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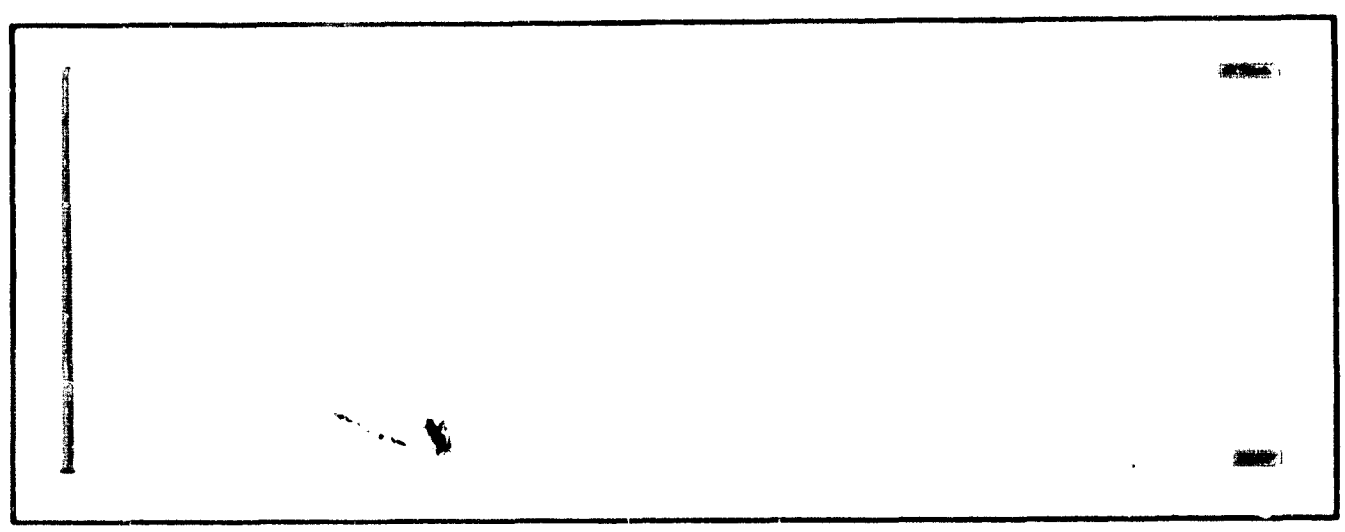

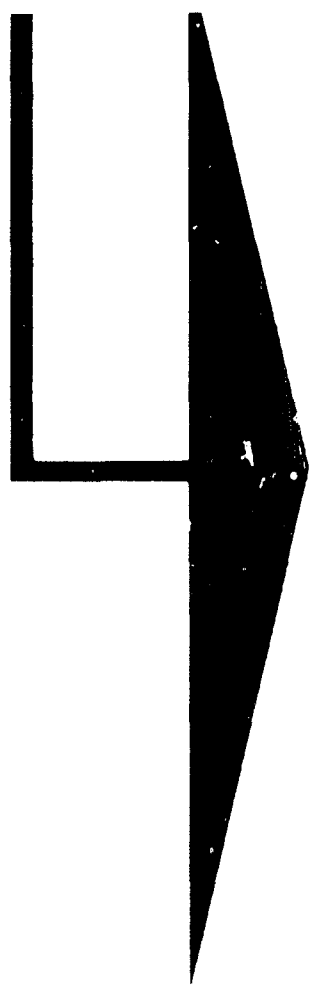

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PHASE I REPORT

STUDY FOR CONTROL OF MICROBIAL GROWTH IN MANNED SPACECRAFT

30 October 1964 - 30 January 1965

Contract No. NAS9-3563

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Prepared for

National Aeronautics and Space Administration
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ABSTRACT

Spacecraft Environmental Control Systems were critically examined with the objective of locating potential sources of difficulty where microorganisms might grow, concentrate, and cause operational problems. Known methods of microbial control were studied with the objective of determining their suitability for application to the environment of spacecraft. Evaluation criteria applied to these methods dealt with power-weight requirements, outgassing properties, mutagenicity, human toxicity, and water potability. Optimum spacecraft locations were determined for the most promising control methods and devices.

It was concluded that filtration methods would be most effective in controlling microbial growth in space suits and spacecraft atmospheres. The use of filters with and without impregnation, in addition to a removable biocide, was judged most appropriate for the control of microbial growth in the potable and condensate water systems.

Several methods were discussed which are capable of rendering a variety of fabrics biocidal or biostatic. These methods, utilizing silver impregnation, allow the metal fabric product to be curative to dermal irritation apparently without causing dermal sensitivity.

In addition to this conceptual evaluation an experimental device, useful in microbial control, was designed specifically for spacecraft water systems. The device employs a series of resins which release a biocidal material in proportion to microbial contamination, then removes the biocide from waters used for drinking or other spacecraft operations.

1. INTRODUCTION

This is the Phase I Report submitted in compliance with Contract NAS9-3563, and describes the work performed during the period 30 October 1964 to 30 January 1965. The contract effort is concerned with (1) a study of methods which are biostatic or biocidal and can be used to control microbial growth in manned spacecraft environments, and (2) the delivery of an item or items which will provide for control of microbial growth in manned spacecraft environments.

The specific constraints on the design of a microbial control system for spacecraft use were taken as evaluation criteria in studies of established methods of microbial control. These criteria were the following:

(a) The microbial control should be effected at spacecraft temperature, humidity, and gaseous environment.

(b) The control should have minimum weight, volume, and power requirements.

(c) The control should be simple to operate and maintain.

(d) The control should add no adverse conditions to the spacecraft environment requiring correction. Adverse conditions would be specifically represented by offgassing, water palatability problems, generation of resistant or virulent microorganisms.

(e) The control should use material nontoxic to humans and incapable of producing sensitivity in humans.

Filtration methods for water and atmosphere with and without impregnation, biocidal coatings for water and atmosphere, and irradiation methods were evaluated both from reports in the microbiological literature

and from experiments in the microbiology laboratory at Melpar. Special emphasis was given to a conceptual and experimental evaluation of the microbial control methods which function by the addition of bactericidal compounds to water followed by their removal after sterilizing action has taken place.

It is felt that the control of bacterial contamination in the space vehicle can be accomplished by the systems proposed in this report. By means of the various proposed methods the space vehicle will be changed from a repository of microorganisms into a self-decontaminating area. These changes will be achieved by the addition and removal of bactericidal substances and the removal of bacteria by filtration. Microbial filtration measures, regardless of microbial viability and pathogenicity, will reduce hazardous concentrations in the spacecraft air and water systems possibly to tolerable dosage levels for astronauts even under conditions of unusual stress.

2. SUMMARY AND CONCLUSIONS

A study was conducted in the first three months of this program to find the most promising methods for controlling the microorganisms in the environmental control systems of spacecraft, the space suit, fuel cell water and condensed water, and the confines of manned spacecraft. Concepts of microbial control appearing in the microbiological literature were analyzed for direct application to the Apollo capsule. Visits were made to NASA Houston, the Air Medical Laboratories at Brooks and Wright-Patterson and talks were held with other people knowledgable in methods of microbial control*. Short term laboratory experiments were also conducted to help illusidate some problem areas and make some preliminary tests of new methods and re-evaluation of old methods.

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* See Appendix

The usefulness of several methods of microbial control were considered from the biological and the engineering aspect of each of the spacecraft components listed above. Methods of microbial control were evaluated for spacecraft use first from the standpoint of health and safety of the astronaut. These methods were then evaluated with respect to efficiency of operation in the closed systems of space vehicles.

Particular attention was given to the control of microbial populations of space vehicles using ultraviolet radiation, bacteristatic coatings, impregnated and nonimpregnated filters. Studies were made both of the bacteristatic properties of water additives and methods for removing such additives following sterilizing treatments of water.

Several potentially useful methods for controlling microorganisms in the air of spacecraft were found during this critical evaluation. For example, it was found that air filters capable of removing bacterial aerosols were highly efficient and have low power input requirements. Furthermore, it appeared possible that high efficiency filters could be impregnated with biocides without significantly altering their filtration characteristics in air purification.

A number of other methods were found to be less attractive for air purification for the following reasons: the power-weight requirements of the electrostatic precipitation methods and impaction/impingement methods were too high for spacecraft application. Ultraviolet light for air purification was considered of marginal value because of offgassing problem, high power, and mutagencity.

Three methods were found useful for the control of microbiological contaminants in the fuel cell water and condensate water of the Apollo spacecraft. These were filters, dye solution irradiated with visible light, and the use of certain heavy metals.

This study found that filters capable of removing bacteria from water did so with a very small pressure drop revealing a low power requirement. This power requirement was not greatly enhanced even with appreciable filter loading from algi and silt. Ultraviolet light was unattractive for microbial control in the water systems because of the power requirement and mutagenicity.

Dye solution irradiated with visible light was tested in the Melpar laboratories and found acceptable for water purification. Killing of test organisms was accomplished by concentrations of dye as low as 10^{-6} molar using a small fraction of the output of a 100-watt light bulb. The quantity of dye used can be easily removed from the system by very small quantities of exchange resins.

By every evaluation criterion employed in this study, silver proved to be the most effective sterilizing agent for use in the water systems of Apollo. Silver coatings and cation exchange resins in the silver form were found to be exceptionally effective devices for killing bacteria and inactivating virus. The silver ion or its compound in water systems was entirely removed by cation exchangers. Silver was not reported to act as a mutagen and it does not cause skin irritation. The quantities of silver used for microbial control are not toxic to man.

A system for microbial control in spacecraft water was designed at Melpar and a breadboard system based on that design was tested. The device employed a series of ion exchange resins containing silver and resin mixtures. When bacterial or viral suspensions pass through resin systems they were killed without power input and without the addition of adverse conditions to the spacecraft environment. Techniques using silver were found also for the control of microorganisms in a variety of fabrics. Impregnated garments produced a bactericidal effect and a currative effect in lessening skin irritation.

As a result of these studies the following specific recommendations should be tested:

1. It was proposed that appropriate filters be located in the CO_2/Odor Absorber in the Apollo ECS. A roughing filter in the Air Recirculating Unit should be considered to minimize loading of bacterial filters under worse case conditions.

2. The use of filters for water purification was indicated at critical locations in the water supply system originating with the oxygen-hydrogen fuel cell and terminating in drinking water supplies.

3. Wicking materials should be silver impregnated and surfaces on which water is condensed, or is stored, should be coated with silver.

4. A Melpar designed ion exchange resin system containing silver and capable of inactivating both bacteria and viruses should be tested for incorporation in series with water flowing in the Apollo water systems. To insure sterilization the water should pass through these resin systems at the port of entry into storage areas and at ports of exit where water is used for drinking or other applications.

5. It is also proposed that a photodynamic method of sterilization be used as a redundant control in drinking water purification.

Table I summarizes the strengths and weaknesses of these control methods and indicates their application in spacecraft design.

From this study the conclusion was drawn also that spacecraft equipment should include methods for detecting the level of microbial contamination in the air and water. It was pointed out that the operation of microbial detection devices would have several advantages in the space vehicle. They would reveal whether or not microbial control measures were functioning properly. Knowledge of failure in these devices could be obtained before challenging doses of contaminating organisms were generated in air or water. The presence of a detection device would also permit the intermittent application of sterilizing agents and thereby conserve both agent and power output.

Future research was proposed to determine the specifications of the developed breadboard microbial control device. The capacity to kill large varieties of organisms at high cell concentration should be determined. Future research should also attempt to identify the chemical state or compound of silver which is responsible for biocidal activity. This information would make possible a broader and more rational application of silver to spacecraft sterilization needs. Additional attention will be given to detection methods useful in spacecraft environments.

From the research performed and from the present state of familiarity with the problem, a tentative scheme of study has been developed and is summarized in table 2.

TABLE I*

SUMMARY - PERFORMANCE OF
MICROBIAL CONTROL METHODS FOR SPACECRAFT

Air Control Methods	Performance Rating & Comments
Filtration (non-impregnated)	Highly effective - no significant penalties
Electrostatic Precipitator	Marginally effective - power & weight penalty
Ultraviolet Light	Marginally effective - power, weight, & mutagen penalties
Filtration (impregnated)	Effective - biocidal function is redundant
Impaction & Impingement	Ineffective - severe power requirement
Water Control Methods	
Silver Coatings and Cation Exchange Resins	Highly effective - meets study criteria
Filtration (impregnated)	Highly effective - meets study criteria
Filtration (non-impregnated)	Highly effective - meets study criteria
Photodynamic Activated Dyes	Effective - small power penalty
Ultraviolet Light	Effective - high power penalty and mutagenic
Chemical Additives Other than Silver	Effective - palatibility penalty

* Table D in the Appendix gives more complete information.

TABLE 2

TENTATIVE OUTLINE OF FUTURE WORK

Phase II

Microbial Control of Air:

a. Selection and Placement of Filters in ECS

b. Power requirements for ECS with filters

Microbial Control in Spacesuit

a. Use of Filters in Air Supply

b. Use of Fabrics Impregnated with Bactericides

Microbial Control in Condensate Water

a. Use of Bactericides for Impregnation of Wicks

b. Application of Silver Resin Column Systems to Condensate Water Purification

Microbial Control in Fuel Cell Water, Waste, and Drinking

a. Screening Study of Bactericidal Activity of Silver Compounds

b. Study of the Control of Silver Compounds in Water Systems

c. Dosimetry determination of Biocidal Agents with Respect to Various Species

Building of Control Methods Based on Metal Buffer Concept and Bactericidal Resin Systems

a. Establish Specifications of Control with Respect to Temperature, Loading Capacity, etc.

b. Modification and Miniturization of Control System

Conceptual Design of Detection Device for Monitoring Microbial Systems in Air and Water

3. AN ANALYSIS OF SOURCES AND ROUTES OF MICROBIAL CONTAMINATION IN THE APOLLO SPACE VEHICLE

In the Apollo spacecraft the respiratory, oral, dermal and lower excretory effluents of man, and contaminated fuel-cell water are considered the primary sources of microbial contamination. From these sources the most critical contamination originates and it is distributed in the spacecraft air and water systems. Because these sources are not subject to absolute control, numerous secondary sources of contamination develop from them during prolonged habitation. Secondary sources of potential hazard in Apollo are illustrated in figure 1 along with the routes by which microorganisms may be distributed.

The Environmental Control System (ECS) of Apollo has been analyzed from the standpoint of determining areas where microbial contaminants most probably would be concentrated in great number. Contaminating particles from the exhaust air of space suits and the capsule will be collected continuously on the filter elements of the Debris Trap and CO_2 Odor Absorber. These ECS elements are shown at points A,B, and C in figure 2. High concentrations of microorganisms and other organic material will collect on the surfaces of these filters. This material will be exposed to the flow of gases of high oxygen concentration and humidity. Thus, areas which collect particulate matter may become themselves breeding grounds for microorganisms and significant sources of secondary contamination.

Another probable area of microbial build-up appears to be in the Air Recirculating Unit shown in figure 1. This unit works to maintain a fixed temperature by recirculating the air of the capsule through the finned surface maze of the heat exchanger. These surfaces contain considerable moisture and therefore facilitate both particle adhesion and efficient impaction.

