BIOCIDAL EFFECTS OF SILVER

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INTRODUCTION

The present study was intended to determine if silver ions can kill or inactivate microbial and viral agents in very pure water. The results are to be applied in the design of future spacecraft water systems. Salts of silver were employed in many of these experiments, but silver ions generated by an electrolytic apparatus were used when possible. Silver was assayed by neutron activation analysis. Bacteria employed as experimental models were selected on the basis of tests of previous spacecraft water systems. Viruses were chosen simply to represent a broad variety of agents. The period of laboratory performance was 1 January 1969 through 31 January 1970.

PHYSICAL STUDIES

Assay system. The basic goal of the physical studies was to adapt and apply the neutron activation analysis (NAA) technique to the measurement of silver in samples from the biological experiments. This technique offers the theoretical advantages of high sensitivity and reproducibility, together with complete independence of the chemical state of the silver at the time that the assay is performed.

The NAA procedure for silver assay is carried out in the University of Wisconsin Nuclear Reactor Laboratory. Silver solutions to be analyzed are encapsulated in 5 ml quantities in heat-sealed polyethylene vials selected for use in the pneumatic sample insertion system of the reactor. Irradiation takes place in a flux of approximately 5×10^{12} neutrons cm^{-2} sec^{-1}. The activated sample is counted with a solid-state gamma-ray spectroscopy system, and the resultant gamma-ray spectrum is read out on punched paper tape for subsequent hand or computer analysis.

The first nuclide selected for study was ^{108}Ag ($t_{1/2}$ = 2.4 min). Gamma ray peaks typical of the nuclide were selected, and a peak intensity evaluation was carried out by identifying the peak channel and the wings of the peak. A background level was then established for the spectral region in which the peak was located.

When the ^{108}Ag nuclide was used, irradiation took 10 min. One and a half min after irradiation, the sample was counted for 200 sec of live time. After counting, the sample's spectrum was stripped with a "no silver" water sample spectrum. The net area under the curve is compared to one obtained with a standard silver sample of known concentration. The primary standard substance selected for this purpose was AgCN: its saturation value (220ppb of the whole compound, which is 180ppb of silver) is within our working range, and it is free of elements which are likely to interfere with NAA.

Several problems have been encountered, and most of them have been surmounted. At one time, we were losing many samples due to failures in heat sealing the vials. A Teflon-lined sealing iron was devised and has virtually eliminated this problem. Another problem was excessive background. Some of this could be reduced by careful cleaning of the outside of the vial to remove residues. An even greater gain was made when assay was based upon a

different nuclide of silver, ^{110}Ag. The half life of this nuclide is shorter ($t_{1/2}$ = 24 sec) than that of ^{108}Ag, so irradiation was reduced to 72 sec. The shorter irradiation time suppresses the sodium background which had been a limiting factor in the precision of measuring ^{108}Ag. A quick-access carrier had to be built, so that there was only a 15 sec delay in getting the irradiated vial into the spectrometer. Counting time was reduced to 1 min. Using the 659 keV gamma peak, the area which represents the "signal" is a factor of ten higher for ^{110}Ag analysis than for ^{108}Ag analysis (Table 1).

Table 1. Comparison of counts obtained with ^{108}Ag and ^{110}Ag at three levels of silver

Sample	Nominal Ag^+ Conc. (ppb)	^{108}Ag 633 keV peak counts	Background counts/channel*	^{110}Ag 659 keV peak counts	Background counts/channel*
1-0-1	150	750	-10	6110	-10
1-1-1	200	885	15	7720	-30
1-5-1	50	250	-10	2000	-40

*Post stripping background

The present estimated accuracy of the NAA technique described is ± 10% or ± 10ppb, whichever is greater. Some of the polyethylene vials that we had been using had proved quite "noisy" and had degraded this performance somewhat, although at best a 10ppb solution would actually give a clear signal above background. Further refinements of the technique have included development of a computer program for least-squares analysis of the peaks and the acquisition of a batch of low-oxygen vials. The latter arrived quite recently and cannot yet be said to have increased the precision of the silver assay, but they have reduced the dead time in counting from 42% to 14%.

Silver standard. The discussion to this point makes it apparent that the measurements being made are quite relative. The assignment of an absolute value to the silver content of any sample is done by comparison to a known standard. A report by Chambers et al. (J. Am. Water Works Assoc. 54:208, 1962) had indicated that silver solutions were hard to store, so we selected AgCN as the standard, added an excess of it to deionized water, and let it form a saturated solution at room temperature. As was stated above, a saturated solution of AgCN at 20 C should contain 180ppb of silver. We began to suspect that the true silver content of the standard solution was higher.

An experiment was performed using $AgNO_3$ solution and a series of low power irradiations to determine the actual silver concentration in the standard cell. The standardizing solution for the experiment was 3.4×10^5 ppb $AgNO_3$. The standard was made up by weighing out 0.00171 g of $AgNO_3$ in a 2-dram polyethylene vial and adding 5 ml of distilled H_2O. The vial was heat sealed. A second solution of about the same concentration was prepared, and a set of concentrations were made by dilution steps. The vials were analyzed and their concentrations calculated based on the 3.4×10^5 ppb standard. The results are given in Table 2. The errors given in Table 2 reflect uncertainty in the actual value of No. 3 and error introduced by the power stepping procedure. It is apparent that the AgCN standard cell concentration is a factor of 3.6 ± 0.2 above the saturation value of 180 ppb. This discrepancy may be due to highly soluble silver compound impurities (1%) in the original AgCN powder. Since AgCN is so insoluble, a small amount of soluble impurity could easily double or triple the Ag ion concentration.

Table 2. Calibration of AgCN standard cell by comparison with $AgNO_3$ solutions prepared by stepwise dilution and assay

Sample	Power level, kw	Ag (ppb)
2	10	3.4×10^5
3	100	3.8×10^4
4	1000	$(3.3 \pm 0.2) \times 10^3$
5	1000	$(3.3 \pm 0.2) \times 10^2$
AgCN standard cell	1000	645 ± 40
1-2-1 NASA Ag ion generator, nominal 250 ppb	1000	290 ± 20

Working with what appear to be very small quantities of silver (though the numbers of ions involved are, of course, astronomic), there was no way to prepare an absolute reference standard. The amounts of silver that we could weigh were larger by several orders of magnitude than what we wanted to measure, and there are obvious uncertainties and chances for error in diluting a stock solution, or in the power-stepping procedure used to obtain some of the data for Table 2. Silver sulfide (Ag_2S) offered a reasonable alternative. About 120 ppb of silver should be present in a solution of Ag_2S which had saturated at room temperature. Silver in two samples from such a solution was reported at 125 and 120 ppb. This is not an irrefutable answer, but it helps.

Container studies. The silver ion-container complexing problem was

studied in a series of experiments using 250 ppb of silver propionate and soft glass, polystyrene, and polyethylene containers. A 250 ppb solution of silver propionate was prepared, and portions of this solution were transferred to containers of each material. Samples of the original solution and samples from each container were taken at intervals, sealed, and analyzed. There appears to be little effect for polyethylene and polystyrene and only a slight effect in glass after about 2 days (Table 3).

Table 3. Effects of storage in various containers upon the silver content of replicate solutions.

Time	Mother solution (ppb Ag)	Soft Glass (ppb Ag)	Poly-styrene (ppb Ag)	Poly-ethylene (ppb Ag)
0	250 ± 25			
0	260 ± 25			
0+		250 ± 25	250 ± 25	250 ± 25
5 min		280 ± 25	250 ± 25	250 ± 25
15 min		NPHS*	240 ± 25	260 ± 25
60 min		240 ± 25	250 ± 25	250 ± 25
97 min		240 ± 25	240 ± 25	240 ± 25
24 hr		240 ± 25	280 ± 25	240 ± 25
48 hr		210 ± 20	240 ± 25	230 ± 25
72 hr		220 ± 20	240 ± 25	240 ± 25

*NPHS = not properly heat sealed; sample lost due to vial leakage.

Further information was obtained by repeated sampling from styrene flasks containing silver propionate or ion generator effluent. These contained from 250 to 350 ppb Ag. Over sampling periods from 2 wk to 2 mo, no change in the silver concentration was detected. In another experiment, there was no measurable loss of silver with at least three transfers in disposable styrene pipettes. These results indicated that container losses were not likely to cause much difficulty in the conduct of these experiments.

Biological samples. The silver used in the biological experiments has been either in the form of silver propionate or of effluents from an electrolytic ion generator. In either case, problems were encountered in attempting to adjust the silver to the selected level. The propionate was stored at room temperature as a saturated solution. Once the assay system was corrected, we found that the silver content of diluted propionate solutions was sometimes far above what it should be. The error was attributed to finely divided, solid silver propionate in the saturation flask, which was being pipetted with the solution. It should have been possible to avoid this by filtering the saturated solution at 0.2 μm porosity just before the use dilutions were prepared, and there are good indications that this succeeded quite well.

The ion generator was another matter. Its output of silver ions was below the predicted levels quite early. After some reworking at the Manned Spacecraft Center, it came back to us producing almost exactly half the predicted level of silver ions when operated at flow rates expected to produce 50 and 250 ppb. This may have been due to differences in the absolute values assigned to assays here and at MSC. More important, we prepared our own calibration curve for the silver output of the ion generator as a function of flow rate; and this, too, has eventually become a poor predictor. Operating the ion generator at throughput rates (with a small positive displacement pump) which are expected to yield a 250 ppb effluent has recently produced too little silver to be detectable above background.

Once we learned that the silver solutions were stable in storage (at least in styrene flasks) at use dilutions, we began to prepare the solutions well in advance of each experiment. It was made a matter of policy to have a NAA report back for each solution before it was used in a biological experiment. This prevented a great many unpleasant surprises. The level of silver in the solution was determined before the bacteria or viruses were added. Additonal samples were taken of the bacteria or viruses without added silver and of the mixture at the end of each experiment. No suspension of bacteria or viruses was found to contain anything measured as silver by NAA unless we put it there. Concentrations of silver in the experimental samples did not differ from those in the "pre" samples by more than the usual sample-to-sample variation. The system outlined here appears adequate to the task of determining how much silver was present in each biological experiment, though there is still some lingering uncertainty about absolute values.

