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 Examining Committee

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Chairman Graduate Committee

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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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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