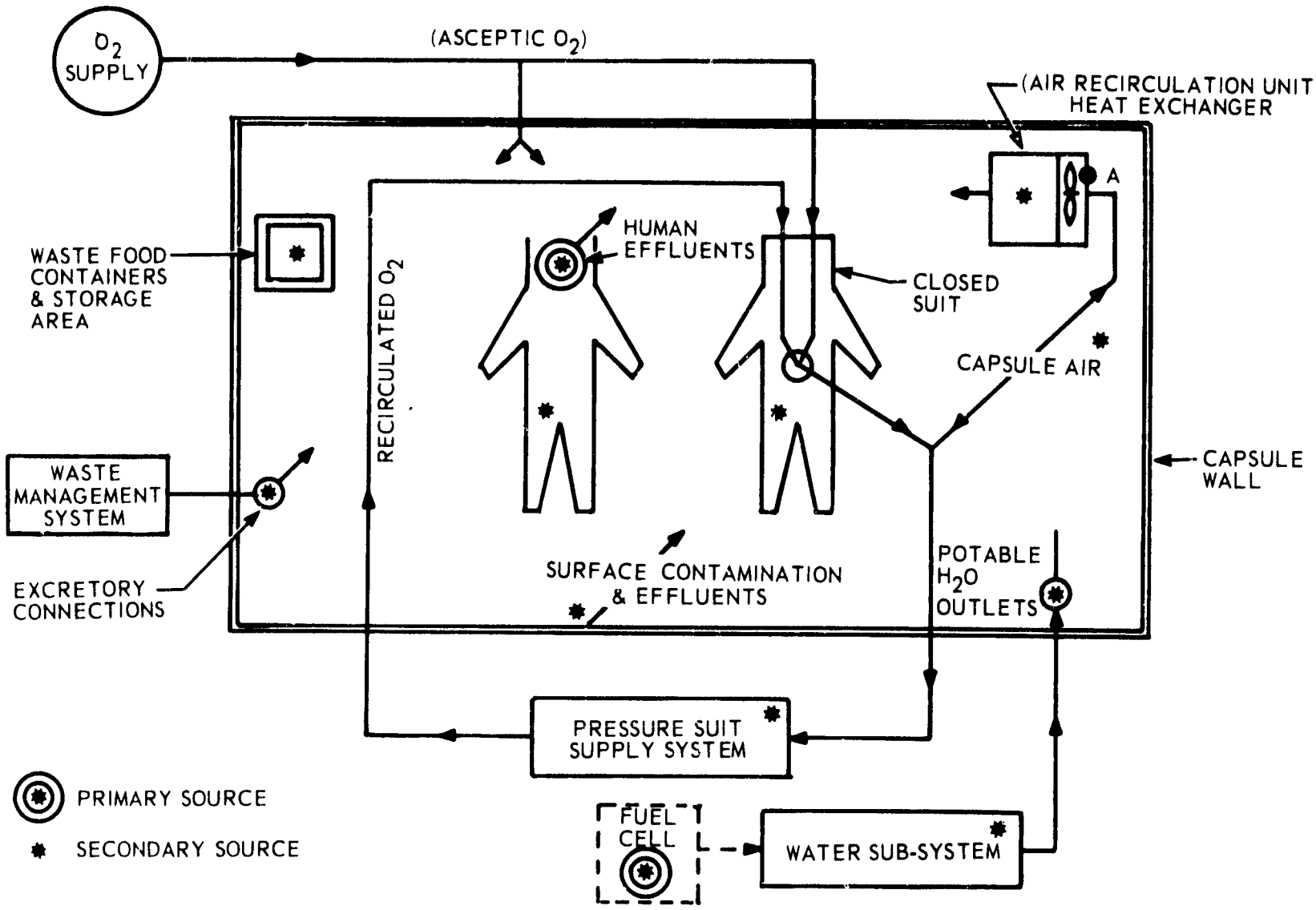


Figure 1. Sources of Contamination - Environmental Control System (Apollo)

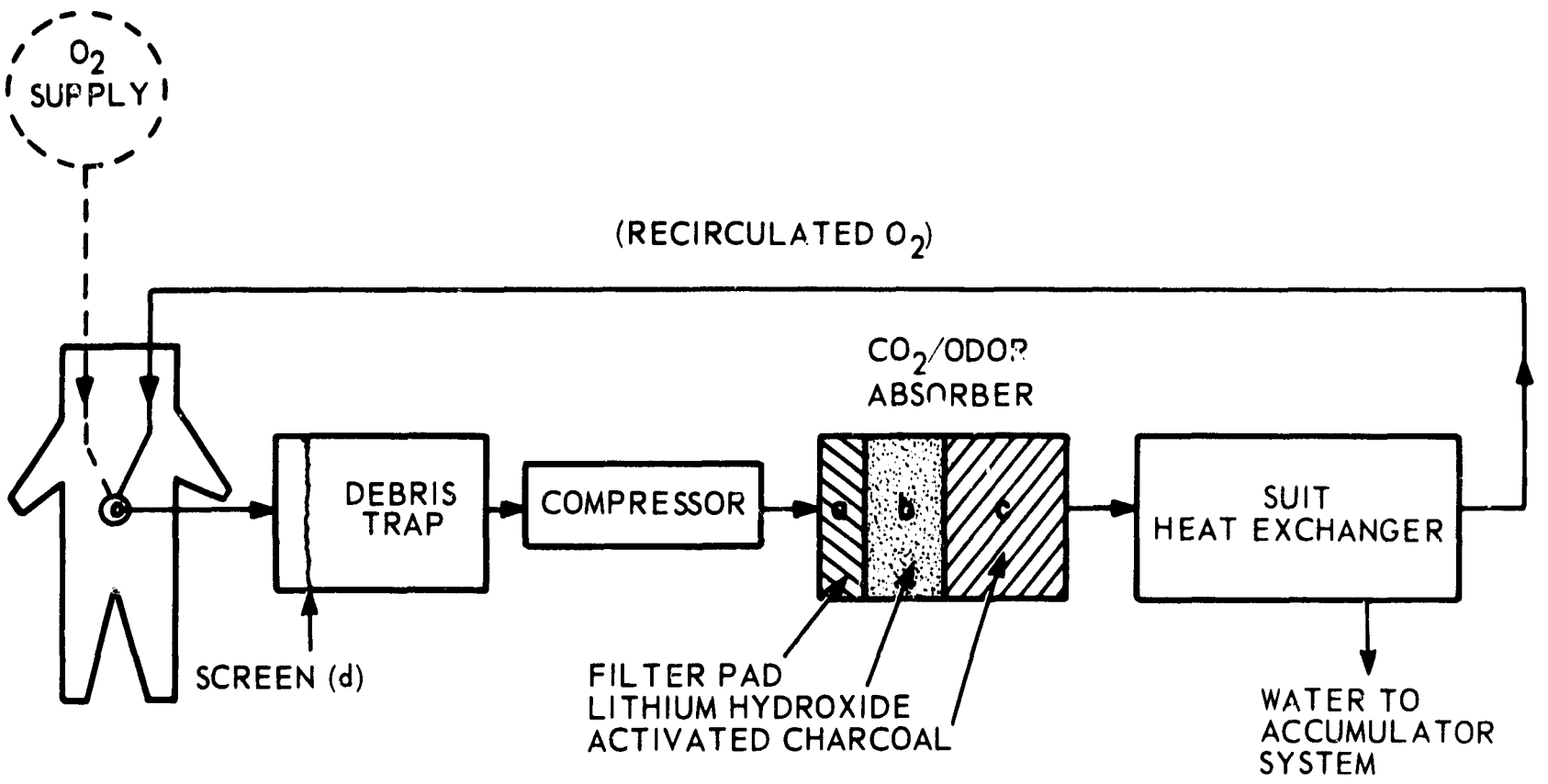


Figure 2. Pressure Suit Supply System Circuit

The sources of water-borne microbial contamination in spacecraft cannot be firmly identified without tests on the operating water subsystem (figure 3). The use of hydrogen-oxygen fuel cells for generating electrical power and potable water is reportedly capable of producing water of good chemical quality but with probable microbial contamination.[4] Also, water from the humidity-control system is of good chemical quality but tests indicate that the probability of microbial contamination is greater than with fuel-cell water. Other water system components may become secondary sources of contamination by trapping and supporting the growth of microorganisms.

The water subsystem for Apollo, shown in figure 3, is essentially a system closed to possible cross-contamination from other system sources. Yet, there may be an exception due to the operation of the suit heat exchanger. Oxygen and free water enter the heat exchanger where wicking material collects and transports the water at a very low pressure head while the oxygen is not passed at low pressure (figure 4). The wicking material, by collecting the water at the point of condensation, will probably collect bacteria present in this water should the CO_2/Odor Absorber pass bacteria from upstream. By holding bacteria on its surface, the wicking material could contaminate the recirculated oxygen flowing past the material into the suit.

In addition to oxygen contamination, there exists a possibility of the wicking material ultimately contaminating the potable water. The routes of possible contamination are shown cross-hatched in figure 3. This contamination would occur if the following series of events took place. Referring to figure 4, the cabin/suit air was contaminated at (a), the CO_2/Odor Absorber passed contamination at (b), the wicking material collected and

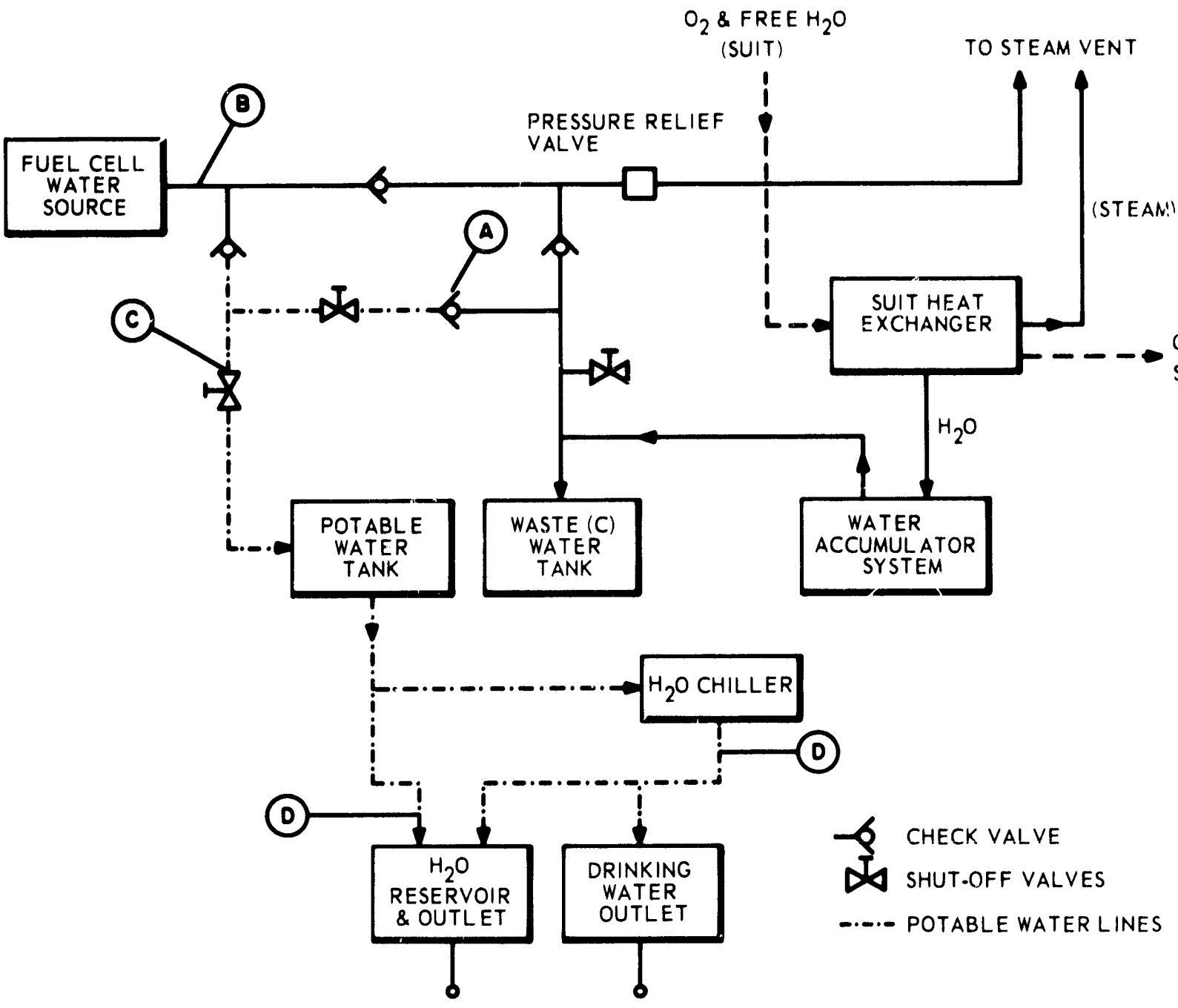


Figure 3. Water Subsystem Circuit

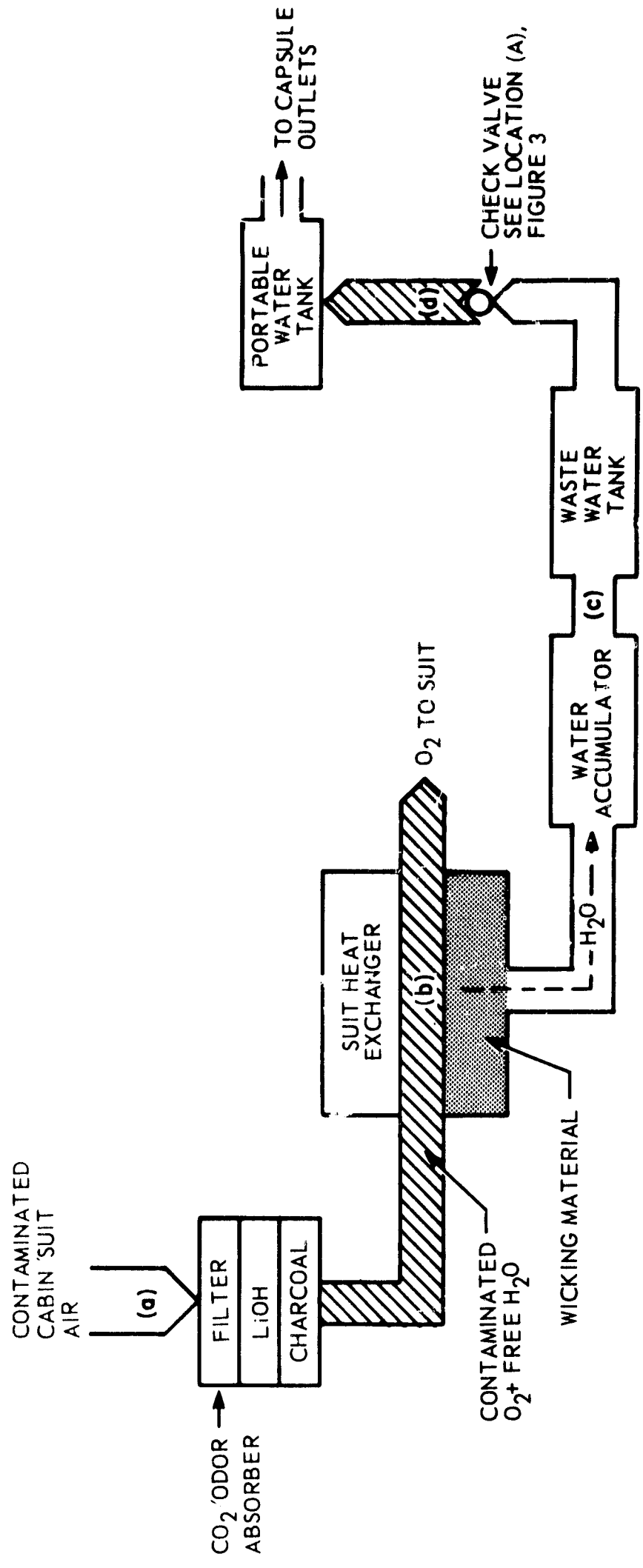


Figure 4. Potable Water Contamination Route

passed contaminated water, ultimately through the waste water tank, at (c), and check valves malfunctioned at (d).

Many of the control measures described in this study appear suitable for confining microorganisms to primary sources or rendering them nonviable in their passages from primary sources to other areas in the space vehicle. The applicability of these methods will be suggested with the knowledge and reservation that the terminal configuration of the Apollo systems continue to be designed.

3.1 The Behavior of Airborne Contaminants

Air environment can be important in the dissemination of microbial cells. These cells, varying in size from a few microns to a fraction of a micron, can become suspended in air through a variety of mechanisms. The organisms once suspended remain in air for long periods of time and they can be dispersed over a wide area by atmospheric diffusion.

A particle 1 micron in diameter, the size of some bacteria, settles downward in air at a rate of only 5 inches per hour, and an aggregate of particles 10 microns in diameter falls at about 0.6 feet per minute. Obviously, small air currents such as those caused by thermal convection are sufficient to propel them in the absence of gravitational attraction.

In a spacecraft atmosphere a certain portion of the organisms suspended will remain viable, and these organisms can be transported from the interior of space suits and carried by cabin air currents originating from the function of the ECS as well as from the motion of the crew.

In this study consideration is given to the theoretical behavior of aerosolized particles. These particles are considered to be either

microbial masses themselves or debris capable of transporting microorganisms. Methods are discussed which are capable of controlling airborne organisms or rendering them nonviable.

3.1.1 The Distribution, Generation, and Deposition of Aerosols

3.1.1.1 Aerosol Distribution: The atmosphere of spacecraft will contain aerosols (both biological and nonbiological) in concentrations which represent a balance between the rates of aerosol particle generation and particle removal by various processes. Physically, the microbial particles while suspended will behave similarly to other particles of the same size and need not be distinguished so far as generation and translocation are concerned. In fact, it can be expected that viable microbial particles will physically adhere to nonviable material quite often. This consideration could be of particular importance at filter surfaces or in impinger fluids because the nonviable material may serve as substrate permitting the growth of large microbial concentrations on these surfaces.

In the absence of gravity it becomes necessary to redefine the term "aerosol." Aerosols consist of particles suspended in a gas, the particles being small enough to stay in suspension for appreciable periods. Thus, a natural fog might be considered an aerosol, while a rain is not. Without gravity, this distinction disappears. However, in the atmosphere of the spacecraft there is still an important difference between the behavior of large and small particles. The small particles have a relatively high ratio of surface-to-mass, and therefore, move through the atmosphere primarily by the influence of viscous drag forces. Large particles, on the other hand, are affected primarily by inertial forces, and are relatively unresponsive to gas flows at moderate velocities.