BACTERIOLOGICAL STUDIES

Preliminaries. The initial experiments were done with two model species: Escherichia coli and Staphylococcus aureus. These were grown in shake cultures in trypticase soy broth and enumerated by pour-plating in the same medium plus 1.5% agar. Cell suspensions were sedimented in the centrifuge and washed twice with deionized water at the start of each experiment. Nothing that assayed as silver by NAA has ever been reported in a control bacterial suspension in these experiments. We wanted a starting count of $\sim 10^4$ cells ml^{-1}. Both the agar medium and the deionized water in the dilution blanks in these first experiments contained 500 ppm of sodium thioglycollate.

Three experiments were done with E. coli. The first two employed silver propionate. Cell populations were quite stable at room temperature in the absence of added silver. The silver killed the cells. The process was not precisely exponential, but there was no indication that killing would not ultimately be complete. The extinction times (10^{-4} killing) might have ranged from < 2 hr to ~ 4 hr at 50 ppb of silver and from < 1 hr to ~ 2 hr at 250 ppb. Silver from the electrolytic ion generator was used in the third experiment, and the probable extinction times were ~ 4 and ~ 2 hr again at 50 and 250 ppb, respectively.

S. aureus was challenged with silver propionate in one experiment.

Extinction times (10^{-4} killing) appeared to be ~ 2 hr at 50 ppb and ~ 1 hr at 250 ppb. The cell population was again quite stable in the silver-free cell control at room temperature.

We also did one experiment to determine if 250 ppb silver, tested undiluted, was capable of inhibiting E. coli colony formation in the pour plates. We found that the silver was not inhibitory, even in the absence of added sodium thioglycollate. On the other hand, fewer colonies were formed when the normal level (500 ppm) of thioglycollate was present in the medium, and fewer still if 1000 ppm were added.

Enumeration of prime test species. Cultures of Achromobacter metalcaligenes, Achromobacter mucosa, Alcaligenes fecalis, Flavobacterium aquatile, Flavobacterium halmephilum, and Pseudomonas aeruginosa were obtained from the American Type Culture Collection. Each was propagated in trypticase soy broth at 36C except for F. aquatile, which was grown in Taylor's M-5 (sodium caseinate) medium at 30 C. Cells were enumerated by pour plating with the medium in which the cells were grown, plus 1.5% agar. We were concerned that silver in the cell suspensions, carried over into the pour plates, might continue to exert an effect. This would have nullified (or greatly complicated) rate measurements where killing in the original cell suspension was treated as a function of time.

Sodium thioglycollate was tested for ability to neutralize silver rapidly. It was added to dilution blanks; but, since some samples were to be tested undiluted, it was also added to the agar media at levels of 500 (1x) and 1000 (2x) ppm. These were compared to no thioglycollate in pour plating cell suspensions in the presence of silver propionate. Silver levels were approximately 500 ppb, or twice the highest which was to be used experimentally, because of some uncertainties in silver assay at the time that these tests were done. Only F. aquatile was inhibited by silver in the absence of thioglycollate, presumably because trypticase soy broth with agar neutralized the silver, while Taylor's M-5 medium did not (Table 4). On the other hand, thioglycollate was strongly inhibitory to A. metalcaligenes and totally inhibitory to F. aquatile at the 1x level. The conclusion was that sodium thioglycollate did not do any good under these conditions, and in some cases might do a great deal of harm. It was used in pour plating in some of the experiments to be described below but was discontinued completely when the last of the results in Table 4 had been obtained.

Table 4. Effects of silver propionate and of sodium thioglycollate in the agar medium upon colony formation in pour plates

Test species	Ag (ppb)	Thioglycollate in agar (ppm)		
		0	500	1000
A. metalcaligenes	0	254*,242	161,160	15,25
	560	226,262	109,108	16,17
F. aquatile	0	352,314	0,0	0,0
	570	109,102	0,0	0,0
F. halmephilum	0	76,87	84,92	58,54
	550	74,87	67,57	64,72
P. aeruginosa	0	259,269	256,261	290,294
	410	258,266	255,250	235,219

*Number of colonies in one plate

APT (All-purpose, Tween) broth (Difco) was used to grow cells, and (with 1.5% agar) to enumerate them by spread plating, in later experiments. A survey of several reagents as alternatives to sodium thioglycollate in neutralizing silver indicated that APT broth was as effective as any of those tested. Thereafter, APT broth served as the diluent for samples as well as the growth medium and the assay medium.

Kinetics of killing from 10^4 cells ml^{-1}. The prime test species listed above were suspended to an initial level of ~ 10^4 cells ml^{-1} and challenged. Silver propionate was used in most of the experiments; silver from the ion generator was used in some but was far below the levels (50 and 250 ppb) for which the experiments were designed. All reactions were carried out at room temperature. Experiments in this and subsequent sections will be reported in alphabetical order by species name, rather than in the exact order in which they were performed.

Silver levels in the first experiment with A. metalcaligenes were estimated at 0,100, and 560 (± 10%) ppb. The kinetic data were not useful, perhaps because of the adverse effects of thioglycollate in the agar medium Thioglycollate was omitted in the second experiment, with the results shown in Fig. 1. The initial cell concentration was higher than intended. They died at the rate (determined by linear least squares) of -0.38 hr^{-1} in the absence of silver. At silver levels determined to be 44 and 290 (± 10%) ppb, killing exceeded 10^{-5} during the first hour. Points of uncertainty in the figure are designated by question marks.

The kinetic data obtained with A. mucosa were not very informative, either. From an initial level of ~$10^{4.1}$ cells ml^{-1}, the logarithmic rate of death was estimated at -0.036 hr^{-1} for the first 24 hr at room temperature in the absence of silver. The first 10^{-4} of killing in suspensions containing silver (140 and 790 (± 10%) ppb) appeared to have occurred within the first hour, but an occasional colony or two appeared in tests of later samples.

Results with A. fecalis were anomalous in somewhat different ways. Starting at a level of ~$10^{4.1}$ cells ml^{-1}, cell death in the absence of silver was at -0.027 hr^{-1} during the first 3 hr. At this rate, the $\log_{10}$ (cells ml^{-1}) at 24 hr should have been 3.47 but was found to be <0.7. Killing in the presence of silver (100 and 540 (± 10%) ppb) appeared to be more rapid at the lower level during the first hour, but no viable cells were detected thereafter. Some further results with this species are reported in a later section.

F. aquatile was a problem to enumerate because it had been shown that silver could carry over significantly into the pour plates and that addition of 500 ppm sodium thioglycollate to the agar medium would suppress the organisms completely. Thioglycollate was used in the dilution blanks only, as a compromise. This was no help when undiluted samples were tested, but it seemed the best that could be done at the time. The cell count in the absence of silver increased initially but had begun to decrease by 3 hr. No viable cells were detected at 24 hr. This is the kind of result one would expect of an aggregated suspension of labile cells. Killing by silver

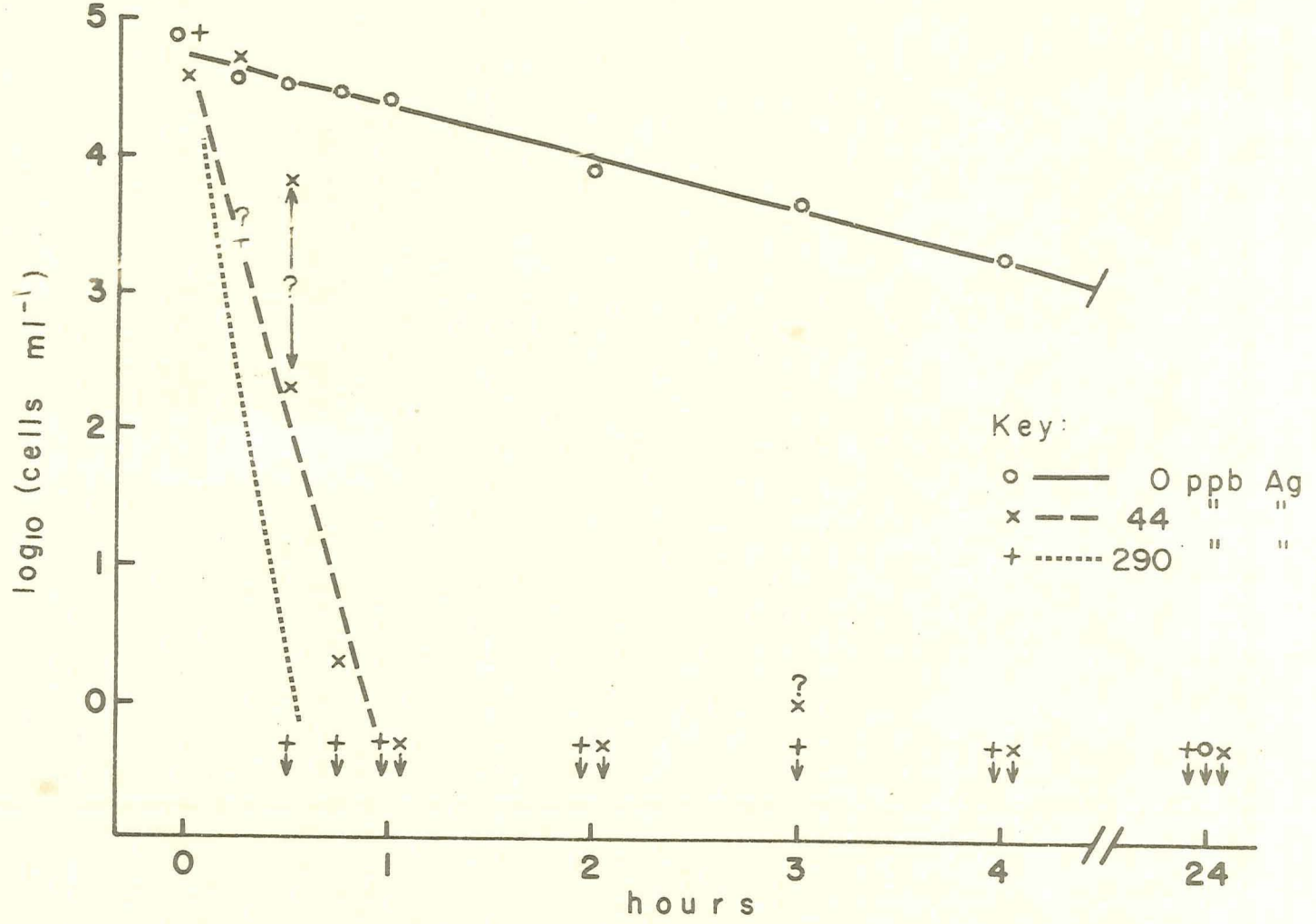

Fig. 1. Achromobacter metalcaligenes experiment 2

(79 and 570 (± 10%) ppb) was rapid during the first hour, but 1 or 2 colonies grew from undiluted samples at 3 and 4 hr, though not at 24 hr.

