II

Fig. 1

Fig. 2

Plate III

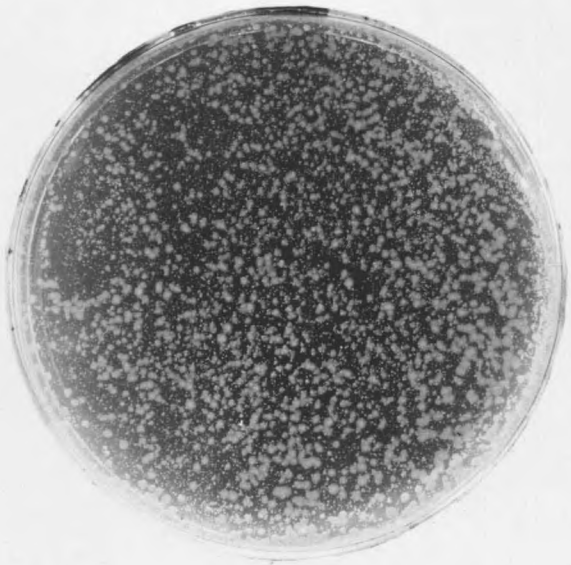

Fig. 1

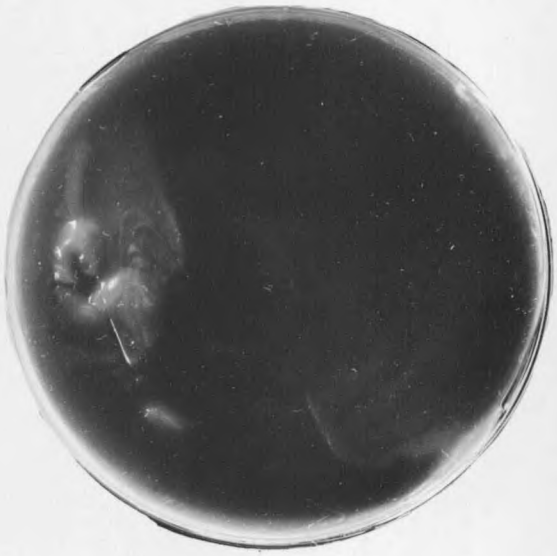

Fig. 2

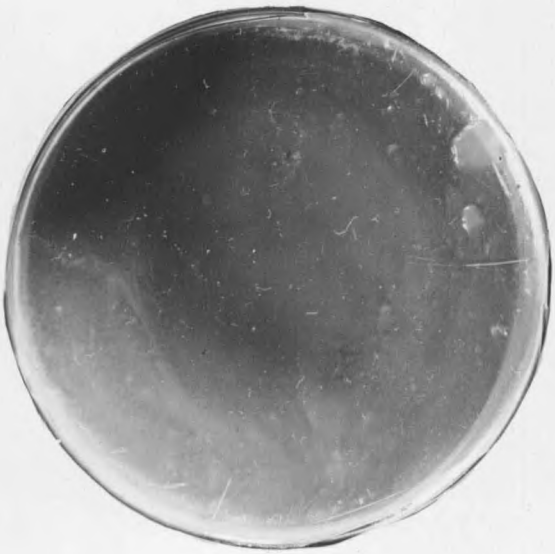

Fig. 3

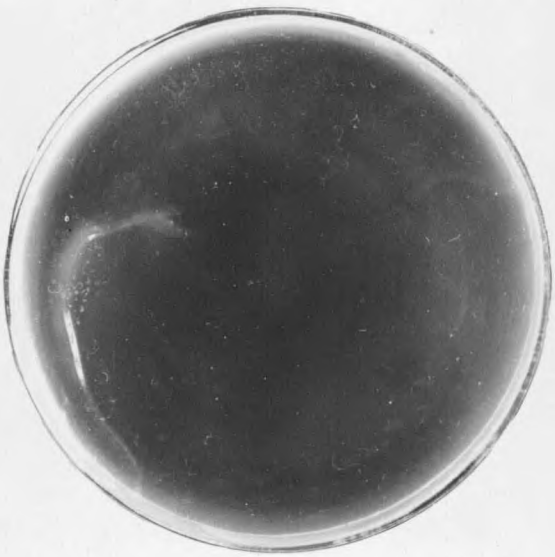

Fig. 4

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