In addition to the small particles characteristic of aerosols, the atmosphere of the spacecraft may at times contain relatively large objects from food or equipment. Some of these could lodge in out-of-the-way locations and furnish sites for microbial growth. Coarse debris also may enter the equipment used for aerosol control, and interfere in the operation of that equipment. This problem is not encountered in dust collectors in gravitational fields. Coarse objects settle out of the air. In designing aerosol control equipment for the spacecraft environment special attention must be given to means for excluding coarse debris and avoiding interference from this source.

3.1.1.2 Aerosol Generation: Aerosol particles are formed by attrition whenever solid surfaces are rubbed or flexed and they are considered here because of their ability to carry microorganisms. In a space cabin the movement of personnel, the manipulation of objects, and any mechanical motion or vibration will produce some aerosol particles. The amount and nature of these particles will depend on the materials involved, and considerable control can be exercised through original vehicle design.

Experience with clean room environments for precision manufacturing operations has shown that people are a prolific source of aerosols and the aerosols may be the vehicle for microbial transport. The aerosols originate from skin and hair shedding, body excretions, and breathing. Even if all reasonable precautions are taken to minimize the contribution of these sources, a certain amount of aerosol product will remain to be dealt with.

Air movement produces aerosols by picking up particles from surfaces and the dispersion of powders and liquids. The atmospheric generation and purification systems of spacecraft could be an important source of aerosol generation. Power and space requirements as a function of the efficacy of aerosol filtration will be discussed.

3.1.1.3 Aerosol Deposition: In the absence of gravity, aerosol particles are effectively removed from suspension by contacting a fixed surface to which they are held by mechanical restraint and by chemical or physical interaction.

Aerosol particles in the cabin will move toward the walls and other exposed surfaces at a rate which depends on several factors. The most important driving force will be furnished by the "air" currents which can easily reach random local velocities of several feet per second. Small particles will be carried about by the gas at essentially the same velocities. Statistically, a certain fraction of the particles per unit time will approach a surface closely enough for the particle to be "captured" and held by surface attractive forces.

The removal of particles on certain sections of the walls may be increased by increasing the "sticking" probability. This may be accomplished by providing a tacky adhesive surface or by using a dielectric material which readily builds up high electrostatic charges. It might also be possible to utilize the "thermal precipitation" effect, whereby small particles are preferentially deposited on a surface which is maintained colder than surroundings.

Further detailed study is needed to determine the advantages of inducing such deposits of aerosols on selected portions of the surface of a space vehicle cabin. The problems of inhibiting microbial growth on the surface and of determining relative collection efficiencies need to be considered in relation to the corresponding problems involved in the use of conventional particle collectors.

3.2 The Control of Airborne Contaminants

Airborne microbial contaminants can be effectively dealt with either by physical removal from the air system or by inactivation in the air by chemical or physical agents.

Methods of inactivating airborne contaminants may require the input of energy and require the addition of substances to the atmosphere such as ultraviolet radiation or biocidal compounds.

Several methods of removing bacteria are discussed. The application of Electro Precipitation Methods to the removal of bacteria from spacecraft atmosphere is outlined, and the removal of airborne organisms by impaction and impingement methods is analyzed. Detailed consideration is given to the use of air filters which require little energy for operation and which neither generate resistant organisms or cause outgassing difficulties.

3.2.1 Ultraviolet Light

Ultraviolet light can be effectively germicidal in air or water in favorable circumstances. A low-pressure mercury arc may return 60% of its electrical input as radiation of wavelength 2537A. This is close to the peak of the germicidal action curve at about 2650A (figure 5). The

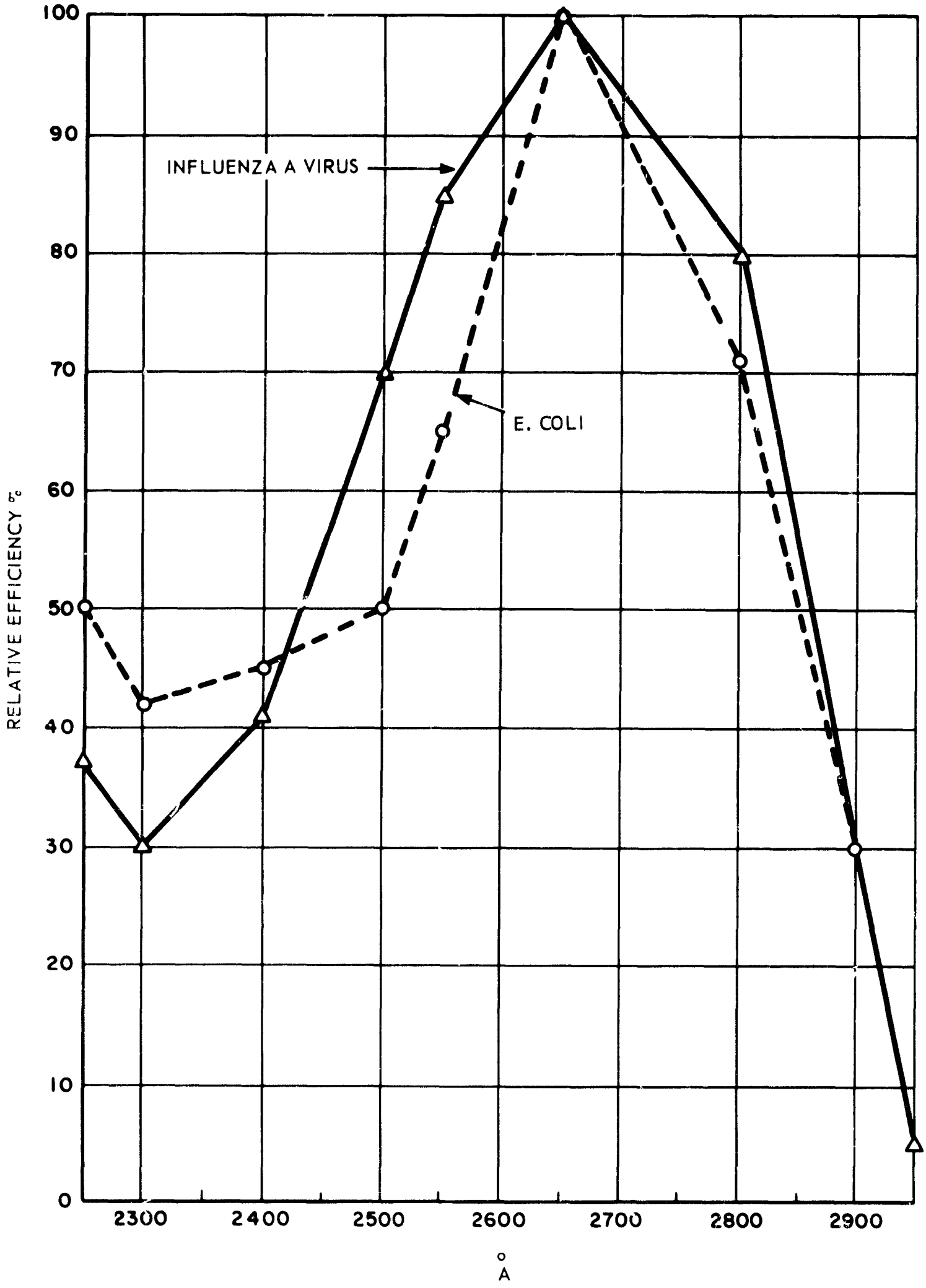


Figure 5. Sensitivity to UV as a Function of Wavelength

relation between effect and wavelength is very similar for a large variety of bacteria, fungi, and viruses. But the ultraviolet dose needed to kill different species in various circumstances ranges over several orders of magnitude (table 3).

TABLE 3

EXPOSURE TO 2537A TO KILL 50% IN ONE MINUTE

Organism	μwatts/cm^2
α- Streptococcus sp. and Staphylococcus aureus	0.75
β- hemolytic Streptococcus sp. and E. Coli	3.4
Mold Spores (typical)	19.
Aspergillus niger spores	250.

The Bunson-Roscoe relationship has been established for ultraviolet radiation and therefore we can convert these figures to 1-second exposure by multiplying by 60, giving a range of 45 to 15,000 μwatts/cm^2.

A range of small tubes is commercially available. Characteristics of 3 tubes are shown in Table 4. It will be seen that these small units are about 20% efficient. The surface area of the G4T4 tube is approximately

TABLE 4

POWER REQUIREMENT AND OUTPUT OF LOW-PRESSURE MERCURY ARCS

Type	Input, watts	UV Output, watts
G4T4	4	0.7
G8T5	8	1.5
G15T8	15	3.3

7 cm^2 and the UV flux at the surface is therefore about 10^5 $\mu w/cm^2$. This diminishes with distance and with passage through air or water, especially if absorbing matter is present in suspension or solution. Loss of power by absorption at the surface enclosing the irradiated area is almost complete unless a suitable reflecting surface is used. This may be specially prepared aluminum or chromium plate, both of which have at least 50% reflectance. It will be seen from comparison between the calculated output of 10^5 $\mu w/cm^2$ and the LD_{50} (50% lethal dose) figures of 45 to 15,000 $\mu w/cm^2$ second that disinfection should be excellent with a dwell time of 1 second in an air duct.

A typical air duct disinfecting intensity for exposures of 1/8 to 1/2 second is 10,000 $\mu w/cm^2$, which is seen to be well within the capacity of the small tubes listed in table 4, provided the linear velocity is appropriate. For a tube length of 6 inches, a dwell time of 1/2 second corresponds to a linear velocity of 1 ft/sec. If the diameter of the enclosure is 6 inches, the volume of throughput corresponding with the velocity of 1 ft/sec is about 1/5 cu ft/sec or 12 cfm. It will be seen that the volume of indicated space with a low intensity source may need to be quite substantial at high flow rates.

Ultraviolet light from mercury arcs has one side effect which may be troublesome in the closed environment. A significant amount of energy is indicated beginning with the 1849A line. This energy is effective in converting oxygen to ozone. Although many factors catalyze the reconversion to oxygen (humidity, visible light, 2537A ultraviolet, surface adsorption), the half-life of ozone is several minutes in practical conditions. Actual

experiment in a system exact in all details of materials and dimensions would be necessary to determine the actual rate of build-up of ozone concentration and whether a catalytic bed would be needed to limit this.

In considering the application of ultraviolet light to spacecraft sterilization it should be recalled that it is highly mutagenic and may exert this effect either directly or indirectly by modifying other substances in aqueous suspensions of microorganisms. The resultant mutants can exhibit not only resistance to ultraviolet light itself but also resistance to other disinfectants.

If ultraviolet light were to be used, an interesting "free" source exists in sunlight. At the earth's surface, very little energy below 3000Å can be measured because of absorption in the atmosphere and especially in ozone layer. In space, the ultraviolet spectrum approximates to the theoretical 6000°K block body radiation within a factor of about 3-fold at 2650Å and is of considerable intensity. The available information has been very fully surveyed by Hinteregger.[11] One cannot make much use of his figures for intensity in the Schumann region and below, because there is practically no information on the microbiological effects of short UV, but the estimated total energy in the 3000-2000Å region of known biocidal efficiency is 1.9×10^4 ergs/cm^2 sec or 2.6×10^{15} photons/cm^2 sec. This is equivalent to about 1.8×10^2 μw/min/cm^2. Figures from a different source for "top-of-the-atmosphere" radiation between 3150A and 2200A give about 1.8×10^3 μw/min/cm^2. The exact value is of no particular importance for it is clear that in this case a highly effective flux of biocidal energy is available. This could be tapped by passing the "air" or water of the

spacecraft through a chamber open to the sun's rays through a UV-transparent window of quartz or suitable hard glass.

It is not possible at this moment to evaluate the practical implications of this idea but it would appear that the use of sunlight has several undesirable features. One of these is the need either to have a multiplicity of "windows" or to keep one area always on the sunward side of the craft. Another is the obvious objection to any breach in the hull which is not essential. These objectives are not offset by getting "free" what can be provided for a few watts of electrical energy.

In summary, it appears that, for the spacecraft atmosphere, ultraviolet light offers no advantage over the simple mechanical filtration that we recommend elsewhere and is at a disadvantage in greater weight, volume, complexity, and lower reliability.

3.2.2 Electrostatic Precipitation

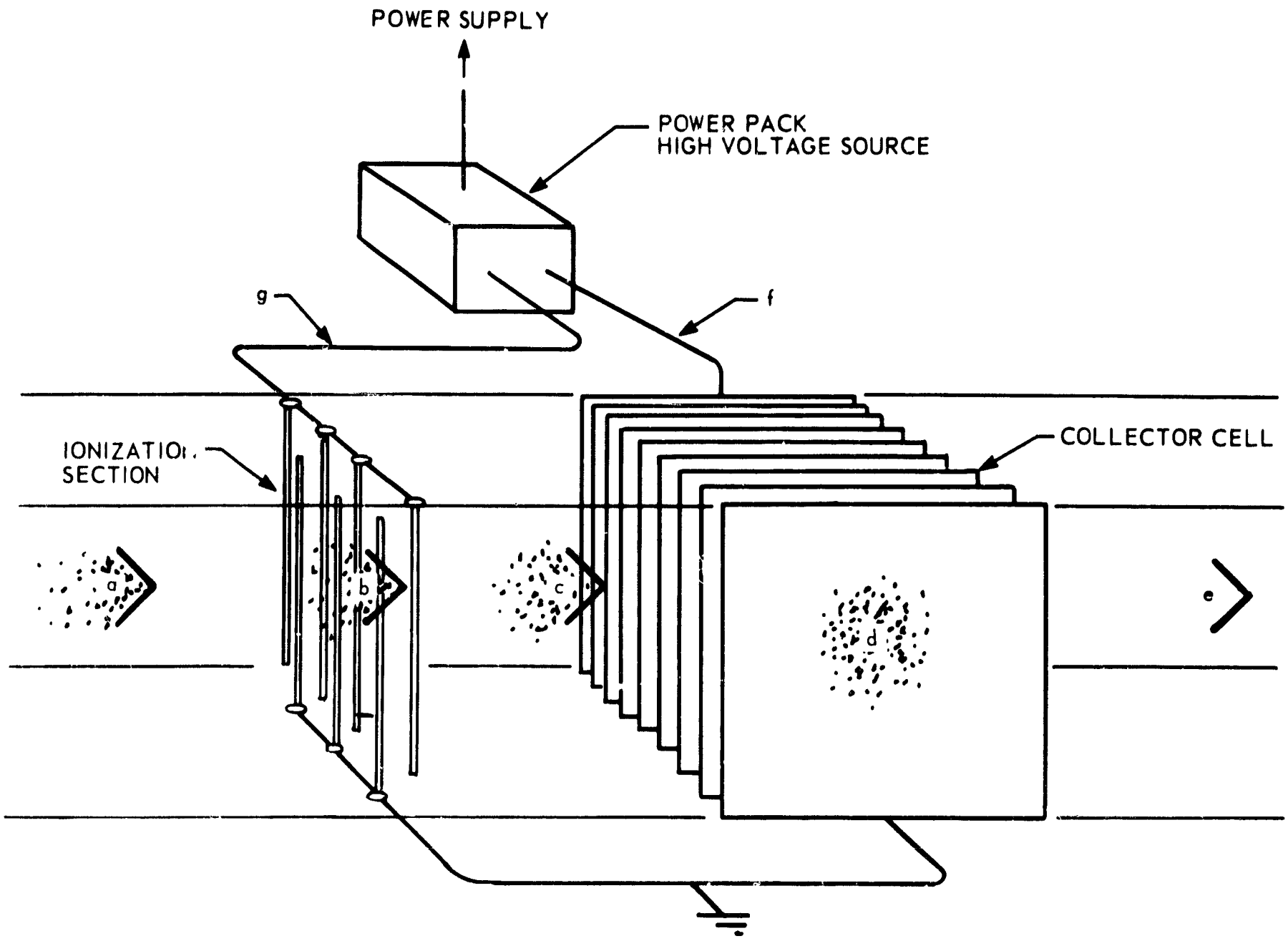
Electrostatic precipitation is a widely used industrial technique for the purification of gases and for reducing air pollution. Since precipitator characteristics typically involve a very low pressure drop, the processing of large flows and the collection of particles in a wide size range extending to the finest aerosols, it is particularly attractive for spacecraft filtration of microbial aerosols.

The general efficiency of electrostatic precipitators is usually over 90% and approaches 100% under carefully controlled conditions. Statements of efficiency are often misleading because information is lacking on methods of assaying samples for the mass and particle size determinations. Theory and practice show the present designs of precipitators cannot reach

100% efficiency, because of turbulence and low migration velocity. Some of the very small particles may never reach the collecting electrodes, and large particles are particularly liable to re-entrainment. Experience suggests that the performance of a precipitator in practice can only be forecast by testing elements of full size. Theoretical experessions of efficiency[1] predict much higher efficiencies for larger particles than for fine, but is generally found that the efficiency is of the same order both for large and fine particles and either one may be higher than the other.