The first experiment with F. halmephilum also gave an initial rising cell count in the absence of silver, and no viable cells at 24 hr (tested at 10^{-2}). Killing in the presence of silver (110 and 550 (± 10%) ppb) was extremely erratic. In experiment 2 the silver-free suspension showed a very consistent death rate of -0.073 hr^{-1} for the first 6 hr at room temperature. This indicated that the $\log_{10}$ (cells ml^{-1}) at 24 hr should be 2.32, but it was found to be < 1.70. Killing in the presence of silver (79 and 330 (± 10%) ppb) was again very erratic. Cell death in the absence of silver was also seen in experiment 3, this time at a rate of -0.137 hr^{-1}, including the 24 hr sample (Fig. 2). Starting at ~10^5 cells ml^{-1}, killing in the presence of silver (53 and 313 (± 10%) ppb) was rapid during the first hour; but residual, viable cells were detected as late as 24 hr. Ion generator effluents were included in experiment 4, to be compared with the results obtained with silver propionate (46 and 320 (± 10%) ppb). Though one of the ion generator effluents had been intended to contain 250 ppb, neither had more than 50 ppb, and the true levels were somewhat uncertain. Cell counts in the absence of silver increased very little over a 24 hr period at room temperature. Very little killing was seen in 2 hr with ion generator silver, but this was to be expected with as little silver as was present. The silver propionate killed rapidly, but not linearly, during the 2 hr sampling period.

Three experiments were performed with P. aeruginosa. Experiment 1 gave relatively orderly results. Cells without silver appeared to die at a rate of -0.056 hr^{-1} (Fig.3). The measured kill rates at 72 and 410 (± 10%) ppb were approximately -0.88 and -1.46 hr^{-1}, respectively, which are the slowest seen among experiments in this section. Some points were missed in experiment 2 because agar in some of the pour plates did not solidify. The very slightly positive slope (+ 0.0075 hr^{-1} for 24 hr by linear least squares) for the 0ppb suspension probably does not differ significantly from zero, but it certainly is not negative. The silver solutions (140 and 750 (± 10%) ppb) appeared to have killed to the extent of 10^{-4} in the first hour. There was generally less than one viable cell per milliliter at 2, 3, and 4 hr; but only one of these samples was completely negative. Ion generator effluents were included in experiment 3, to be compared with the killing effects of silver propionate (55 and 310 (± 10%) ppb). Ion generator effluents intended to contain 50 and 250 ppb of silver were found to contain ~ 22 and ~ 20 ppb, respectively. Though the propionate suspensions, with their higher silver assays, appeared to kill more rapidly during the first hour, differences were doubtful at 2 hr. Cell death in the absence of silver was negligible through 24 hr. The results of these experiments, taken together, lead to no obvious conclusion. More useful information was derived from experiments to be described below.

Extinction time as a function of temperature. Since the kinetic data for suspensions which initially contained ~ 10^4 cells ml^{-1} were often difficult to interpret, we decided to try 5-log death time (t_{10}-5) as a comparative statistic when determining the effect of temperature upon cell killing. We planned to begin with ~10^4 cells ml^{-1} and to determine the