Electrostatic precipitators can operate over a wide range of conditions of temperature and pressure. Lapple (1950)[1] has reported units working up to 1200°C. Providing the gas is dry, temperature may be as low as -100°F without undue loss of efficiency. Humidity/temperature can have pronounced effects on the ionization process in precipitators and give rise to condition which may be favorable to precipitation (Sproull and Napada, 1951)[1]. The high humidity, and especially the high oxygen concentration of spacecraft atmospheres, will heavily influence the design of the ionization section of a spacecraft unit to minimize arcing.

The power and weight requirements of any electrostatic precipitator utilized in spacecraft cannot easily be determined by this study. This is because the power and weight will be primarily a function of how well the essential operating elements of the electrostatic precipitator can be integrated with existing components of spacecraft systems. As a mirimum, a simple two-stage precipitator requires ionization and collecting sections, a power pack (source of high voltage) and blower unit. (See figure 6). The ionization elements of precipitators consists of wire electrodes that are



a. UNCHARGED PARTICLES
b. CHARGING PARTICLES
c. POSITIVELY CHARGED PARTICLES
d. PRECIPITATED PARTICLES
e. CLEAN AIR
f. 6,000 V D-C TO POSITIVE PLATES
g. 13,000 V D-C TO IONIZER ASSEMBLY

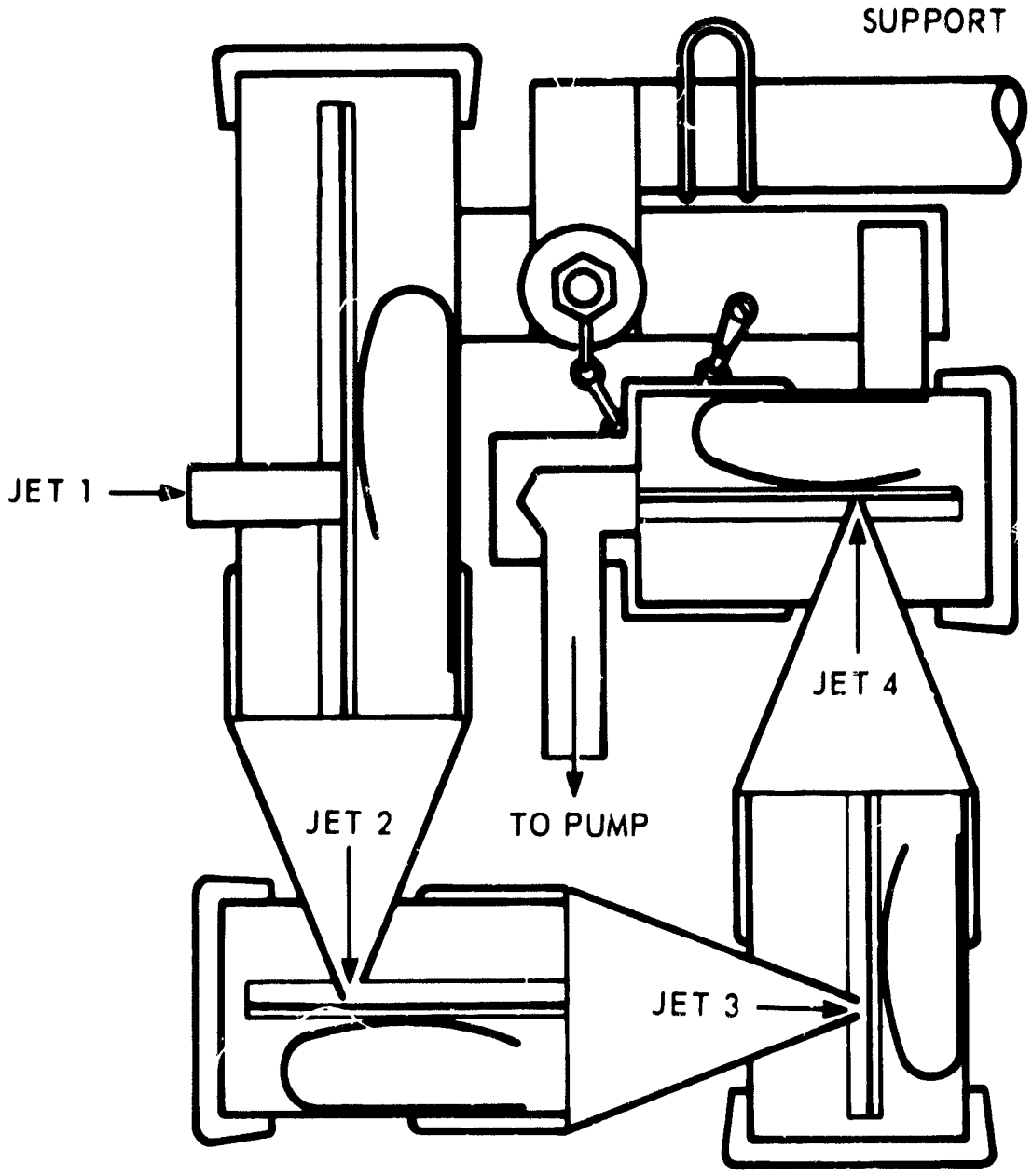
Figure 6. Ionizing and Collecting Sections of a Typical Two-stage Electrostatic Precipitator

raised to a high, usually negative potential, of 25 to 100 Kv. The particles first pass these electrodes and are charged in the corona discharge from the wire before passing on to the collection system – a system of electrodes which are alternately charged and grounded. Disregarding possible dangers with such an ionization element in spacecraft atmospheres, this element and its high voltage source would probably represent an addition to existing spacecraft systems. Only the blower unit and collection sections might be conveniently integrated with the cabin recirculation blowers and the cabin heat exchanger structure of the Air Recirculation Unit (figure 1). This unit appears most suitable for the integration of a precipitator to reduce particulate loading in the capsule atmosphere.

3.2.3 Impaction and Impingement

The collection of aerosol particles on surfaces or in liquid media can be accomplished by a large number of devices utilizing inertial deposition techniques. It is desired here to discuss their performance in the light of their dynamic principles so that an evaluation can be made of the suitability of using these devices to control microorganisms in a space vehicle.[6]

The Cascade impactor is a particle sampling device utilizing inertial principles to efficiently collect small particles ($\leq 20\mu$). (See figure 7). It is typical of a series of impaction devices (Owens Jet Dust Counter, Bausch and Lomb Dust Counter) in which the sample is drawn in at a very high speed through a long narrow slit to impinge on a flat plate. Impaction of the particle arises primarily from inertia carrying it across streamlines so that it hits and, in many cases, sticks to the obstruction



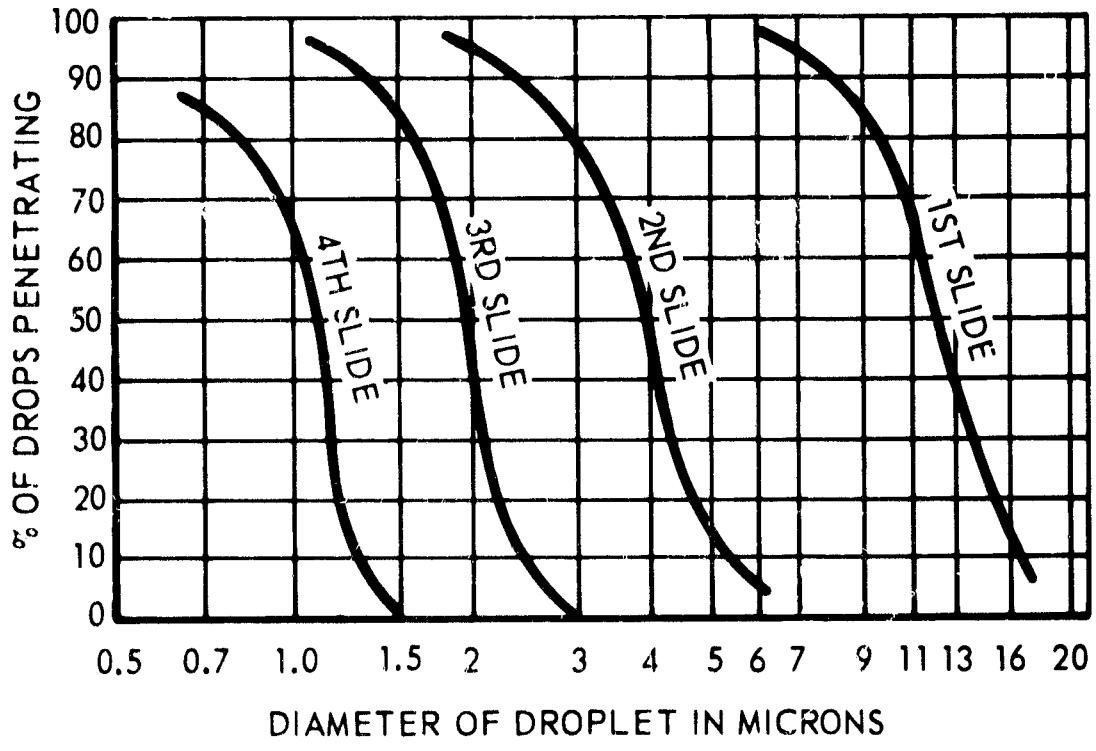


Figure 7. Diagram of Cascade Impactor and Efficiency Curves of the Four Stages

which has caused disturbance of the streamlines. Impaction under conditions of turbulence occurs only in extreme cases of very high velocity.

Flow is maintained through the Cascade impactor at a fixed rate by means of suction while impaction takes place in four stages. The sample is drawn in through a large orifice, so that impaction is at low velocity in the first stage and this velocity is progressively increased at the other stages through reduced openings of jets. The particles are deposited ultimately at high velocity on glass plates.

Although the efficiencies of this instrument depend greatly upon the nature of the particle it collects, most liquid particles of 0.05 to 20μ diameter are collected at high efficiency. To accomplish efficient impaction the device must process air at the rate of 0.6 ft^3/min with a total pressure drop of 60 inches H_2O. To move air against a static pressure of only 14 inches H_2O (45 cfm) requires 180-200 watts from a 1.8-lb miniaturized multistage vaneaxial blower (Globe Industries).

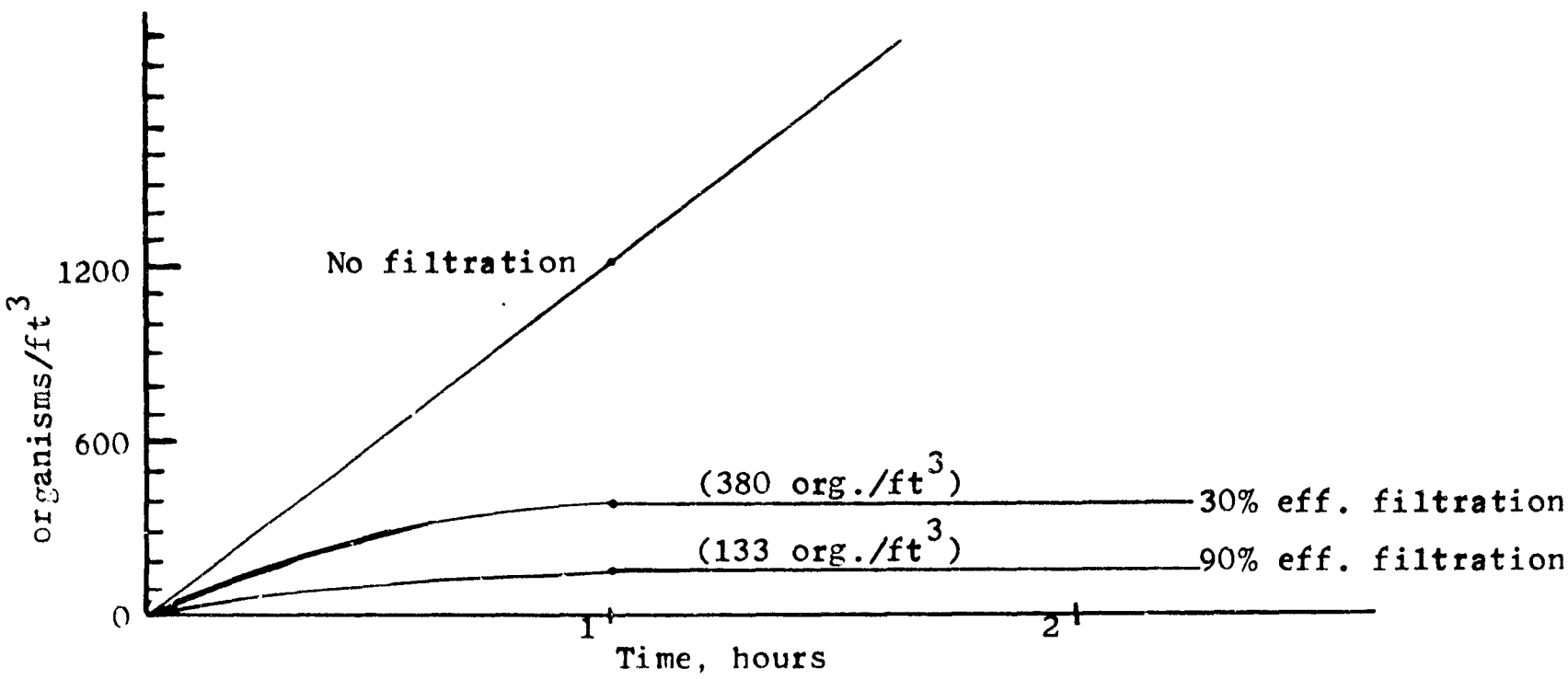
The power requirements of impaction or impingement devices do not recommend their use as components of air purification systems in spacecraft vehicle. Generally, this would be true with the application of any inertial deposition technique known to Melpar. Impingement methods and instruments also fall in this category. Impingers collect small aerosol particles by jetting the sample into liquid media. Again, small flow rates (2-28 liters/min), small orifices, high particle velocities (near sonic) are required to efficiently collect particles of $<10\mu$ diameter.

3.2.4 Filtration – Characteristics and Available Materials

The concentration of aerosol in a closed system at any time may represent either a terminal steady-state concentration or the rate at which

a system is approaching aerosol saturation. In a closed spacecraft in which aerosol generation and removal occurs steady-state kinetics would prevail, but it would nonetheless appear prudent to determine the time when a steady-state concentration would prevail. The curves from the example that follows show the way in which aerosol concentration in a closed system changes with time in the presence and absence of filters. The change here is due only to the turnover of air volume in the capsule and not to concentration changes because of re-entrainment of particles from various sites.

Changes in aerosol concentration may be represented by the following example. A closed space of 5000 ft^3, (the air to be clean at the start) 10 changes of air per hour with filtration continuous through all changes, and complete mixing. If organisms are generated within the space at the rate of 100,000 per minute, then at the end of 1 hour the number of organisms per cubic foot would be 1200 without filtration. With filtration, a 90% efficient filter can be calculated to reduce the concentration of 133 organisms/ft^3 at the end of 1 hour where it will not be exceeded. Similarly,



a 30% efficient filter produces a concentration of 380 organisms/ft^3.

The steady-state concentration can be shown to vary directly with the generation rates when using a particular filter.[2] In most instances this concentration will not vary greatly if either a 90% or a 99% efficiency filter is used. Therefore, even for critical areas such as operating rooms, the use of a high efficiency filter (90%) is quite satisfactory and consumes less power than filters of higher efficiency.

An example of the materials, low resistance characteristics, and high-volume capacities of several classes of commercial filters for small particle filtration (1 to 5 microns) is apparent in tables A, B, and C in the Appendix.

Selection of filters for spacecraft application will involve consideration of material composition, flow and pressure characteristics, filter area, frequency of replacement, and dust loading capacity. Choice of the highest dust loading capacity is, of course, desirable since power consumption required by the filter depends directly upon the loading capacity. The time required for the filter to become loaded depends upon the concentration and size of particles in the air and the volume of air being cleaned.

3.2.4.1 Filter Impregnation: The impregnation of filters with biocidal agents may have conceivably little effect on the efficiency of the filter but such impregnation might serve at least two functions: (a) the viability of escaping organisms would be diminished, and (b) the filter matrix with entrapped microorganisms and organic debris would not support microbial growth.

The need for biocidal coatings on filters of airborne material in spacecraft is probably not required if filters are used correctly and are

employed in relatively stable air flows. In practice, it has been demonstrated (1) that microbial particles are not easy to dislodge from filter fibers when sufficient moisture is present to affect a strong bond, (2) that unless very small leaks develop in or around the filter they operate at their specified retention efficiency quite reliably and mechanical strength is seldom a problem within specified flow rates, and (3) that heavy microbial loading and growth of filters will not constitute a hazard if proper safeguards are used to protect the filters from careless handling during disposal, excessive vibration, and reverse flows.