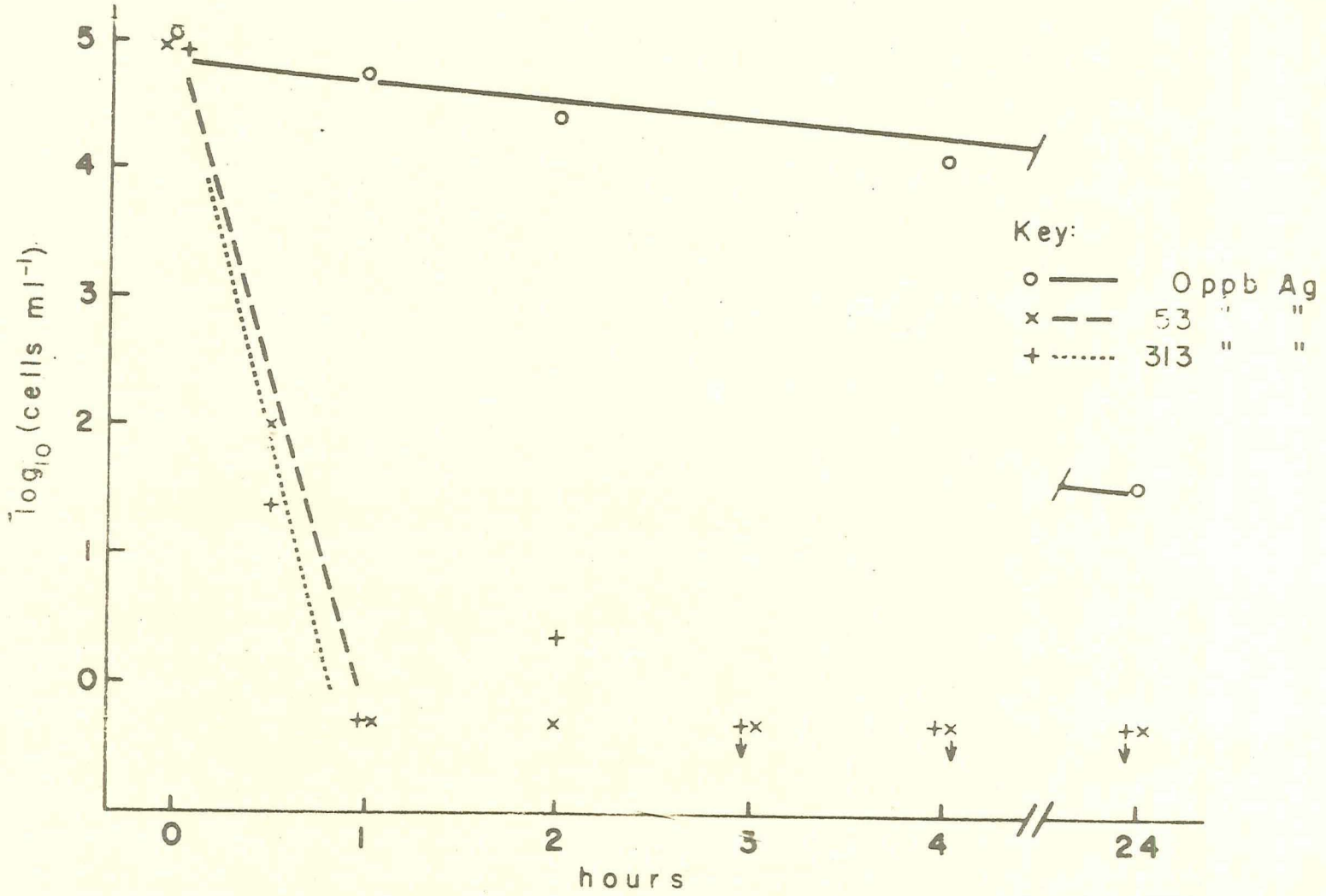

Fig. 2. Flavobacterium halmephilum experiment 3

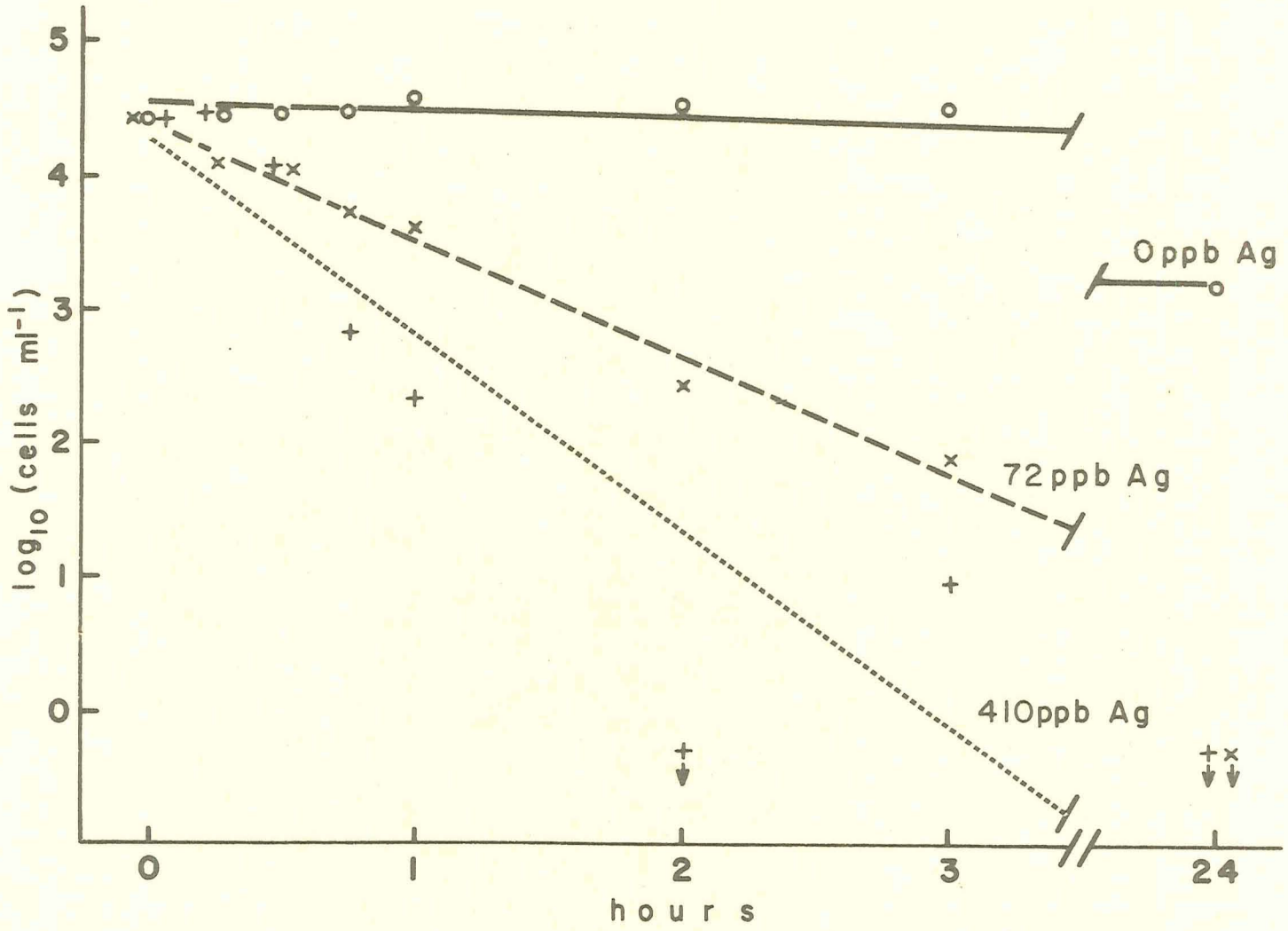

Fig. 3. Pseudomonas aeruginosa experiment I

time required to reach 10^{-1} cells ml^{-1} by collecting viable cells on 0.45 μm porosity filters and incubating these on agar medium to permit colony formation. As much as 100 ml could readily be sampled at once by this method. We feared, however, that neutralizing substances in the agar might reach the cells too slowly to arrest the action of silver promptly when the sample was taken. Two possible answers came to mind: adding thioglycollate to the sample or rinsing free silver ions through the filter with an additional volume of sterile, deionized water.

A three-factor experiment was performed using F. aquatile as the model species. The factors were silver (0 or 250 ppb), sodium thioglycollate (0 or 500 ppm), and rinsing (none or 100 ml of deionized water after the sample). The only forthright finding was that F. aquatile is quite intolerant of even brief exposures to thioglycollate. Further work with F. aquatile was deferred until another sampling method could be devised.

A similar experiment was performed with P. aeruginosa as the model, except that the filter porosity was 0.22 μm. Colony formation, in the absence of silver and other factors, was approximately as efficient on the filters as in pour plates (145 and 138 colonies on filters vs. 138 and 160 colonies in 2 plates). The combination of thioglycollate with rinsing seemed to do the best job of mitigating the silver effect (Table 5). This combination was selected for use in further experiments, though it may have been somewhat deleterious in the absence of silver ions.

Table 5. Effects of three factors upon colony formation on filters by P. aeruginosa

Thioglycollate	Rinse	Silver 0ppb	Silver 250ppb
−	−	145*,138	97,87
−	+	101,111	76,82
+	−	133,143	109,96
+	+	69,110	116,123

*Number of colonies on one filter

Results of three experiments indicated that temperature exerted a significant effect upon t_{10}-5 (Table 6). It also appeared that F. halmephilum might be somewhat more resistant to silver than P. aeruginosa under these conditions and that t_{10}-5 might be somewhat dependent upon initial cell count. Some results to be reported below appear to support these notions at a higher level of certainty.

Activity of silver from different sources. A further experiment was intended to determine the effect of silver source upon t_{10}-5 for P. aeruginosa at various temperatures. Starting at ~ 8×10^3 cells ml^{-1}, all combinations of temperature and silver level were included for the ion generator silver, but only the points reported as inequalities in Table 6 were determined for silver propionate. Every t_{10}-5 for ion generator silver was shorter than its counterpart observed with silver propionate

(Table 7). Little was made of this at the time, because the differences were not great and some might be attributable to the difference in initial cell numbers in the two experiments.

Table 6. Effects of silver propionate upon bacteria at 3 temperatures, expressed as t_{10}-5

Test species	Initial cell count	Ag^+ level	TEMPERATURE 14-15C	24C	36C
F. halmephilum	~$4x10^2$ cells ml^{-1}	50ppb	>>3 hr	>>3 hr	~2 hr
		250ppb	>3 hr	~2 hr	1-2hr
	~10^3 cells ml^{-1}	50ppb	>>5 hr	>5 hr	~ 3 hr
		250ppb	~ 5 hr	~ 4 hr	~ 3 hr
P. aeruginosa	~$4x10^4$ cells ml^{-1}	50 ppb	>> 3 hr	>3 hr	~ 1 hr
		250 ppb	1.5-2 hr.	1.5-2 hr	~ 1 hr

Table 7. Effects of silver propionate and ion generator silver upon P. aeruginosa at different temperatures, expressed as t_{10}-5

Silver Source	Level	Temperature 14-15C	24C	36C
Propionate	50ppb	5-6 hr	3-4 hr	—
Ion generator	50ppb	4-5 hr	3 hr	0.5- 1 hr
	250ppb	~ 1 hr	1-2 hr	<0.5 hr

Higher cell numbers have been used in more recent experiments. Two test species have been added: Flavobacterium sp (IIb) and another organism designated as group IIIa. These had been isolated from spacecraft water systems and supplied to us from the Manned Spacecraft Center. Their designations are based upon a taxonomic system used by the National Communicable Disease Center. An inquiry to NCDC yielded no more information about them than that group IIIa has not been assigned to a genus. Another, equally obscure organism (group IIIb) was received with these from MSC but experiments on this organism have produced no results worth reporting. All cell counts in this series of experiments were obtained by spread plating on APT agar.

F. aquatile began to be killed by silver propionate only after the first hour (Table 8). By 3 hr, more cells had been killed by the propionate than the ion generator silver. This was the only result of its kind: each of the other test organisms had been killed to a greater extent at 1 hr and thereafter by the ion generator silver. The other test organisms showed a definite tailing in killing by ion generator silver at the end of the second or third hour. The difference at the end of the fourth hour was greatest with Flavobacterium sp. (IIb), least with group IIIa, and intermediate (at approximately 20-fold) in two experiments with P. aeruginosa. We are hesitant to conclude from the data in Table 8 that ion generator silver will kill more cells than the same amount of silver as the propionate. It may be that the ion generator silver takes effect more rapidly, though the number of cells ultimately killed does not differ.

Effect of initial cell count upon kill rate. The higher cell numbers used in the experiments just described had permitted more extensive comparisons than could be made by beginning at 10^4 cells ml^{-1}. However, it appeared that the number of cells which could be killed by a given quantity of silver was exceeded in some experiments with high cell numbers. For instance, the t_{10}-5 for F. halmephilum had been estimated at 2 hr at room temperature in 250 ppb silver (propionate), starting with 400 cells ml^{-1} (Table 6); in 200 ppb, with an initial cell count of 9×10^7, killing was only $10^{-2.3}$ in 3 hr. A further example was provided by P. aeruginosa at initial levels in excess of 10^4 and 10^7 cells ml^{-1}. Kill rate was a function of silver concentration at the lower cell level, and there was no indication of a "tail" in the kill curve (Table 9). At the higher cell level, a tail appeared within 90 min at 220 ppb of silver, and very little killing occurred at 58 ppb.

Rapid killing of dense cell suspensions might still be achieved if higher silver concentrations were employed. A series of trials of ion generator silver with Flavobacterium sp. (IIb) is reported in Fig. 4. This organism appears to be more sensitive to silver than is P. aeruginosa, for there is extensive killing ($\sim 10^{-4.5}$) at 58 ppb. However, the kill rate is approaching zero at 4 hr in this silver suspension and has definitely declined within 1-2 hr at 220 and 250 ppb. Only at 370 ppb is the kill curve approximately linear over the entire range of observed values.

Relative silver sensitivity. We wished to know which of our test cultures was most susceptible to the action of silver. Seven experiments which bear upon this question are summarized in Table 10. It is unfortunate that the starting concentrations of silver and of cells were not identical in all of these, but the conditions seem similar enough to permit comparisons. These and other experiments described elsewhere in this report suggest that F. halmephilum is the least sensitive to silver of the species compared. P. aeruginosa and group IIIa are comparable to each other and are followed by F. aquatile, Flavobacterium sp. (IIb), Achromobacter metalcaligenes, and Alcaligenes fecalis in apparent order of increasing sensitivity. The use of higher initial cell numbers facilitates this kind of comparison; it also leaves one to assume that a similar rank order would have resulted if the cell numbers had been in the range that has occurred in spacecraft water systems. Unfortunately, experimental

Table 8. Relative effectiveness of ion generator and propionate silver in killing cells at room temperature

Test organism	Silver Source	Silver Level (ppb)	Sampling time (min) 0	30	60	90	120	180	240
F. aquatile	Generator	197	1.2×10^{7}*	–	4.1×10^{5}	–	1.2×10^{3}	5.8×10^{2}	–
	Propionate	187	1.1×10^{7}	–	1.2×10^{7}	–	1.3×10^{2}	7.5×10^{1}	–
F. sp. (IIb)	Generator	58	1.6×10^{6}	5.6×10^{5}	8.5×10^{3}	5.6×10^{3}	2.1×10^{3}	4.3×10^{2}	5.5×10^{2}
	Propionate	49	5.2×10^{6}	2.7×10^{5}	1.2×10^{5}	2.5×10^{5}	1.7×10^{5}	1.4×10^{5}	3.2×10^{4}
Group IIIa	Generator	51	2.7×10^{6}	–	1.6×10^{6}	–	9.1×10^{5}	7.8×10^{5}	6.5×10^{5}
	Propionate	51	2.5×10^{6}	–	2.5×10^{6}	–	1.5×10^{6}	1.9×10^{6}	7.6×10^{5}
P. aeruginosa	Generator	219	4.6×10^{7}	4.5×10^{6}	2.9×10^{5}	5.1×10^{4}	–	7.3×10^{4}	7.1×10^{4}
	Propionate	226	4.2×10^{7}	2.5×10^{7}	1.4×10^{7}	1.0×10^{7}	–	–	1.3×10^{6}
	Generator	246	6.5×10^{7}	–	4.8×10^{5}	–	6.0×10^{4}	2.1×10^{4}	1.9×10^{4}
	Propionate	246	4.0×10^{7}	–	1.4×10^{6}	–	8.0×10^{5}	3.3×10^{5}	4.7×10^{5}