For the possible locations of airborne filters described in Section 3.2.5, it is felt that all of the above problems in filter application can be easily avoided through proper design.

3.2.5 Filter Location in Apollo Air Systems

The atmosphere reaching the interior of the closed space suit is supplied continuously from only two sources--the Oxygen Storage System and the Pressure Suit Supply System (figure 1). Although microbial contamination can exist in both of these sources, the purity of the O_2 supply may be carefully controlled and its purity verified prior to use. Consequently, it is believed that all "in flight" microbial control measures for the oxygen supply, including filtration, may safely be ignored.

The Pressure Suit Supply System, on the other hand, can contaminate space suit air without proper microbial controls. This system (figure 2) already possesses some measure of control due to the filtration capability of the debris trap and the CO_2/Odor Absorber. The limitations of these filter elements is best evaluated by their ability to efficiently filter both coarse and fine microbial aerosols. Most microbial filtration

systems, such as those found in closed laboratory cabinets and laboratory rooms, successfully employ a roughing filter in line with a high efficiency unit.[1] In this manner, the roughing filter relieves the more efficient filter of the burden of large particles which would otherwise shorten its useful life.

The Debris Trap (figure 2), currently designated for the Apollo Pressure Suit Supply System, cannot be considered a "roughing" filter for microorganisms because it would not remove these small particle sizes (1 to 5 μ). The trap consists of a stainless steel screen designed to block particles larger than 0.040 inch, and a filter bypass value which will open at 0.5 inch water differential pressure when heavily loaded.[5] Thus, when the bypass value opens <u>both large and small airborne particulates will pass through the system</u>. These particles are then carried at high velocity through a centrifugal suit compressor (figure 2) probably with some fractionation of the particles but with negligible attendant kill of microorganisms. The particles subsequently pass from the compressor into the canisters containing orlon filter pads and filtering beds of lithium hydroxide and activated charcoal. These elements are designed primarily to absorb CO_2 and other gas from the suit and cabin. To be effective in retaining microbial aerosols, these elements must act as both "roughing" and high efficiency filters.

Filter materials for removing microbial particles from the air have been classified[2] in terms of their removal of particles (of unit density) in the diameter range of 1 to 5 microns.

Class	Efficiency
Roughing	10-60%
Medium Efficiency	60-90%
High Efficiency	90-99%
Ultrahigh Efficiency	over 99.99%

3.1.1.1 Application and Location of Filters: Melpar's preliminary analysis of the Apollo ECS systems for control of airborne contamination suggests three locations of particulate filters within the spacecraft. Firm recommendations for filter application in these areas obviously cannot be made until the degree of hazard and control requirements are quantitatively defined.

Cabin Air Recirculating Unit

The installation of a roughing filter here, probably on the downstream side of the cabin blower (point A, figure 1), will help maintain a generally low level, steady-state concentration of microbial particles in the capsule air.

Debris Trap (Pressure Suit Supply System)

For coarse particle filtration of suit and capsule air, the modification of the present screen filter element (point D, figure 2) to a roughing filter would serve to lengthen the life of the CO_2/Odor Absorber elements under adverse loading conditions.

CO_2/Odor Absorber (Pressure Suit Supply System)

If required, this unit (figure 2) may be modified to retain a high percentage of aerosol particles less than 10 microns contained in the air from the suit and capsule. Even without modification, the fiber pads and beds of lithium hydroxide and activated charcoal contained within the

replaceable cartridge may already serve as adequate filters for this size range.

If particles less than 10μ (microbial aerosols) are not presently trapped in the cartridge, the felt pad, for example, may be replaced with a higher efficiency glass fiber filter. This would add little if any weight and create no outgassing problems. The additional power required would, of course, depend largely upon the presently unknown concentration of particles, approximately 10μ in diameter, coming from the space suit and capsule exhaust air. Other factors needed for estimating power would be the loading capacity (grams/Δp) of the filter element chosen and the volume of air being cleaned.

4 THE BEHAVIOR OF WATER-BORNE MICROBIAL CONTAMINANTS

Water-borne and airborne microbial contaminants differ primarily in that the former can multiply significantly, whereas, the environment of the latter brings about microbial death. Water is also invariably the repository of sufficient mineral and organic material to support some microbial growth, unless elaborate precautions are taken to prevent and maintain sterility. Indeed the microbial population of a water container is as much a function of the quantity of organic material present as it is a function of the initial concentration of microorganisms present.

Methods are evaluated for spacecraft use which are capable of removing bacteria and viruses from water systems. Other methods are considered which chemically inactivate microorganisms. Both chemical and physical methods of microbial control in water function in the control of mineral and organic contaminants; in this way they assist in the regulation of microbial growth.

4.1 The Control of Water-Borne Microbial Contaminants by Physical Means

Potable water in Apollo is generated on the catalytic surfaces of a hydrogen-oxygen fuel cell. This water is absorbed to wicking material within the cell. The absorbed water is transported by pipe to the water storage tank containing drinking water and waste water.

The fuel cell is a primary source of microbial contamination and, therefore, the entire drinking water system is subject to contamination. In considering microbial growth in this closed system it should be pointed out that both moving and static waters are encountered. Therefore, filtration may be used for retarding microbial growth in flowing systems and chemical agents can be used at low concentration and permitted to act for long periods of time in static systems.

Established methods for controlling microbial growth have been analyzed with respect to their usefulness in Apollo. In the following the comparative value of several methods is discussed with respect to power-weight requirements, and possible contribution to adverse conditions in the spacecraft environment.

4.1.1 Non-Impregnated Filters

Filtration of water-borne microbial contamination extending to viral size particles can be accomplished by commercially available filter materials. In many cases, much data exists relative to the applications sought in this study. For example, bacterial removal efficiency of light to heavily contaminated water is shown in tables 5 and 6 for a filter manufactured by the Pall Trincor Corporation. The filter (ULTIPOR.15)

TABLE 5*

BACTERIA REMOVAL EFFICIENCY OF ULTIPOR.15 FILTERS

Organism	Non-Filtered Water Bacteria/ml	Filtered Water Bacteria/ml		
		1	2	3
Escherichia coli	490	0	0	0
Staphylococcus aureus	540	0	0	0
Shigella dysenteriae	580	0	0	0
Streptococcus pyogenes	490	0	0	0
Diplococcus pneumoniae	620	0	0	0
Vibria comma	560	0	0	0
Salmonella typhosa	490	0	0	0
Mycobacterium tuberculosis	518	0	0	0

*Pall Trincor Corporation Data

TABLE 6*

BACTERIA REMOVAL EFFICIENCY OF ULTIPOR.15 FILTERS
ON HEAVILY CONTAMINATED WATER

Organism	Non-Filtered Water Bacteria/ml	Filtered Water Bacteria/ml
Bacillus subtilis	10,000,000	0
Serratia marcescens	27,000,000	0
Escherichia coli	3,730,000	0

*Pall Trincor Corporation Data

efficiency when filtering liquids is rated for 98% (nominal) removal of particles $>0.15\ \mu$ and 100% absolute removal of particles $>0.35\ \mu$. The loading (dirt holding) capacity of this filter of 1.1 ft^2 area per MIL-F-27656A is illustrated in figure 8. The filter material has been demonstrated by rat feeding tests to be nontoxic. It may be furnished with a biocidal coating.

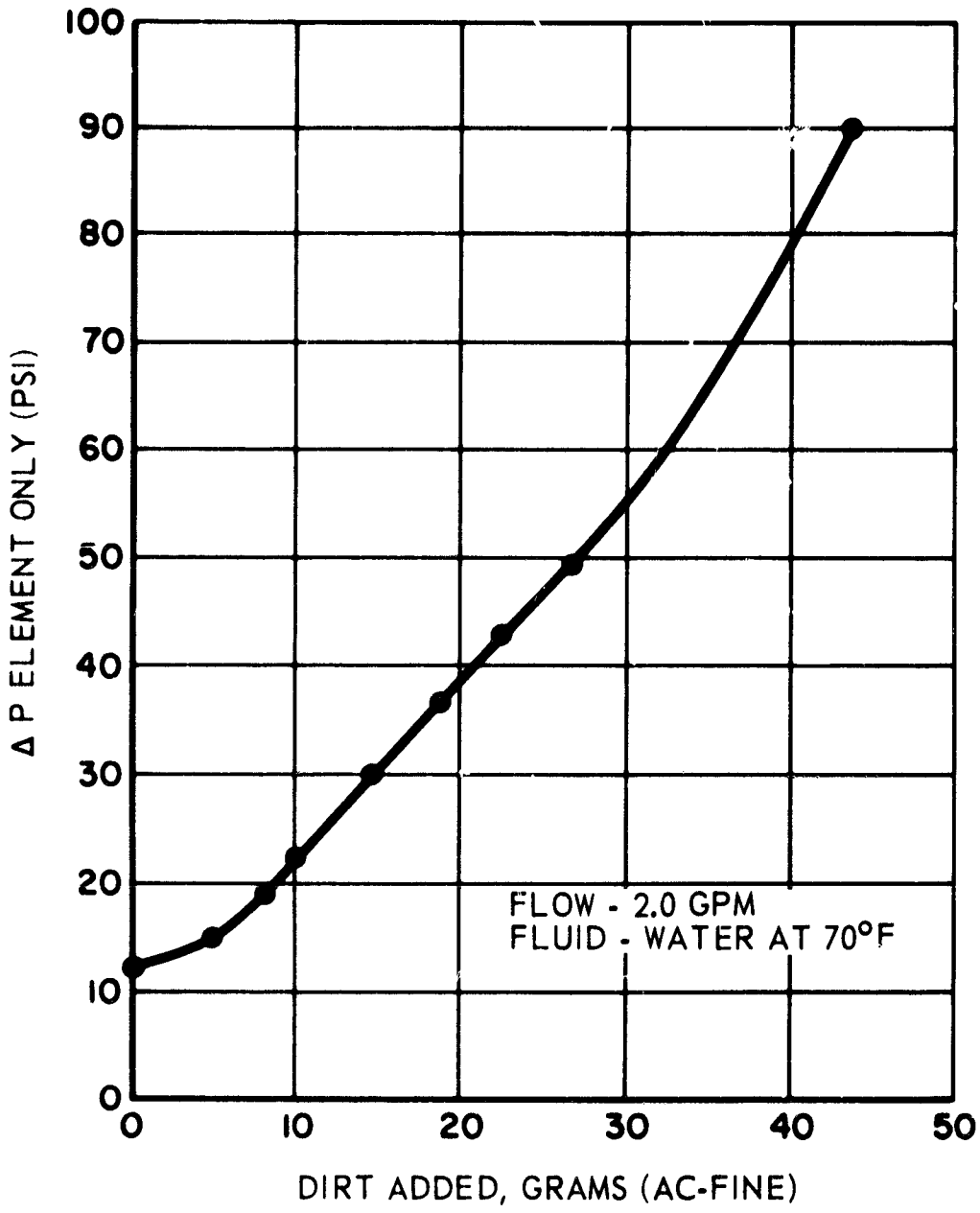


Figure 8. Dirt Capacity (MIL-F-276ɔ6A) for ULTIPOR.15

The energy requirements of this particular filter are estimated to be reasonably low for the Apollo water system application. This is based upon a relatively low pressure drop of 0.1 psi at 0.5 gal/min flow recorded using turbid water with algae present. The filter's loading capacity for small particles is relatively high provided roughing filtration is employed. Therefore, one can expect a small energy differential up to about 5 grams of material over approximately 1 ft^2 of filter area.

4.1.2 Membrane Filters

A recent development in sterilization techniques has been the introduction of membrane filters designed to separate bacteria, fungi, and viruses from liquids and gases. Some of the current high-volume applications of these filters include sterilization of such products as pharmaceuticals, soft drinks, beer, etc., and removal of particulate matter from recirculating air systems for "clean" rooms and ultrafiltration of fluids in hydraulic lines.

Constructed of biologically inert cellulose esters, the filters possess a high degree of uniformity of pore size and perform as simple screen-type filters or sieves. In operation, the filters retain upon their surface all particles whose dimensions are greater than the pore size. It is believed that the filters retain an appreciable percentrage of particles smaller than the pore size because of electrostatic charges generated on the filter surface during passage of gases and because of secondary valence (Van Der Waal) forces produced during passage of liquids. In addition, there is some retention of smaller particles due to build-up of larger particles on the filter surface.

The filters are available in a variety of sizes and shapes with pore sizes ranging from 5μ down to 0.1 mμ. For separation of bacteria and viruses, only those with pore sizes of 1μ or less are suitable. Table 7 illustrates the flow rates obtainable with various pore sizes at a pressure differential of 13.5 psi.

TABLE 7

MEMBRANE FILTER FLOW RATES AS A FUNCTION OF PORE SIZE

	Flow Rates	
Pore Size	Water $\frac{ml}{min/cm^2}$	Air $\frac{l}{min/cm^2}$
0.45 μ	65	4.9
0.22 μ	22	2.5
100 mμ	3.0	1.0
50 mμ	1.5	0.7
10 mμ	0.5	0.3

These filters can be used in the spacecraft water supply circuit for the sterilization of water. It is estimated that a 10-mμ pore size filter on the order of 10 cm^2 could provide about 7 liters of bacteria and virus-free water every 24 hours.

4.1.3 Impregnated Filters

As with air filters, water filters impregnated with biocidal agents might serve two functions--to diminish the viability of escaping microorganisms and to inhibit microbial growth. Should these functions assume importance in a particular application, it will probably be

necessary to develop the impregnated filter. This is due to the limited number of commercially available filters and also to the limitations on biocidal properties posed by specific organisms, and the outgassing and toxicity requirements.

A commercial filter with a biocidal coating intended for home water purification has been developed by Pall Corporation, Glenn Cove, Long Island. The coating incorporates a silver salt which produces a low silver ion concentration, 15-25 parts/billion, to inactivate bacteria over relatively long periods of time (hours to days).[3] This low concentration meets PHS 1962 Drinking Water Standards that are concerned with long-term (30 years) toxicity effects.

4.1.4 The Location of Filters in the Water Systems of Apollo

Preliminary study of the Apollo water system suggests filter locations either near the source or the outlet, or both. Possible source locations are indicated as B and C in figure 3. The B location filters all water from the fuel cell, whereas the C location filters only the potable water and any water accidentally entering the potable water lines from the waste water system. Outlet locations are shown as points D in figure 3. Two filters would be necessary and would serve to divide the load. This location may also facilitate filter maintenance for replacement or emergency operations. Filter locations at points of low flows are generally preferred to allow finer filtration, reduced filter size, or reduced energy requirements.

4.2 The Control of Water-borne Contaminants by Chemical Additives

The suitability of biocidal water additives for use in the potable and condensate water systems of Apollo have been evaluated with respect to weight, power requirement, human toxicity, and spacecraft contamination potential.

In this study antibiotics have not been considered because of their ability to select mutant microbial strains of high resistance, because of their individually limited fungicidal and bactericidal spectrum, because they are not effective against viruses, and because of their ability to produce occasional sensitivity and shock reactions in man. Compounds such as the halogens and heavy metals have been used effectively in the past in controlling microbial growth in water supplies. These elements have considerable germicidal activity and the absence of many of the undesirable features of antibiotics noted above.

In this study particular attention was given to the usefulness of chlorine, iodine, mercury, silver, and heavy metals other than silver as biocides in the water supplies of Apollo. It was concluded that microbial control by silver offered the most advantages for spacecraft application. Several seemingly extravagant claims in the literature regarding the bactericidal properties of this element or its coumpounds were reinvestigated and found to be correct.

A device was designed by Mulpar which is capable of maintaining silver in a form that kills both bacteria and viruses. This device is light in weight, its effect is exerted without imparting toxic or chemically measurable quantities of silver to water, and it has either a very small or no input power requirement.

4.2.1 Chlorine and Iodine

Several environmental factors determine the level of microbial contamination in a water storage system and control the effectiveness of biocides added to the system. The temperature, organic content, mineral content, and pH of the water determine the rate of growth of microorganisms and the rate at which compounds such as iodine or chlorine react with cells and kill them. The effectiveness of biocidal agents must be evaluated with respect to a given environment and the changes that occur therein.

The technology of water purification by the addition of chemicals has not changed much in the recent past, especially in small-scale operations.[7] The continued use of chlorine or iodine or compounds which release these elements is justified on the basis of their effectiveness as biocides at concentrations which are innocuous to man. The development of microorganisms that are resistant to these agents is not a frequently reported phenomenon. The biocidal properties of these agents appear to depend on reactions which grossly denature microbial protein rather than reactions directly with nucleoproteins or active centers of enzymes.

Chlorine and iodine are generally not removed from water following the completion of germicidal activities. They therefore impart taste to waters and may render it sterile but not potable.

In sterilization of waters "available chlorine" may be supplied to the system by calcium hypochlorite or by chlorine gas. The compound deriving from these reactants with bactericidal properties is believed to be hypochlorous acid. The reaction leading to the generation of this acid from these sources are the following:

$$Cl_2 + H_2O = HCL + HOCl$$

$$Ca(OCl)_2 + 2HCl = CaCl_2 + 2HOCl$$

The acid formed is weak and is almost entirely dissociated at pH 9. Such solutions loose their bactericidal properties. It appears that the disadvantages of chlorine as a method for controlling microbial growth far outweighs the advantages in the water systems of a space vehicle. If hypochlorous acid is generated from its salt then a small concentration of the cation evolved must be dealt with in the water system. Also, the hypochlorite ion can breakdown to form chlorine gas. The use of chlorine gas as a source of hypochlorous acid appears to lack advantages because of the implied necessity of transporting a toxic gas in a space vehicle and the implied necessity of metering its use in the vehicle water supply. It appears probable that chlorine compounds could be used only in closed containers such as water systems, whereas, silver, as will be shown, can be used in numerous components of both the potable water and condensate water systems.

Several properties of iodine recommend it as a bactericide superior to hypochlorous acid but sufficient information has not been obtained by us to permit its recommendation for spacecraft utilization. Some properties of iodine which should be considered in evaluating its usefulness as a microbial control in Apollo are the following. First, iodine is a solid at room temperature but it can spontaneously enter the vapor state without first passing through a liquid state. Second, the chemical form of iodine is strongly determined by hydrogen ion concentration, and iodine dissolved in water can be changed to iodate or iodide by alterations

in pH; these ions are not strongly bactericidal. Third, the bactericidal form of iodine is indeed, I_2. Fourth, we have not seen quantitative data from studies dealing with the efficiency of I_2 absorption from aqueous solution. Fifth, if iodine is added to water intended to be potable in the form of an iodophor, a system may be needed to remove the reaction product of the iodophor.

4.2.2 The Action of Metals as Bactericides

In this study three classes of metallic bactericides are distinguished on the basis of their relative toxicity to man and to bacteria. Table 8 taken from data in Reddish[10] exemplifies this difference in activity. One class of compound is exemplified by mercuric chloride which is highly toxic to both bacteria and man. Another class is exemplified by zinc chloride which is mildly toxic to man and to microorganisms. A third class of metals most important in this study is represented by silver, a compound highly toxic to bacteria but lacking an acute toxicity to man.

The use of metals as bactericides and viricides in the water systems of Apollo seems particularly appropriate. By means of ion exchange resins the metal concentration of spacecraft of spacecraft water could be controlled accurately. This control could be exerted by a system small in weight and volume and requiring no direct energy input.

The feasibility of using metals as bactericides has been established for a long time but questions still remain unanswered as to the chemical form of the metal most potent as a bactericide. This question has been considered in the research performed during this period, and from this research an item has been designed to control microbial growth in the water systems of Apollo using a cation exchange resin in its silver form.

TABLE 8

MAXIMUM DILUTION OF SALTS REQUIRED TO KILL SALMONELLA TYPHOSA AND MICROCOCCUS PYOGENES VAR. AUREUS IN 10 MINUTES BUT NOT IN 5

Salt	Maximum Dilution	
	S. typhosa	M. Pyogenes var. aureus
$AlCl_3\ 6H_2O$	1:3	*
$CoCl_2\ 6H_2O$	1:20	1:6
$Co(NO_3)_2\ 6H_2O$	1:5	1:5
$CuCl_2\ 2H_2O$	1:300	1:7
$Cu(NO_3)_2\ 3H_2O$	1:50	1:6
$CuSO_4$	1:40	---
$Pb(C_2H_3O_2)_2\ 3H_2O$	1:3	---
$Pb(NO_3)_2$	1:3	---
$NiCl_2\ 6H_2O$	1:5	1:3
$Ni(NO_3)_2\ 6H_2O$	1:3	1:3
$Zn(C_2H_3O_2)_2\ 2H_2O$	1:3	---
$ZnCl_2$	1:8	---
ZnI_2	1:30	1:10
$ZnSO_4\ 7H_2O$	1:3	---
Phenol	1:90	1:60
$HgCl_2$	---	1:16,000
Ag^+	---	1:1,000,000

*Indicates that a saturated solution of the salt failed to kill.

Ag^+ concentration in water contained in a flask coated by a silver mirror. This datum was obtained at Melpar, Inc.

5. THE CONTROL OF SURFACE MICROBIAL CONTAMINANTS

Surfaces likely to experience great microbial contamination are those in the interior of the space suit. These areas present unique control problems for several reasons. They are continuously contaminated by both the nonviable and viable exudates of the crew and their proximity to the skin imposed special restriction where the application of bactericides is considered. Yet, it is believed that control of microbial growth in the space suit is of first order importance because it is within this area that possibly the largest concentration of organisms are generated and spread to the environment of the space ship.

It is felt that a large measure of control over microbial growth can be exercised in the space suit by the use of clothing by the crew which has been impregnated with biocidal materials. Biocides for this purpose may also function as preservatives as well as compounds capable of diminishing skin irritation associated with the growth of organisms indigenous to the skin of man.

5.1 The Control of Microbial Growth by Fabric Impregnation with Metallic Compounds

Many organic compounds containing copper are used in preservation of cotton fabrics. Some of the more widely used compounds are Copper Naphthenate, copper-8-hydroxy quinoline, and a large number of copper salts of organic acids. These compounds are sparingly soluble in water and are therefore susceptible to being leached from impregnated fabrics. Some of them also have a slight toxicity to man and a vapor pressure such that an odor is invariably associated with their presence.

The disadvantages associated with copper organic compounds are not inherent in compounds formed with silver which may be used to impregnate fabrics and render them germicidal.

Silver impregnation compounds are not widely used industrially because of their expense, but for the limited application of space suit impregnation they appear unusually suited. Patents exist on processes of impregnating cotton, wool, silk, nylon, plastics, and latex with silver compounds.[8,9] The impregnated material exerts a germicidal activity not only within its own matrix but in the immediate environment. Such materials may be washed repeatedly without loss of germicidal properties and without discoloration, and do not cause skin irritation or toxic manifestations in man.

From the standpoint of space suit requirements, it appears that silver impregnation would be the method of choice in controlling space suit contaminations. It is recognized, however, that the application of silver impregnation in space suits should be contemplated following an impartial laboratory evaluation of the germicidal and toxicological properties of the impregnated product.

6. EXPERIMENTAL EVALUATION OF MICROBIAL CONTROL METHODS

Select methods of controlling microbial growth were studied in the laboratory of the Microbiological Section of Melpar to determine their efficiency and suitability for spacecraft application.

A part of this experimental work was accomplished to evaluate claims and reports of the bactericidal activity of known control methods. Another part of the experimental evaluation dealt with the usefulness of new methods of microbial control which were developed at Melpar over the past several years for possible application in Apollo.

6.1 Microbial Killing by Photochemical Agents

Methods described for water system sterilization were generally designed for processing very large volumes of water intended for human consumption over long periods of time. The design of such methods have therefore stressed economy of operation and avoidance of the introduction of substances which might have either an acute or chronic toxic effect. These restrictions are desirable but they are not necessarily essential in the design of microbial sterilization methods for spacecraft utilization. For example, a highly toxic material such as mercuric chloride could be used to kill microorganisms in water supplies and storage containers. This material could be effectively removed from water before it was used for drinking or other applications. Also, a material which produces a chronic toxicity might be acceptable for spacecraft utilization where flight duration is about 1 year.

With these considerations in mind tests were performed to give some preliminary information on the utility of dyes as water sterilization agents. The dye chosen for this test was itself not highly toxic to the test organism but it was capable of bringing about microbial death at extremely low concentrations when irradiated with small doses of visible light. The sterilizing effect of the dye is attributed to its ability to produce a photodynamic effect, that is, to cause an oxidation of organic material of the bacteria in the presence of adsorbable radiant energy. Also, the dye is a cation and can be removed from solutions of drinking water by cation exchange resins. But to render water potable by removing the low quantities of the dye

needed to bring about a photodynamic effect would not require the utilization of large quantities of exchanger.

Data presented in Table 9 make possible an order of magnitude calculation of the energy and dye concentration required to inactivate E. coli cells suspended in distilled water with different concentrations of a dye, acridine orange. The cells used were from 18-hour-old cultures grown at 37°C. The cells were harvested by washing one time in 50 volumes of distilled water, and resuspended to 10^8 cells per ml in water or acridine orange solutions of different concentrations.

From these determinations it is apparent that irradiated concentrations of this dye as low as 10^{-7} M exert a measurable influence on cell multiplication, while the dye at 10^{-5} M kills 99.94% of the 10^8 cells per ml present after 1 hour of exposure to visible light. It seems improbable that the water system of Apollo would develop or maintain this relatively high concentration of microorganisms.

The concentration of acridine orange used per liter of water can be absorbed by 0.000452 gram of Dowex-50-H resin. Therefore, the quantity of resin needed to remove a concentration of acridine orange which proves germicidal (10^{-5} molar) from 1000 liters of water would weigh only about 0.4 grams.

In considering the power requirement for a sterilization system using acridine orange it whould be pointed out that the peak visible absorption of this compound is at 4900Å, while only a small fraction of the visible output of the source used in this study is at this wavelength. Therefore, the energy requirements for sterilization by the use of this dye

could be substantially reduced by using either sunlight or a discharge bulb with radiations predominantly in this wavelength region.

TABLE 9

THE SURVIVAL OF E. COLI IN ACRIDINE ORANGE SOLUTIONS AND IN WATER IN THE PRESENCE OF VISIBLE RADIATION*

Time	H_2O Control (%)	10^{-5}M (%)	10^{-7}M (%)	10^{-9}M (%)
0 Time	100	100	100	100
1 Hour	550	0.06	150	620
3 Hours	900	0.00001	450	880
pH after 3 Hours	5.8	6.0	5.9	5.9

*Irradiation was by a 100-watt incadescent bulb 16 inches from the surface of a 50 ml solution 1/2 inch deep.

NOTE: Acridine orange at 10^{-5}M in the absence of light did not cause appreciable loss in viability.

The use of acridine orange could be used in redundancy with sterilizing mechanisms which employ exchange resins as an integral component part.

6.2 Bactericidal Activity of Silver Salt

The silver ion is characterized by its ability to form many insoluble compounds with certain anions. But, to bring about bacteristatis a compound must be soluble and capable of moving from the solvent into the bacterial cell. It was surprising, therefore, to read reports of the very great bactericidal activity displayed by surfaces of the metallic form of this element. Accordingly, attempts were made to verify experimentally

in our laboratory the bactericidal activity of silver salts and of silver-covered surfaces. Finally a device was designed for use in the water systems of Apollo using a silver-saturated cation exchange resin. The work reported in this section dealt with the bactericidal activity of the silver ion in its simplest form as silver nitrate.

The data in table 10 show the time concentration dependence of bactericidal activity of silver against E. coli. The culture used for inactivation was 18 hours old. It was grown on nutrient broth at 37°C. The cells were harvested by washing three times in 100 volumes of water for each washing. The harvested cells were then suspended in water or solutions of $AgNO_3$ at the concentrations indicated. Incubation in these suspensions was of 37°C for the time intervals shown. All suspensions were initially at 2.4×10^9 cells per ml and at pH 5.2 to 5.4. All calculations of percent survival were based on the viable cell count of suspensions in water only at zero time.

TABLE 10

SURVIVAL OF E. COLI IN $AgNO_3$ SOLUTIONS

	Molarity of $AgNO_3$ Solutions			
Incubation Time (Hours)	0 % Survival	10^{-6} % Survival	10^{-4} % Survival	10^{-2} % Survival
0	100	100.00	$\ll 0.004$	$\ll 0.004$
1	160	0.090	$\ll 0.004$	$\ll 0.004$
4	125	0.004	$\ll 0.004$	$\ll 0.004$

Silver ion is indeed extremely toxic to microorganisms. It is observed that essentially 11 of a very concentrated suspension of microorganisms was killed by Ag ion at 0.0188 ppm at 37°C in less than 1.0 hours. This molar concentration of silver represents only 0.108 mg of silver per liter of water. The daily silver intake from foods and cooking utensils is estimated to be 0.06 to 0.08 mg of silver per man per day.[12,13] While there is not a well established quantity of silver which causes acute toxicity it has been estimated that 0.01 to 2.5 grams of silver salts may be ingested at one time without causing acute symptoms. On the other hand, chronic silver poisoning is represented by the disease argyria which is a cosmetic anomaly resulting from the deposition of ingested silver on the skin. Argyria results from the silver ingestion over many years, but it is not accompanied by impairment of known physiological activities.

The actual addition of soluble silver salts could be used as a method for controlling microbial growth in Apollo water systems because both the anion and cation of such salts could be easily removed by exchange resins. Here again only about 1.0 mg of Dowex-50 resin is required to remove the silver in 1 liter of water rendered sterile by 10^{-6} moles of silver.

6.3 Bactericidal Activity of Silver Surfaces and the Effect of Sodium Chloride, Glucose, and Albumin on that Activity

The presence of metallic silver in bacteriological media has been reported to inhibit bacterial growth. Because of our preconceived notions concerning the possible validity of these reports, tests were made of the bactericidal effect of silver hydroxide or oxide placed in plates of TGY agar with and without E. coli.

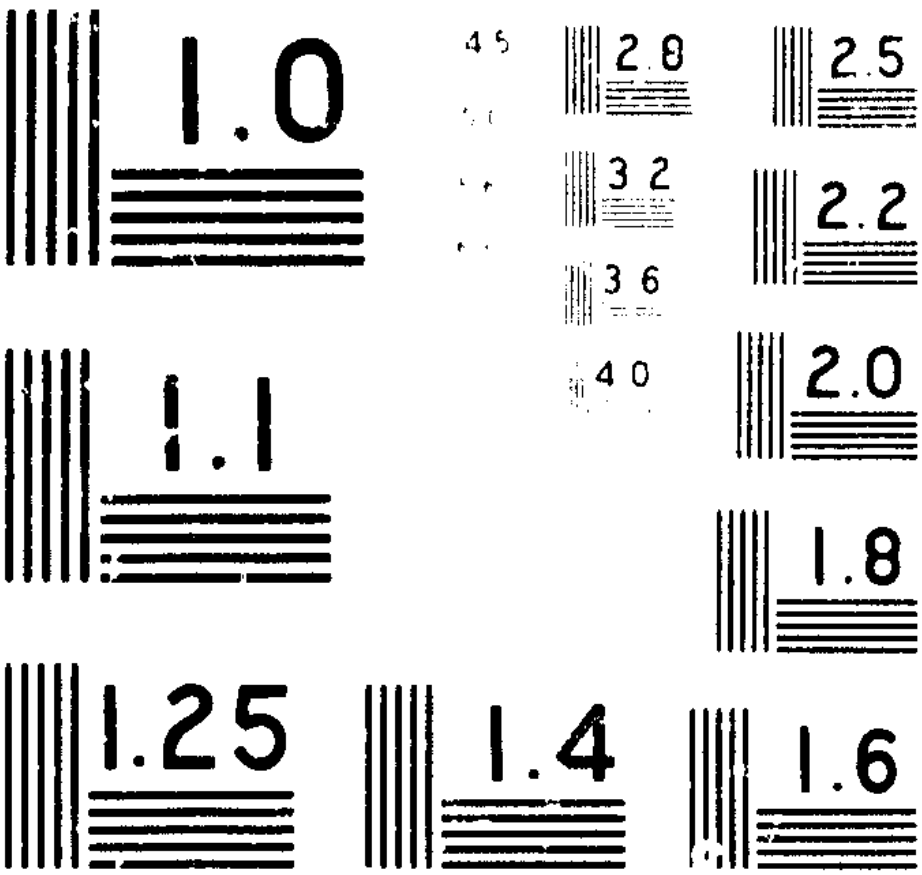


Figure 9 shows zones of silver diffusion in uninoculated plates, Figure 10 shows zones of silver diffusion and inhibition of E. coli growth and Figure 11 shows the same zone phenomenon and inhibition. The concentration of microorganisms in Figure 11 was five times that used in Figure 10. The plates were incubated at 37°C for 48 hours.

The diffusion patterns assume a form in Figure 11 suggesting that silver is reduced or perhaps transported by some metabolic products of the growing organisms present. This material could be as simple as hydrogen ion or it might be an organic acid silver complex. From Figure 10 it appears that sufficient silver compound in its colorless form diffuses from the silver precipitate to produce growth inhibition.

These observations suggested to us that surfaces of silver may indeed possess considerable bactericidal activity. To test this possibility the bottom and sides of 250-ml erlenmeyer flasks were plated with silver by the following method. To a flask containing 3.0 gram of $AgNO_3$ in 40 ml of H_2O, concentrated NH_4OH was added until a precipitate formed and then dissolved. This solution was added to a flask to be silver plated. To the solutions of silver ammonia complex in each flask was added 0.4 gram of tartaric acid in 40 ml of H_2O. The solutions of silver and tartaric acid were warmed slowly. With stirring and with the reduction of silver ion a silver mirror formed on the interior of each flask.