* Cells ml^{-1}

Table 9. Killing of P. aeruginosa by ion generator silver, as a function of silver level and of initial cell count

Experiment	Silver (ppb)	Time (min) 0	30	60	90	120
1	54	3.4×10^{4}*	1.3×10^{4}	1.1×10^{3}	5	< 5
	260	4.1×10^{4}	3.0×10^{2}	2.0×10^{1}	< 5	< 5
2	58	3.7×10^{7}	2.5×10^{7}	3.0×10^{7}	3.0×10^{7}	–
	220	4.5×10^{7}	4.5×10^{6}	2.9×10^{5}	5.0×10^{4}	7.0×10^{4}

* Cells ml^{-1}

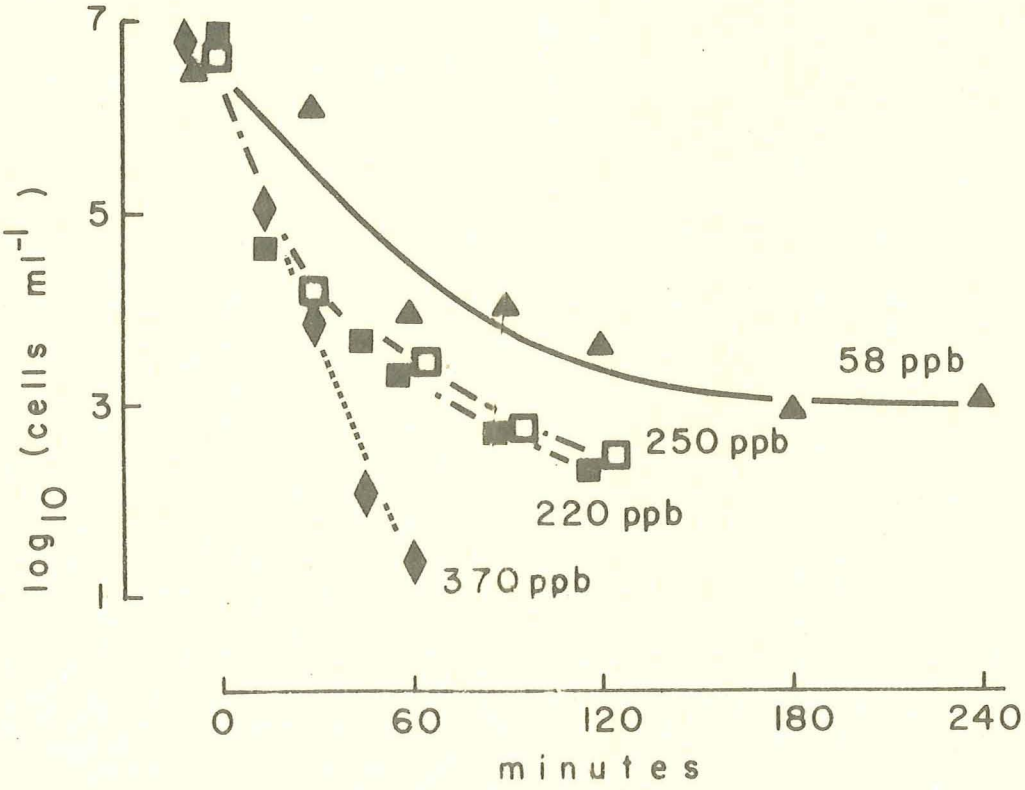

Fig. 4. Killing of large numbers of Flavobacterium sp. (IIb) cells by ion generator silver

conditions are almost never identical with the conditions under which the results are to be applied.

Table 10. Killing by ion generator silver of seven test bacteria

Test organism	Silver (ppb)	Time (min) 0	60	120	180	240
A. metalcaligenes	200	2.2×10^{7}*	$<10^{4}$	$<10^{3}$	5×10^{2}	3×10^{1}
A. fecalis	200	6.8×10^{5}	$<10^{1}$	$<10^{1}$	–	–
F. aquatile	200	1.1×10^{7}	4×10^{5}	1.3×10^{3}	5×10^{2}	–
F. halmephilum	200	9×10^{7}	2.3×10^{7}	9×10^{5}	4.5×10^{5}	–
F. sp (IIb)	250	4.4×10^{6}	2.8×10^{3}	2.0×10^{2}	–	–
Group IIIa	250	4.3×10^{6}	5.6×10^{4}	7.3×10^{3}	2.5×10^{3}	2.2×10^{3}
P. aeruginosa	260	4.4×10^{7}	2.0×10^{6}	2.6×10^{4}	1.2×10^{4}	1.1×10^{4}

* Cells ml^{-1}

Selection for silver resistance. Having determined the sensitivity of an organism to silver, one would like to believe that this would remain relatively constant. However, we had seen "tails" on kill curves and not known whether to attribute them to depletion of the silver, or to the presence of a more resistant fraction, or both. If a resistant fraction were present, subculture after the more susceptible cells had been killed should select for this property.

A suspension of washed P. aeruginosa cells was used in a first experiment. This was treated with silver (260 ppb) for 120 min at room temperature, and 0.25 ml of the remaining suspension was transferred to 9 ml of APT broth and shaken for 20 hr at 30C (Table 11). The cycle was then repeated: cells were again washed and challenged with 260 ppb of silver at room temperature. The degree of killing may have been somewhat greater in this second cycle. The change in cell count was positive in the third and fourth cycles. We have no explanation for these increases, but they do appear to be significant. We also noted that in plating cells that had been treated with silver, pinpoint colonies were often formed, indicating a late start or slow growth, and that such atypical colonies were absent in assaying the cells in cycles 3 and 4. Great selective pressure was involved in producing the silver-resistant derivative. Whether a comparable result is possible in a spacecraft water system is uncertain, but it would seem that means of preventing it should be sought.

Flavobacterium sp. (IIb) was used in a second experiment. Two cycles of selection were carried out, based upon 30 min at room temperature with 250 ppb of silver. The parent strain had shown more than 10^{-4} killing in 120 min

Table 11. Effect of repeated silver challenge - subculture cycles upon the silver susceptibility of P. aeruginosa

Cycle number	0 ppb Ag		260 ppb Ag	
	0 min	120 min	0 min	120 min
1	4.5×10^{7}*	4.3×10^{7}	4.5×10^{7}	3.5×10^{4}
2	2.8×10^{7}	2.2×10^{7}	1.5×10^{7}	2.1×10^{3}
3	5.3×10^{6}	6.9×10^{6}	4.7×10^{6}	1.5×10^{7}
4	1.3×10^{8}	1.4×10^{8}	1.5×10^{8}	6.1×10^{8}

* Cells ml^{-1}

at 250 ppb, whereas the derivative strain after two selection cycles was killed 10^{-2} at 260 ppb (Fig.5). The parent strain of this organism was more sensitive to silver than that of P. aeruginosa. One cannot tell from these results whether the fact that the derivative strain of Flavobacterium sp. (IIb) is not as resistant is due to this or to the decreased selective pressure which resulted from subculturing after just 30 min of silver challenge. This distinction may or may not be worthy of further investigation.

Silver complexing by cells. One would expect that the silver ions must associate with the cells in order for killing to occur. Still, this had not been demonstrated directly. The question is of more than theoretical concern: if the silver is irreversibly associated with the cell it kills, it will not be available to kill other cells which may be introduced later.

Two experiments were performed which illustrate this possibility. In the first, 2 ml of Flavobacterium sp. (IIb) washed cell suspension were added to 18 ml of ion generator effluent to give a level of 250 ppb and sampled over a period of 120 min. Then another 0.2 ml of the same cell suspension was added, and sampling was continued for another 120 min. The added cells were definitely killed more slowly than the initial inoculum at zero time, but it is difficult to say that killing is slower than for the initial inoculum after 120 min (Fig. 6). That is, if the second segment of the curve were transposed downward, it would form a fairly smooth continuation of the first segment. The second experiment employed P. aeruginosa; and although the procedure was the same, the results were rather different (Fig. 6). The slope of the second segment of the curve is a distinct departure from that of the end of the first segment. There is also a "shoulder" at the beginning of this curve. This is usually thought to indicate that the cells in the suspension are aggregated, but there was no indication of this when another portion of the same suspension was added at 120 min. Though killing was definitely slower in the second segment, it did occur.