The silver-plated flasks were washed vigorously and repeatedly with water. Then to each flask 100 ml of water was added and following this the flasks were placed on a rapid rotary shaker for 18 hours at 37°C.

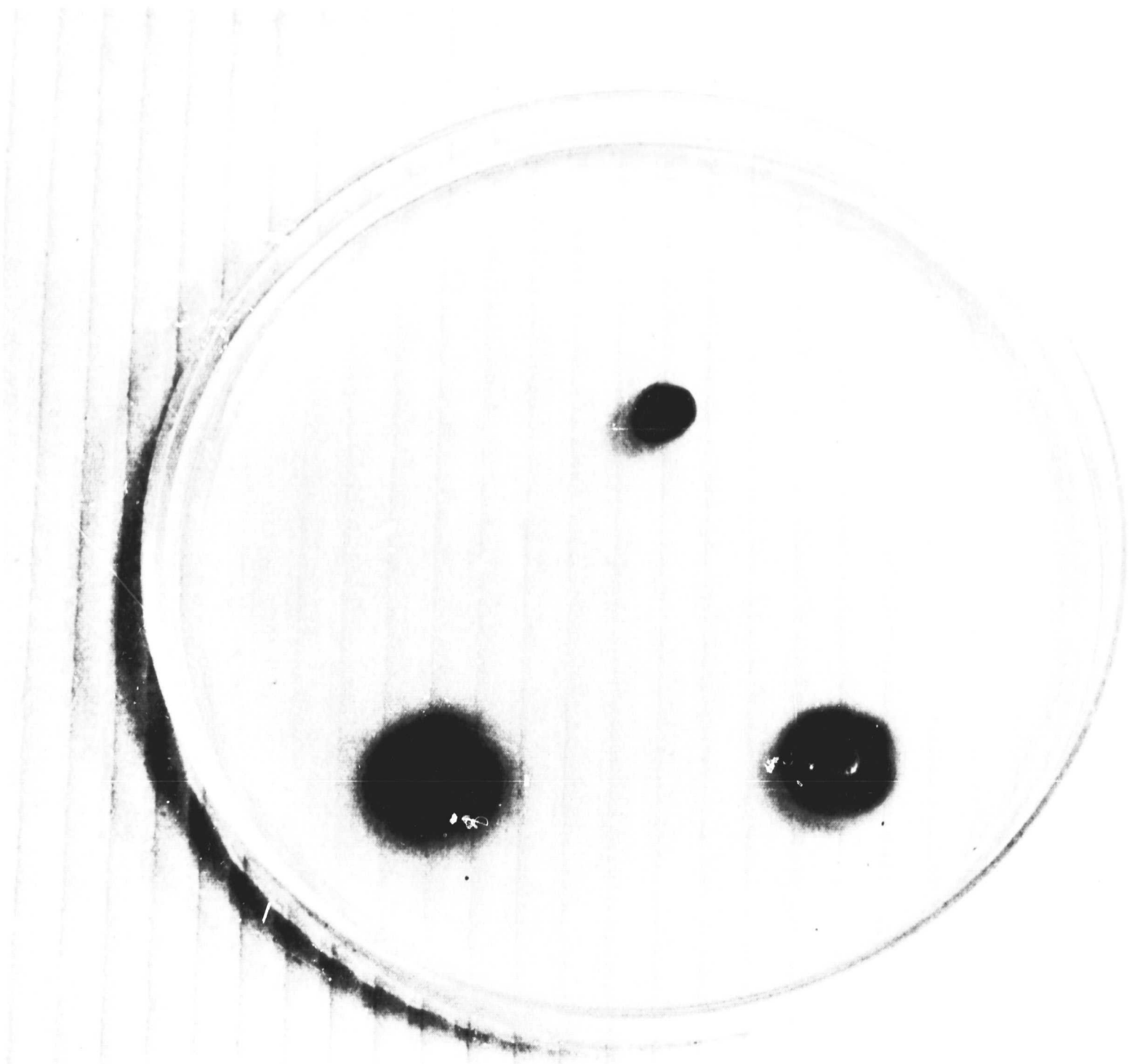


Figure 9. Uninoculated Control

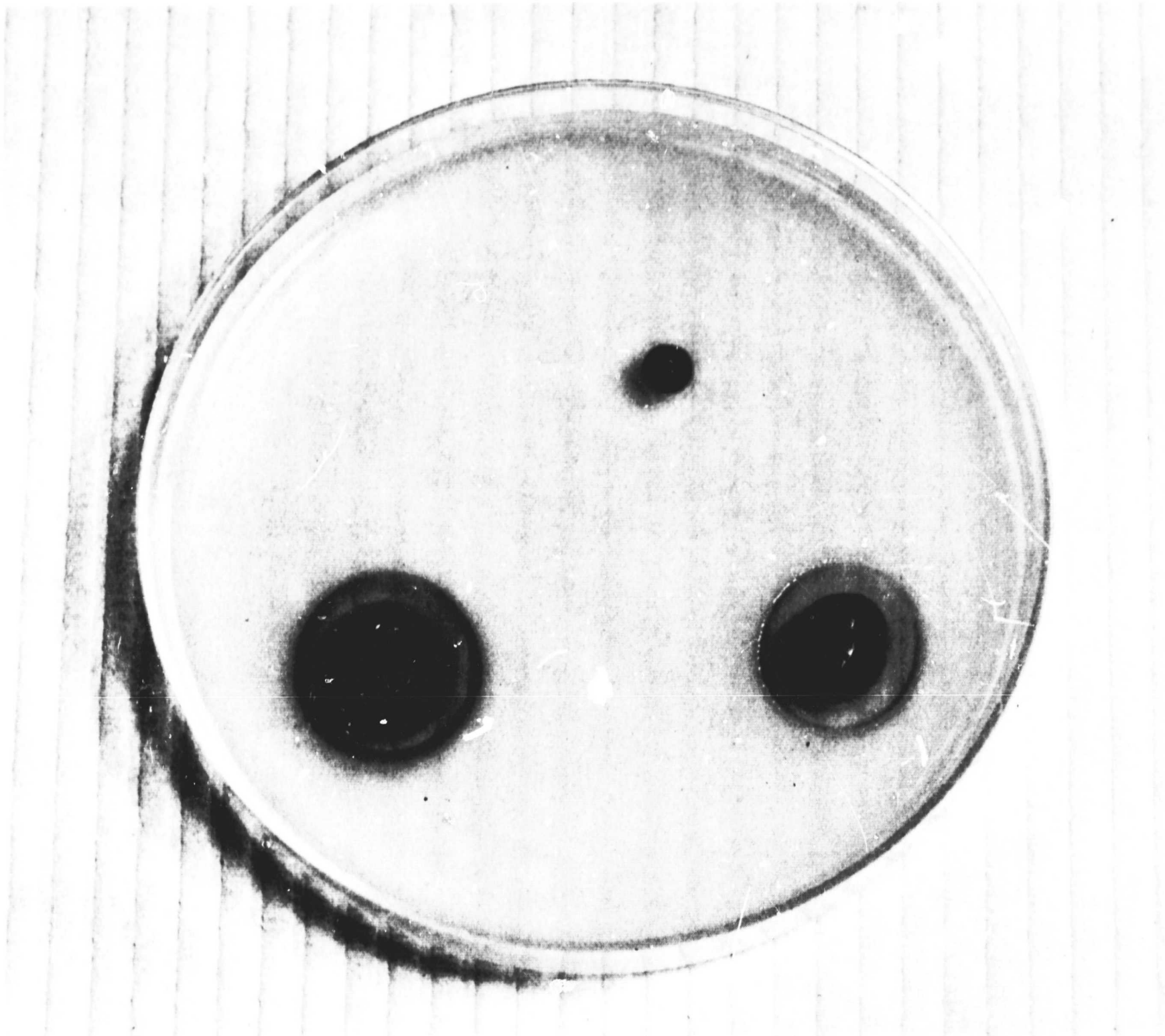


Figure 10. Inhibition of Microbial Growth by the Diffusion of Silver in Agar Plates

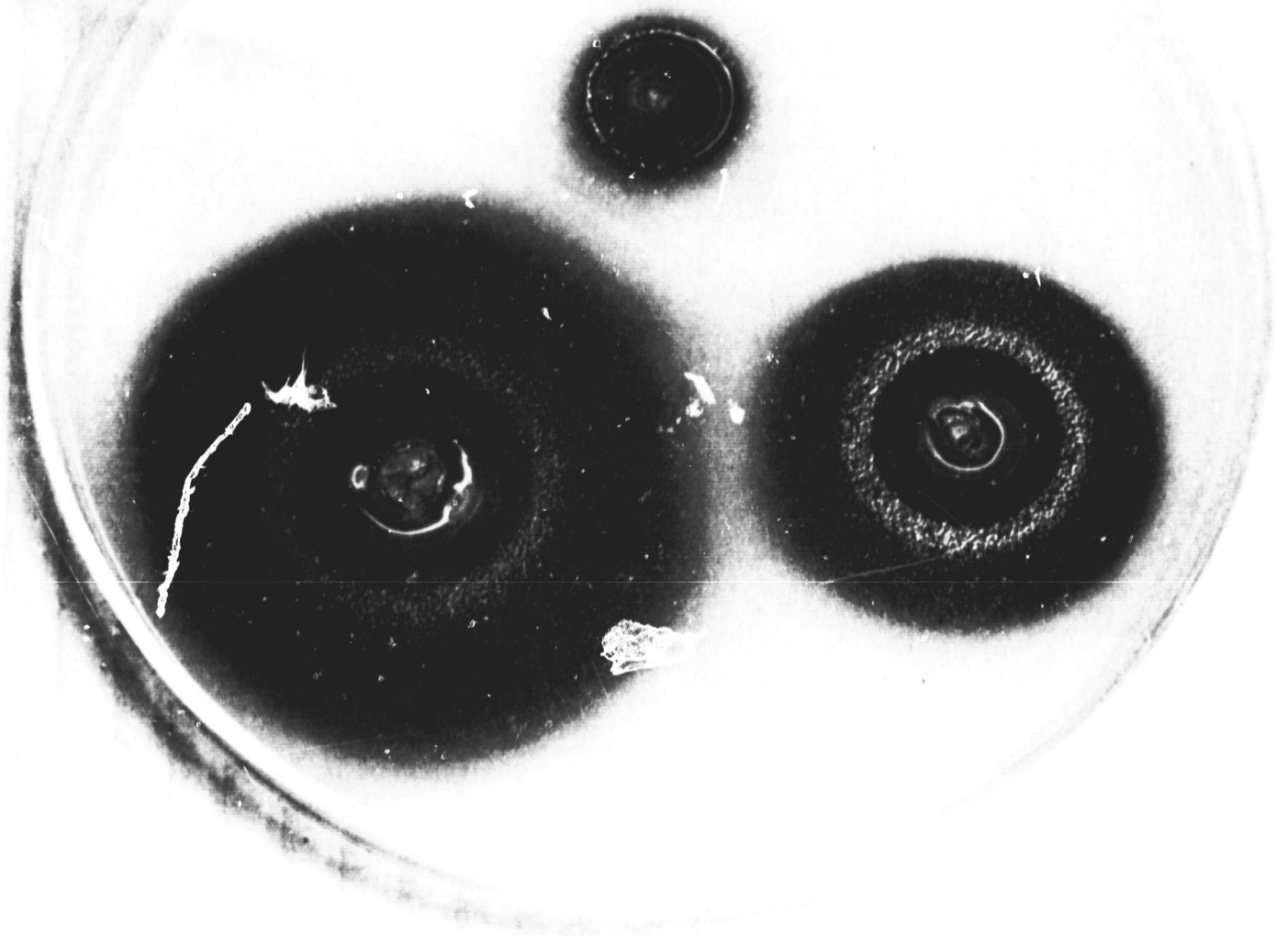


Figure 11. Inhibition of Microbial Growth by the Diffusion of Silver in Agar Plates

At the end of this time the water in the flask used as a wash was discarded. To each washed flask a 100-ml suspension containing 10^7 organisms per ml was added. These suspensions were then made to the various indicated concentrations of NaCl, glucose, or albumin. It was believed that any of the additive might act as an inhibitor of the bactericidal activity of silver.

The data in table 11 shows the concentration of silver in water or water solutions suspending the organisms, the survival of organisms in the absence of silver (controls), and the survival of organisms in various solutions contained in flasks with silver mirror interiors.

From table 11 it appears that considerable killing occurs in solutions containing less than 1 ppm Ag^+ in the presence of sodium chloride. Killing also occurs in water suspensions with glucose or albumin. In all cases the silver in the suspending medium can either be removed by cation exchange resins or probably ingested directly without harmful effect.

The amount of silver leached from the silver mirror depends to some extent on the rate of deposition and, therefore, on the rate of reduction or heating of the tartaric acid silver ammonia mixture. These differences are not well understood, but their contribution is shown by variations in the concentration of silver leached by microbial suspensions. This concentration varies from about 1 ppm to a concentration considerably less than 0.1 ppm as shown by the silver content of the various suspensions studied.

Three organisms were tested for their susceptibility to the bactericidal activity of silver surfaces. Table 12 shows that all organisms

TABLE 11

SURVIVAL OF E. COLI SUSPENDED IN VARIOUS SOLUTIONS IN FLASKS WITH AND WITHOUT SILVER MIRROR INTERIORS

Suspending Solution	Container	Incubation Time (hours) 0 %S	2 %S	4 %S	5 1/2 %S	Silver Concentration (ppm)
H_2O	Mirror	100	0	0	0	1
	Glass	100	52	21	11	0
0.01% Glucose	Mirror	100	0	0	0	2
	Glass	100	48	15	7	0
0.1% Egg Albumin	Mirror	100	0	0	0	3
	Glass	100	53	24	11	1
10^{-2} M NaCl	Mirror	100	0.01	0	0	0
	Glass	100	87	87	75	1

Incubation was at 37°C and initial cell count was $\sim 10^7$ cells per ml. The cells were grown on TGY agar and washed with water before use in this determination.

TABLE 12

SURVIVAL OF THREE SPECIES OF BACTERIA IN WATER CONTAINED IN PLA[illegible] WITH AND WITHOUT SILVER MIRROR INTERIORS

Organism	Incubation Time (min) 0 %S	45 %S	90 %S	300 %S	Silver Concentration (ppm)
E. coli					
Silver mirror	100	0	0	0	1
Control	100	90	64	24	1
S. aureus					
Silver mirror	100	0.5	0	0	1
Control	100	98	78	68	1
Ps. aeruginosa					
Silver mirror	100	0	0	0	1 1/2
Control	100	96	70	65	1

are readily killed by these surfaces at an unexpected low concentration of silver. Thus, silver coatings of certain portions of the water system of space vehicles may be used to great advantage in the control of microbial growth in these areas.

Experiments with silver-coated flasks raised the question as to whether the bacte·icidal silver compound resided in the aqueous suspension or was picked up by bacteria as they struck the silver surfaces of the coated erlenmeyer flask. To determine the location and possible concentration of the bactericidal compound(s) water was placed in a silver-plated flask and shaken for 18 hours at 37°C. The water was removed, centrifuged, filtered, and diluted as described in table 13. Each dilution was made to 5×10^8 cells per ml and incubated for the time shown. Cells in the 1:10 diluted water were killed at almost the rate of cells in the undiluted water. The killing of cells by the silver compound extracted from the silver mirror showed a definite dose response curve both with respect to concentration and exposure time.

Once again the low concentrations of silver from mirrors exercising bactericidal effects suggest that water from such sources could be drunk directly or the silver concentration present could be easily controlled by cation exchange resins.