We had also hoped that we could measure silver complexing by collecting

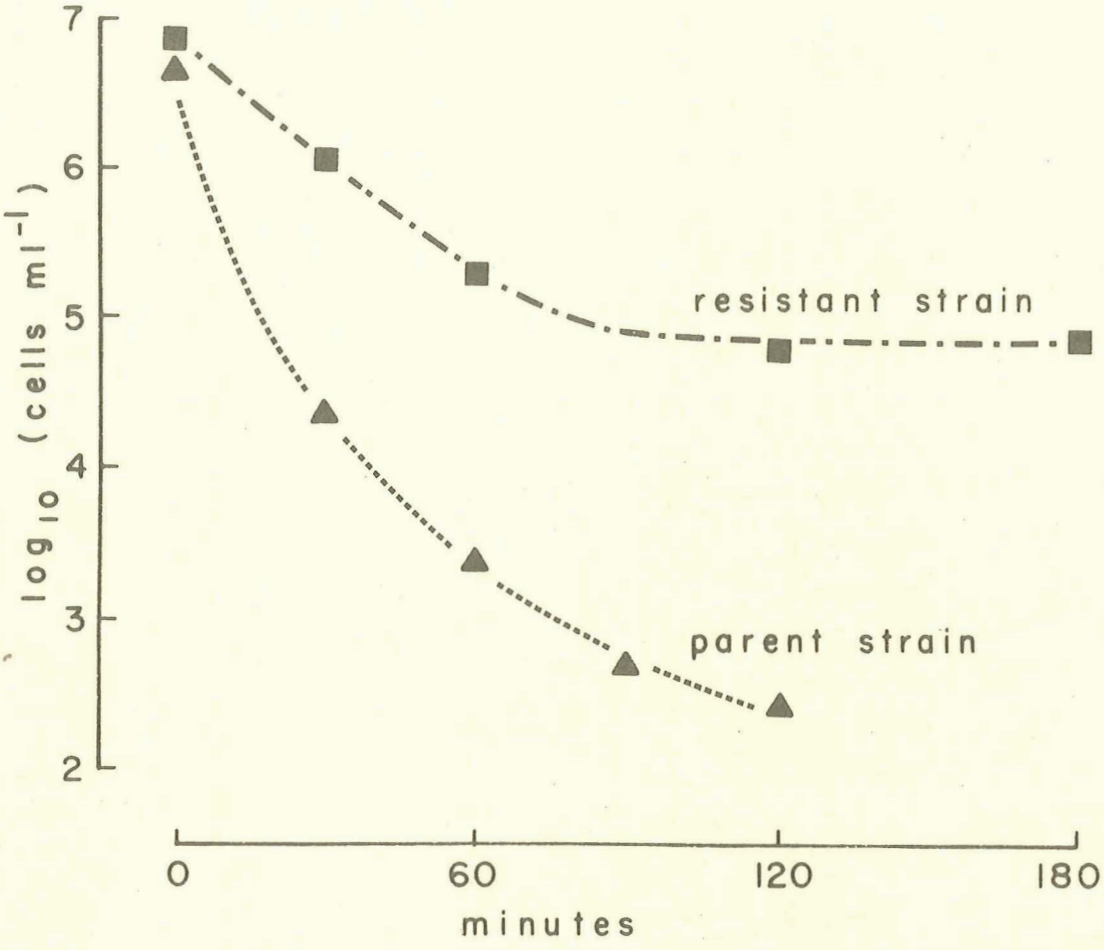

Fig. 5. Killing by silver of two strains of Flavobacterium sp. (II b)

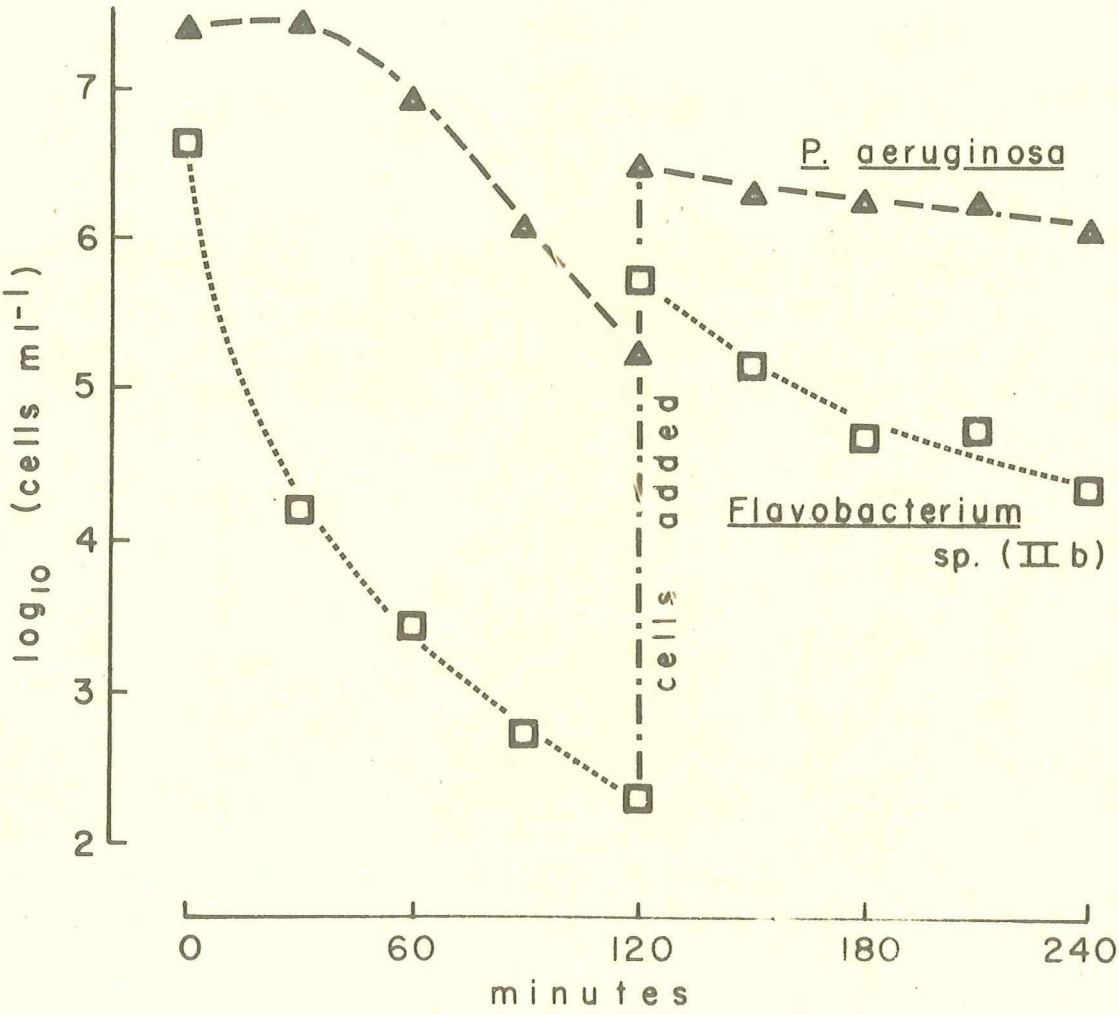

Fig. 6. Effects of adding live cells after many have been killed by silver

the killed cells on a membrane filter and subjecting this to NAA. Some preliminary tests indicate that this may be possible: the control filters show no silver activity by NAA, though they tend to adsorb silver from solutions they filter. Further work will be necessary if this approach is to yield any meaningful data.

VIROLOGICAL STUDIES

The viruses used in these experiments have been quite diverse and have been handled rather differently from each other. The methods of propagation and assay in tissue culture have been based upon those described by Cliver and Herrmann (Health Lab. Sci. 6:5, 1969.), with a few necessary exceptions. Silver propionate was used in most of the challenge experiments.

Coxsackie virus type B-3 (CB-3; representing the enteroviruses) was propagated in primary rhesus monkey kidney (PMK) cells under L-15 medium plus 2% agamma calf serum. It was clarified at 16,500 rpm for 30 min and concentrated at 50,000 rpm for 120 min. In a preliminary trial, there had been no loss of titer with filtration at 50 nm porosity. However, the cleaned virus suspension used in the first silver challenge experiment lost 28%, which may be taken as a rough indication of the degree of aggregation.

The silver levels in this experiment were found to have been 0, 72, and 410 (± 10%) ppb. Samples were assayed in PMK cells by the plaque technique, with the results shown in Fig. 7. These are expressed logarithmically as plaque-forming units (PFU) ml^{-1}. Two points of uncertainty have been indicated with question marks. Least squares analyses gave estimated slopes of -0.0058 (for 0ppb), -0.19 (for 72 ppb), and -1.48 (for 410 ppb) hr^{-1}. At 410 ppb, t_{10}-5 would be estimated at 3.4 hr.

Influenza virus type A, strain PR 8 (representing the myxoviruses), was propagated in the allantoic sacs of embryonated chicken eggs. A sample of the stock was clarified at 5,000 rpm for 30 min and concentrated at 15,000 rpm for 30 min. The final suspension had a titer of $\sim 1.4 \times 10^5$ PFU ml^{-1} and was ~ 80% aggregated as determined by filtration at 200 nm porosity. The original suspension was found to be > 90% aggregated by this criterion, but some of the aggregates were removed during clarification.

Virus from the same stock suspension, clarified and concentrated in the same way, was used in the silver challenge experiment. Filtration at 300 nm porosity (0 time, 0ppb suspension) resulted in a 26% increase in plaque count, while a similar test with a 24 hr sample showed a 30% increase. Such increases are unusual among viruses in general and are usually taken to indicate that aggregates are breaking up at the filter surface. Since some aggregates are breaking up while others are almost certainly being retained by the filter, there is no absolute basis for estimating the degree of aggregation in these preparations. It does seem likely that the two were pretty much the same, however (i.e., little or no net change in degree of aggregation during 24 hr of incubation), and must certainly have been aggregated to > 26 to 30%. No pH readings were recorded. The silver levels were found to have been 0, 59, and 260 (± 10%) ppb. The results are shown in Fig. 8. Least squares estimates of the curve slopes are -0.0099 (0ppb), -0.029 (59 ppb), and -0.11 (260 ppb) hr^{-1}; fits are neither outstandingly good nor bad. Clearly, influenza virus is quite limited in its susceptibility to silver ions.

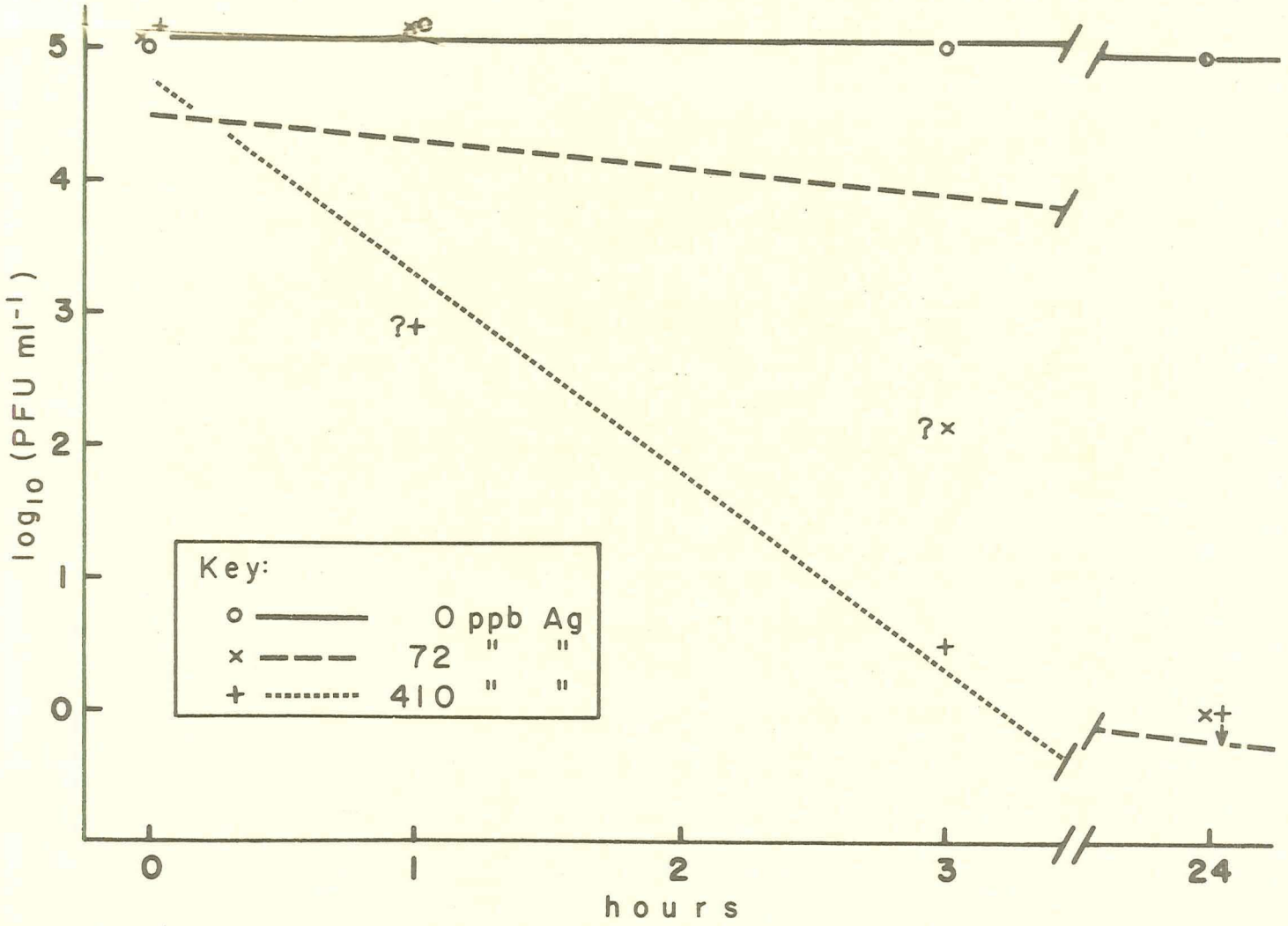

Fig. 7. Coxsackie virus type B-3 experiment I

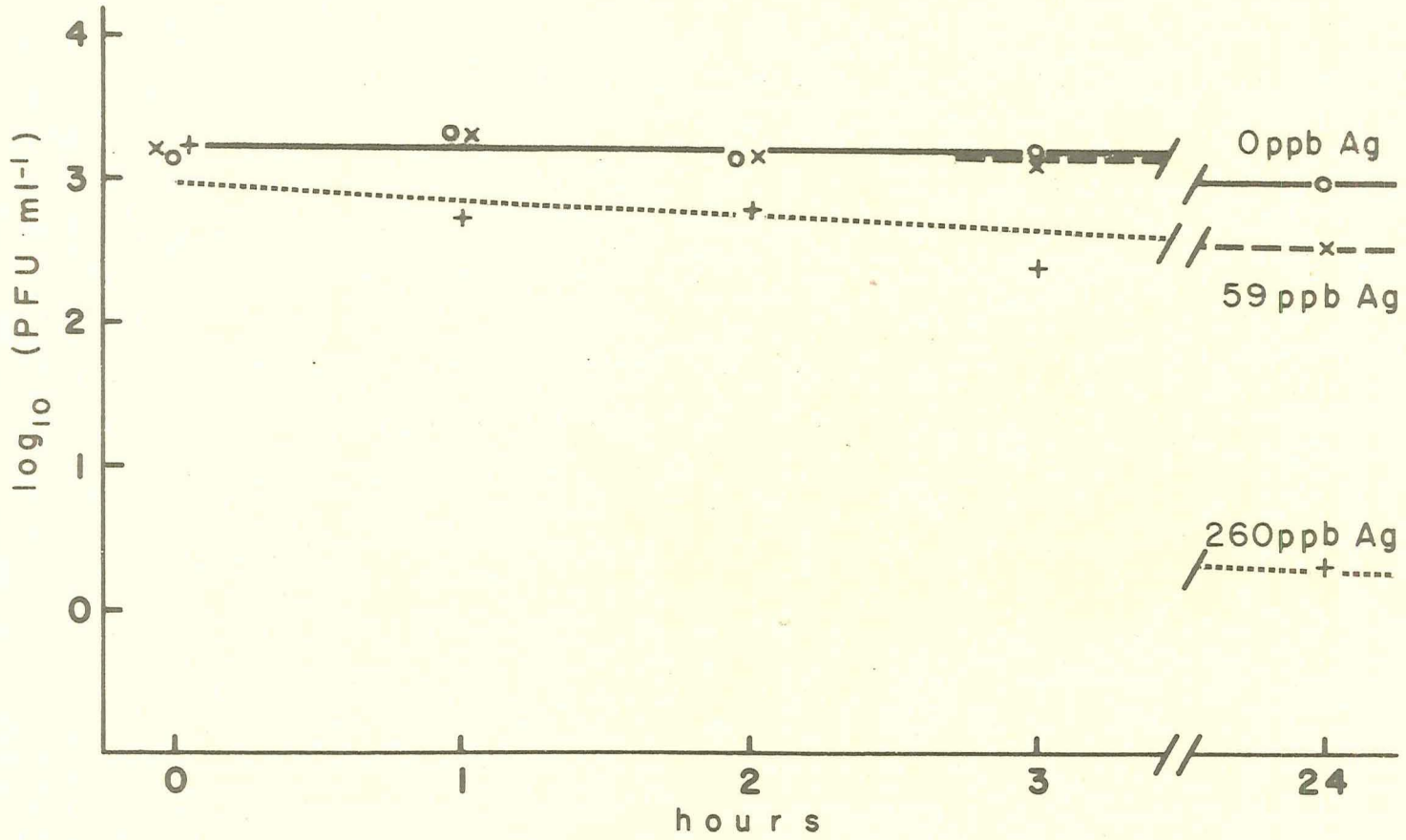

Fig. 8. Influenza virus type A experiment I

Reovirus type 1 (representing the reoviruses) was propagated in PMK cells under L-15 medium plus 2% agamma calf serum and was assayed by the plaque technique in PMK with pancreatin overlay. It was clarified at 7,500 rpm for 30 min and concentrated at 50,000 rpm for 30 min. Filtration of the clean, silver-free suspension at 0 time at 100 nm (0.1 μm) porosity resulted in a loss of 84% in the plaque count, while filtration of a 24 hr sample caused a 72% loss of titer. This difference probably is not significant. The silver levels were found to have been 0, 580, and 2700 (± 10%) ppb, or roughly a factor of 10 greater than were intended. The virus appeared to have been quite rapidly inactivated. No residual plaque-forming units were detected in samples taken at 1 hr or later.

Rhinovirus type 1A (representing the rhinoviruses) was propagated in WI-38 cells and was cleaned up by the same method as was described for CB-3. Filtration of a 0 time, 0ppb suspension at 50 nm porosity caused a 31% decrease in plaque count (determined in PMK cells), which may provide a rough estimate of the degree of aggregation. The levels of silver in the suspensions were found to have been 0, 46, and 280 (± 10%) ppb. The 0 time, 0ppb virus suspension had slightly less than 10^3 PFU ml^{-1}, which was lower than we wanted by a factor of more than 10. Rhinovirus titers are consistently lower than enterovirus titers, so perhaps this experiment is representative. The 0 time samples with silver had titers < 10 PFU ml^{-1}, which is approximately a 100-fold reduction (or more). The least squares slope for the 0ppb points was approximately -0.036 hr^{-1}. The suspensions which contained silver were tested undiluted at 1,2,3, and 24 hr without detecting any residual infectivity. Even though this experiment was begun with less than the desired level of virus, it demonstrates that this rhinovirus is extremely sensitive to inactivation by silver ions.

Vaccinia virus (representing the poxviruses) was propagated in primary rhesus monkey kidney (PMK) cells under L-15 medium plus 2% agamma calf serum. The titers observed under various agar media in PMK were all > 10^7 plaque forming units (PFU) ml^{-1}. A plaque was subcultured and grown up to prepare a virus stock. Some of this was cleaned by centrifuge clarification at 3,000 rpm for 30 min, concentrated by centrifugation at 8,000 rpm for 30 min, and resuspended in deionized water. The clean-up process was ~ 50% efficient, by comparison of the final titer to that of the stock virus suspension. Filtration of a 0 time, 0ppb suspension at 450 nm resulted in a 74% decrease in plaque numbers; this may not be an ideal indicator of the degree of aggregation, but it is all we have. The levels of silver were found to have been 0,600, and 2400 (± 10%) ppb. There was an apparent early decline in the titers of the suspensions which contained silver; but despite the ten-fold excess of silver over the desired concentrations, there was no significant difference at 24 hr. That is, approximately the same proportion (5-10%) of virus "survivors" was present over a range of silver concentrations of 0-2400 ppb. Filtration of the 24 hr samples at 450 nm showed less aggregation among these than in the 0 time sample, so aggregation probably was not the basis on which these plaque-forming units escaped inactivation. Vaccinia virus apparently is almost totally resistant to silver.

Silver source. CB-3 had been found to be inactivated by silver ions at a moderate rate. It was therefore selected as a model agent for studies

of another question: the relative effectiveness of silver propionate and of ion generator silver. CB-3 was cleaned up and incubated with silver from each of these sources at room temperature. Inactivation by silver was seen at 24 hr, but not during the first 3 hr of the challenge, in the first experiment. When this experiment was repeated, inactivation was demonstrable beginning with the 2 hr sample (Table 12). The inactivation at 3 hr seemed to be greater with silver from the ion generator, but by 24 hr the difference appeared insignificant. One might well be tempted to read more into the results of this experiment than is actually there: control of the time dimension in the reaction was rather uncertain. We had not developed a way to turn it off promptly.

Table 12. Inactivation of Coxsackie virus type B-3 with time, as a function of the source of the silver ions.

Silver Source	Level	PFU ml-1 at 1 hr	2 hr	3 hr	24 hr
–	0ppb	$4.0x10^4$	–	$6.4x10^4$	$4.0x10^4$
Ion generator	40	$4.0x10^4$	$2.7x10^2$	86	2.7
	240	$5.0x10^4$	$1.6x10^2$	89	< 1
Propionate	66	$6.4x10^4$	$2.2x10^2$	$3.2x10^2$	6.4
	190	$7.3x10^4$	$2.2x10^2$	$1.2x10^2$	1

Neutralization of silver. We were hesitant to try strong chemical neutralizers for silver, for the tissue cultures in which the samples had to be tested might be injured by the neutralizing reagent. Either dilution or addition of serum seemed a likely way to arrest inactivation by silver. A preliminary experiment indicated that both were effective: dilution to 10^{-3} in phosphate-buffered saline plus 2% agamma chicken serum arrested the process promptly, and addition of 10% serum after 24 hr with 250 ppb of ion generator silver may even have reversed some of the inactivation. There was another innovation in this experiment: the CB-3 was washed in a Diaflo filter apparatus, rather than in the centrifuge. The suspension of tissue culture virus was placed in the filter holder, and deionized water was added from the top while the diluted tissue culture medium was being drawn through the filter and out of the bottom, with continuous stirring. We hope that, as experience is gained with this method, it will prove much easier to control than centrifugation, particularly from the standpoints of virus loss during clarification (which is worse with the larger viruses) and aggregation during concentration.

Results of a more extensive experiment led to a different interpretation. In the presence of 250 ppb ion generator silver, the addition of 10% serum at the time of sampling might preclude some undefined carry-over effect on CB-3 in samples taken at 0 and 2 hr (Table 13). The serum was without effect in samples taken at 4 and 24 hr, even though these were tested

without dilution.

Table 13. Arresting silver inactivation of Coxsackie virus type B-3 by adding serum

Silver (ppb)	Serum* (%)	PFU ml^{-1} at 0 hr	2 hr	4 hr	24 hr
0	0	$2.6x10^4$	—	$1.2x10^4$	$9.0x10^3$
250	0	$1.3x10^4$	$2.3x10^2$	34	1
250	10	$2.7x10^4$	$4.6x10^2$	35	1

* Added at time of sampling

Effect of impurities. As is indicated above, we had taken some pains to remove impurities from the virus suspensions used in these experiments. We know, however, that viruses are not shed from the infected host in pure form, so the virus contaminants we had used in the previous experiments probably were not truly realistic. Further, the experiments with serum indicated that here was one impurity which prevented silver ions from inactivating CB-3.

Enteroviruses are shed in feces, and feces might well prove a significant impurity. Though CB-3 had been selected to represent the enteroviruses, it was not available to us as shed in feces. We did have frozen some feces from infants who had received trivalent oral poliovirus vaccine and were shedding one or more of the virus types. We had selected those specimens whose titer exceeded 10^6 PFU ml^{-1} but had not bothered to determine which types of polioviruses they contained. Poliovirus type 1 (Po-1), strain CHAT, produced in PMK cell cultures, served as the comparison. The feces were suspended at 1% (w/v) in deionized water, and the virus from PMK cells was simply diluted 10^{-4} in deionized water or was washed in the Diaflo filter apparatus after dilution. The experimental results were not extremely orderly, but they did indicate that only washed virus was susceptible to inactivation by silver ions (Table 14). The test of the propionate was not really germane to the question, but it suggested again that the silver from the ion generator might work somewhat more rapidly at least during the early period of inactivation.

Table 14. Effects of virus source and purity upon inactivation of poliovirus by silver ions

Virus		Silver		PFU ml^{-1} at	
Source	Purification	Source	Level	0 hr	4 hr
Feces	none	—	0ppb	$9.5x10^3$	$1.6x10^4$
		Ion generator	250ppb	$9.5x10^4$	$1.4x10^4$
PMK cells	none	—	0	$5.9x10^4$	$5.9x10^4$
		Ion generator	250	$9.5x10^4$	$2.1x10^4$
	Diaflo	—	0	$3.5x10^4$	$9.5x10^4$
		Ion generator	250	$8.2x10^4$	$6.2x10^1$
		Propionate	250	$3.6x10^4$	$1.4x10^2$

DISCUSSION

The physical studies have shown us that the electrolytic silver ion generator generates silver ions (though not always) and that ion generator effluents and silver propionate solutions can be stored for long periods in polystyrene containers without measurable loss of silver activity. The finding that polyethylene did not adsorb silver significantly is at odds with that of Chambers et al. (op. cit.). We do not know why, but containers have not proven to be much of a problem in this study.

The neutron activation analysis (NAA) technique gives reproducible (± 10%) assays fairly rapidly. Though we have attempted to identify the technical pitfalls of the procedure, a very great deal must be included under the heading of "operator experience." We would still like to obtain some irrefutable evidence that our judgment is correct with regard to absolute silver concentrations.

The bacteriological studies have indicated that organisms of the genera associated with spacecraft water supplies are killed by silver ions at room temperature. Bacterial concentrations in these experiments have ranged from initial levels of 10^4 to nearly 10^8 cells ml^{-1} in different experiments. Silver concentrations have been intended to be 50 or 250 ppb. It had already been noted that, at 10^4 cells ml^{-1} and 50ppb of silver, there are $\sim 2.8 \times 10^{10}$ silver ions per cell. This is a commentary on the use of the term "oligodynamic." In the most extreme situation (10^4 cells ml^{-1} with 250 ppb of silver), if one estimates the dry weight of a bacterial cell at $2.5x10^{-13}$g, there should actually be more than one silver ion in the system for every atom (other than those in water) in every bacterial cell. At the other extreme (10^8 cells ml^{-1} and 50 ppb of silver), the ratio of weight of silver to dry weight of cells should be approximately 0.002. These are, of course, only extremes within the constraints imposed by our chosen experimental conditions. Though the source of the bacteria that have been found in spacecraft water systems is not known, there is no reason to suppose that

contamination occurs initially at a level of at least 10^4 cells ml^{-1}.

The range on which bacterial concentrations might vary is much more extensive than that for the silver ions, assuming that the bacteria can find any substrate on which to multiply in the spacecraft water system and are not immediately suppressed by the silver. They evidently have found an adequate substrate on occasions in the past, and the substrate has yet to be identified. Thus, while the silver might well affect the organisms' ability to use the substrate, the substrate might also influence the silver's ability to act upon the organisms. In the light of these considerations, it is surprising how limited a variety of results has been obtained. Viable cells were usually recoverable for at least an hour after the challenge was begun (at room temperature), regardless of the concentration of silver. Except for intensively selected cell strains which might have no counterpart in practice, any level of silver ions that we could measure killed at least some cells. Between these two extremes, there have been differences in curve shapes (usually as to the presence or absence of a "tail"). There must be some critical factors: for instance, there is the repeated indication that silver from the ion generator kills more rapidly than the propionate, though the ultimate number of cells killed by a given quantity of silver may be the same. This may have to do with the state of ionization in which these solutions exist, but we really do not know.

The differences in susceptibility of bacterial cells are as yet totally unexplained. If we knew what silver ions did to the cells they kill, we might have some idea of how the selected strains could be resistant. No matter how rigorously the mode of action of silver might be approached, there will undoubtedly always be some lingering area of uncertainty which is dismissed as "species differences."

The differences among viruses are more extreme than those among bacterial species. The two experimental agents which have a lipid envelope (influenza A and vaccinia) are largely or completely insensitive to silver. The model reovirus and rhinovirus were inactivated very rapidly, and the enteroviruses were inactivated at an intermediate rate. The ratio of weight of silver to weight of virus is often very large. A picornavirus (enterovirus or rhinovirus) particle may have a mass of 7×10^6 daltons, and a plaque-forming unit might represent from 1 to 100 physical particles. This means that a suspension of 10^4 PFU ml^{-1} of picornavirus in 50 ppb silver may have a ratio of 4,000 to 400,000 g of silver per gram of virus. Perhaps it is more surprising that the model enterovirus was inactivated at a rate we could measure than that the rhinovirus was inactivated before we could detect it. It is also surprising that even a hint of more rapid inactivation by ion generator silver could be detected against the background of this great ratio. The finding that impurities can protect an enterovirus from inactivation by silver is of uncertain practical significance. More information is needed with regard to this and other silver-sensitive viruses and the effects of various classes of impurities.

SUMMARY

Bacteria and viruses in very pure water were treated with silver ions (added as the propionate salt or by electrolysis of elemental silver). The silver in the suspensions was assayed by neutron activation analysis, using the 659 keV gamma peak emitted by the ^{110}Ag nuclide. Precision was of the order of ± 10% or 10 ppb, whichever is greater. Bacteria were enumerated by colony counts, and viruses were quantitated by the plaque technique in tissue cultures. The bacteria tested were principally of the genera Achromobacter, Alcaligenes, Flavobacterium, and Pseudomonas. Each test species was killed by silver both at nominal 50 and 250 ppb concentrations, but some were apparently more susceptible than others. The rate of killing was found to vary with cell numbers, with silver level, and with water temperature. Killing by silver from the electrolytic ion generator may have been somewhat more rapid initially than by that which was added as the propionate. Silver apparently is bound to the cells during the killing process. Silver resistant strains of two species were obtained by a rigorous selective process. Five unrelated test viruses were inactivated in silver solutions at room temperature at rates which ranged from faster than we could measure to essentially nil. The viruses which were least sensitive to silver had lipid envelopes.

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