6.4 The Bactericidal and Viricidal Activity of a Styrene Cation Exchange Resin Saturated with Silver

The low human toxicity and considerable bactericidal activity of silver recommend it as the agent of choice for the control of microorganisms in the water systems of Apollo. However, it has been frequently pointed

TABLE 13

SURVIVAL OF E. COLI IN DILUTIONS OF WATER TAKEN FROM A SILVER-COATED FLASK

Incubation (Time (hrs)	Control No Ag^+	No Dilution	1:1	1:10	1:100	1:1000
0	100	100	100	100	100	100
1.0	130	<0.001	0.001	4	120	130
5.5	75	<0.001	<0.001	2	75	60

Incubation was at 37°C for 48 hours. Cells were diluted in water and plated on TGY agar.

out in the literature that the most bactericidal form of silver is not the silver ion but rather some compound in equilibrium with silver ion which is available to microorganism. Strong evidence for this oligodynamic form of silver could not be adduced from our experiments but it was reasonable to attempt to add silver to a water system in a form which would rapidly equilibrate. Such a form was thought to be a styrene resin saturated with silver ion. The equilibrium at any point in a column of the silver saturated resin might be represented by the equation:

$$RSO_3^{\ominus}\ Ag^{\oplus} \longleftrightarrow RSO_3^{\ominus}\ H^{\oplus} + Ag^{\oplus}$$

The presence of bacteria in a column of the Ag-saturated resin would shift the reaction to the right as follows:

$$Ag^{\oplus} + (\text{bacterium})^{\ominus} \longleftrightarrow Ag(\text{bacterium})$$

The silver bacterium complex would be nonviable and the depletion of $Ag^{\oplus}$ from the resin, $RSO_3^{\ominus}\ Ag^{\oplus}$, would depend entirely on the number of bacteria present in a given column. The number of silver sites on the bacterium saturated by silver would depend on the concentration of free silver, and this concentration, for large quantities of the Ag-saturated resin would be quantitatively governed by the distribution coefficient...

$$K_d = \frac{(Ag^{\oplus})\quad (RSO_3H)}{(H^{\oplus})\quad (RSO_3^{\ominus}\ Ag^{\oplus})}$$

It is apparent that the silver could be removed from a microbial suspension by passage over a cation exchange resin in the hydrogen or sodium form by means of the reaction

$$Ag\ (\text{bacterium}) + RSO_3^{\ominus}\ H^{\oplus} \longleftrightarrow RSO_3^{\ominus}\ Ag^{\oplus} = H^{\oplus}\ (\text{bacterium})$$

This reaction would also remove Ag^{+} from the water suspending the bacterium. The amount of Ag^{+} absorbed by the bacterium cannot be predicted

but it appears unnecessary to remove all of the bacterial silver, which exists in a highly unavailable form. Under anticipated loading conditions silver-treated organisms could be easily removed from the system by filtration.

In summary, the system described using a silver-saturated resin column acts as a metal buffer thus tending to maintain a constant concentration of free silver. The Ag-saturated resin column is in series with a system consisting of a cation exchanger in the sodium or hydrogen form. This exchange is arranged as a column designed to progressively lower the Ag concentration of a solution or suspension passing over it. The function of these two systems in series suggests that the silver binding sites on bacteria could be saturated in the metal buffer system thus killing the bacterium, and that a large part of the silver taken up by the bacterium could be removed subsequently by the column operation. The bactericidal effect of silver at very low concentrations indicated that the silver binding site responsible for the killing of the organism holds the metal tenaciously; therefore diminishing the likelihood that cells rendered nonviable by silver could be reactivated by means of its removal through column operation.

The bacterial control system proposed in this study would consist of three parts as illustrated in figure 12. The top column would contain a mixture of anion and cation resins respectively in the hydroxyl and hydrogen ion forms. This column would remove ions which might interfere with the operation of the silver-saturated resin by causing the displacement of silver. Such interfering ions would be represented by chloride ion, which is readily absorbed by such a mixed resin. The second column in series with the first would be the silver-saturated cation exchange resin, and in series with

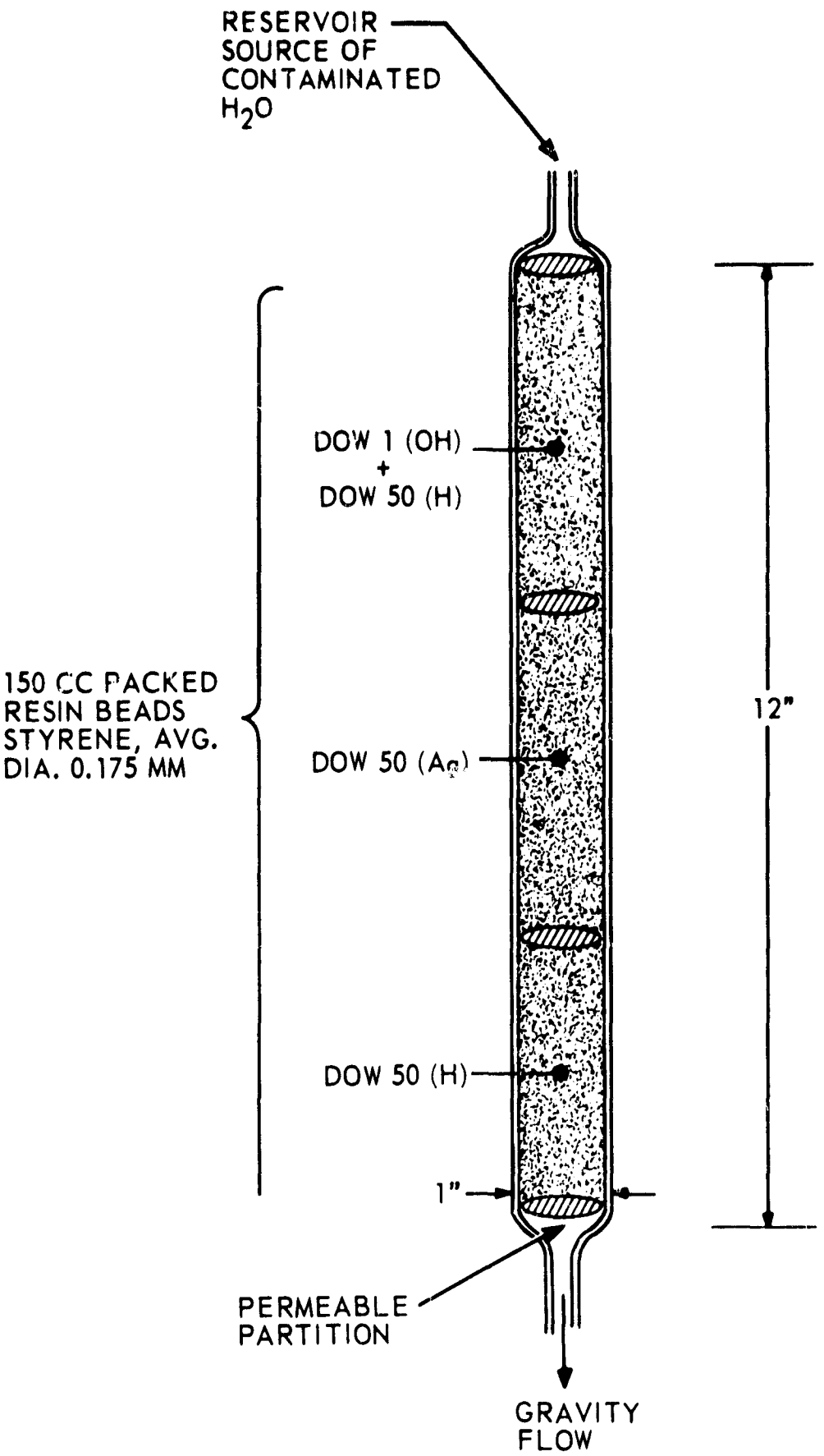


Figure 12. Experimental System for Inactivation of Microorganisms in Spacecraft Water Subsystems

this would be a cation exchange resin in the hydrogen or sodium form. The latter resin would remove a certain fraction of the silver from the microorganisms passing through the column, and any of the silver spuriously displaced from the Ag-saturated resin.

Tests described in the following were performed to determine the validity of a few of the above assumptions and to determine the efficiency with which a silver-saturated ion exchange resin can kill bacteria.

A mixed resin bed was made up of equal parts of Dowex-50-H^+ and Dowex-1-OH^-. This was packed in a 30-ml glass column. A batch of Dowex-50-H was washed successively in 1.0 N and 0.1 N HCl and then in distilled water. A portion of this resin was used as a control and another portion was saturated with Ag ion using a concentrated solution of $AgNO_3$. The silver-saturated column and the control were extensively washed with distilled water until the effluent of each was between pH 5 and 6. The titratable acidity of this effluent was negligible and fewer than 0.1 ppm of silver was present in the distilled water effluent. The silver-saturated resin was packed in a 30-ml glass column and the top of this column was attached to the bottom of the mixed resin column. The bottom of the silver-saturated column was in turn connected to the top of a 30-ml column containing Dowex-50-H. The control system without silver was prepared in the same way except that the silver-saturated column was replaced by Dowex-50-H.

Suspensions of microorganisms were prepared in water and passed through these systems of columns with and without silver. Cultures of *E. coli* were grown for 18 hours at 37°C in TGY broth. They were then centrifuged at 5°C and resuspended in cold, sterile, distilled water. The final volume was always 500 ml. A microscopic count and a plate dilution

count were made for each batch. The counts were always between 4 to 5×10^8 cells per ml.

These 500-ml suspensions of the E. coli were passed through the column systems. The suspensions at $0^{\circ}C$ were fed by gravity onto the top bed of the resin at room temperature. The flow rate through the column was maintained at about 5 ml/min. Fractions were collected in 100-ml volumes and packed in an ice bath.

A 1-ml aliquot was then removed from each fraction and serially diluted so that the tube receiving the first aliquot had a possible dilution of 10^{-2} or 10^{-8} cells/ml, the tube receiving the last aliquot had a dilution factor of 10^{-8}, or approximately 1 cell/ml. A series of tubes so prepared was chilled. After chilling, 0.1-ml aliquots were taken and mixed with TGY agar in petri dishes and incubated for 24 hours at $37^{\circ}C$.

Cells were gram-stained before and after passing through the column and a microscopic count was made on the first and last fractions in all cases. Gram-stain characteristics were not altered and essentially all cells were recovered as judged by microscopic count.

An analysis of the viability of cells passed through the control columns without silver revealed that this passage did not result in the loss of viability. However, when samples of the same cells suspension were passed through the column system containing a silver-saturated resin all bacteria were killed. Table 14 shows the viability of cells passed through a column system without silver and table 15 shows the viability of cells passed through the column systems containing silver-saturated resin. The high cell concentration and large volume of cell suspension inactivated by the silver resin should be noted. It seems improbable that the level

TABLE 14

VIABILITY OF *E. COLI* SUSPENSIONS PASSED THROUGH A RESIN SYSTEM WITHOUT Ag^+

Effluents	Dilution						
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
	Number of Colonies						
Untreated	TN	TN	TN	TN	TN	44	5
1st 100 ml	TN	TN	TN	TN	TN	40	5
2nd 100 ml	TN	TN	TN	TN	TN	45	3
3rd 100 ml	TN	TN	TN	TN	TN	42	4
4th 100 ml	TN	TN	TN	TN	TN	45	6
5th 100 ml	TN	TN	TN	TN	TN	50	4

TN = too numerous to count

Untreated: sample of suspension before passage through.

TABLE 15

VIABILITY OF *E. COLI* SUSPENSIONS PASSED THROUGH A RESIN SYSTEM WITH AG^+

Effluents	Dilution						
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
	Number of Colonies						
Untreated	TN	TN	TN	TN	411	47	5
1st 100 ml	0	0	0	3	0	0	0
2nd 100 ml	0	0	0	0	0	1	0
3rd 100 ml	0	0	0	0	0	0	0
4th 100 ml	0	0	0	0	0	0	0
5th 100 ml	4	2	1	0	0	0	0

TN = too numerous to count

Untreated: sample of suspension before passage through column.

of microbial contamination in the Apollo water systems would ever approach the quantity of organisms used in this study.

Further attempts were made to determine the upper limits of the capacity of the column systems with silver to kill bacteria. The susceptibility of three species of bacteria to silver resin inactivation was also determined.

E. coli, pseudomonas, and staphylococcus were processed by the the method described above, and cell concentration were made to 6×10^9, 5.8×10^9, and 4.4×10^9, respectively. Table 16 summarizes viability determinations made on the effluent of the column system with and without silver. It is apparent that essentially all organisms are killed by passage through the silver resin column system.

Large volumes of this high cell concentration mechanically block the flow rate after passage of the first 600 ml. The flow rate began at 5 ml/min and then stopped. This problem was remedied by either a slight back-washing or an increase in the hydrostatic head of the sample.

As the cell suspensions pass down the column its normal light throw color changes to that of cloudy white.

Tests were made of the ability of the silver resin column system to inactivate viruses. In this preliminary study the coliphage T_2 was used. A 100-ml suspension of the phage containing 10^8 particles per ml was passed through the column system with silver at the rate of five ml per minute. Phage passed through the silver containing column were killed but those passed through the same resin column system without silver were not killed, neither were phage killed in containers with silver surfaces.

TABLE 16

THE KILLING OF THREE SPECIES OF BACTERIA IN CONCENTRATED SUSPENSION BY PASSAGE THROUGH A RESIN SYSTEM CONTAINING SILVER

Effluents	Dilution						
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
	Number of Viable Cell Colonies						
E. coli sample	TN	TN	TN	TN	TN	597	60
Pseudomonas sample	TN	TN	TN	TN	TN	520	58
Staphylococcus sample	TN	TN	TN	TN	TN	562	44
1st 100 ml	0	0	0	0	0	0	0
2nd 100 ml	0	0	0	0	0	0	0
3rd 100 ml	0	0	0	0	0	0	0
4th 100 ml	0	0	0	0	0	0	0
5th 100 ml	0	0	0	0	0	0	0
6th 100 ml	0	0	0	0	0	0	0
7th 100 ml	0	0	0	0	0	0	0

TN = too numerous to count.

The concentration of silver in all eluates was determined by emission spectroscopy. In the absence of solute the quantity of silver eluted by water from a saturated silver column was much less than 0.1 ppm.

When organisms were passed through the silver resin column system in large concentration their silver content could be determined. It was found that such organisms contained approximately 5.8×10^{-14} gm of silver per cell. These organisms could be easily removed by filtration and the bactericidal system yielded water essentially silver free.

It should be pointed out that 5.8×10^{-14} gm in all likelihood saturated the binding sites for Ag^{+} in these dead organisms. But the killing of the organisms could be accomplished probably by silver bound to a small fraction of the total number of sites involved. If indeed this were the case then resins with a greater avidity for silver could be used with the result that even smaller quantities of Ag^{+}would be removed from the solution.

6.5 Experiments on the Loading of Microbial Filters Under Worse Case Conditions

The purpose of the following experiment was to discover the gram loading capacities of a number of particulate filters using a worse-condition bacterial aerosol (a worse-condition aerosol being one that would not be encountered in a space capsule). A number of particulate filters were obtained from Mine Safety Appliance, Inc. and the Pall Company for investigational use.

For this study a plexiglass chamber having a 100-liter capacity was used. Coupled to this chamber was a glass nebulizer with a filtered air supply. An air-tight filter chamber was located between the plexiglass

chamber and a portable vacuum pump. Four in the filter, two in front and two in back of the filter cartridge, allowed for manometric measurements and the collection of the bacterial aerosol by liquid impingement. The air pressure across the nebulizer was 5 cubic ft/min at 6 lb pressure. Outflow from the filter chamber was 44 liters per min.

Manometric measurements during an experimental run were made every half hour. Flow meter measurements were made concurrently. Liquid impingement samples were taken every 2 to 3 hours with an impinger volume of 44 ml of tryptone-glucose-yeast extract (TGY) broth.

Bacillus globigii (BG) was cultivated on TGY broth in 2-liter batches so that a final sample of washed cells would have a concentration of 10^9 to 10^{10} cells.

Twenty-ml portions of the above suspension was used in a 5-hour run from 2 M.S.A. filters.

The filter used in the first run was of the glass wool type. No pressure drop across this filter was observed throughout a 4-hour run and the bacteria began passing through this filter immediately.

The second filter used had a rating of 95% for all particles down to 0.3 μ. The particle size of the aerosol generated in these experiments was from 1 to 10 μ. As in the first case no pressure drop was recorded, but unlike the first case all bacteria were held by this filter in a viable condition. It may be noted here that for both runs at least 10^8 cells either hit and passed through or hit and were held to the filter every 90 seconds.

Although no loading of filters was obtained in these experiments it is conceivable that filter loading is possible. As dust, food particles, and moisture (in the form of urine, sweat, saliva, and fecal material) collects on a filter's surface, bacteria already present will multiply using these agents

for a nutrition supply.Thus, the filter surface will have a breeding ground for bacteria and molds. This collection of debris mixed with growing colonies of bacteria will not doubt clog any filter and hasten its breakdown.

It may be concluded then, that "pure" microbial aerosols of very high concentrations may be adequately filtered and will result in negligible loading and power requirements. But loading by microbiological particulates, together with microbial growth on filter fibers, are the significant loading factors.

MICROBIAL DETECTION DEVICES FOR MONITORING MICROBIAL CONTROL MEASURES

An effective system for controlling the level of microorganisms in the spacecraft environment would consist of a method for removing or killing the microorganisms and a method for monitoring the effectiveness of the control measure. Melpar has been working for three years on devices for detecting bacteria and viruses and some of these methods could be used as monitoring systems for the potential control measures described in this report. The use of a simple, small, light weight low power detector in combination with a control measure would permit much greater flexibility to be incorporated into the microbial control system. For example, in cases where excessive microbiological loading occurs, the astronaut could divert the air or water into another controlling device or make the appropriate change of control device and thus eliminate the potential problem.

A number of detection methods developed in the Melpar laboratories show sufficient promise to warrant consideration for incorporation into spacecraft and microbial control systems because of their specific characteristics and abilities. These methods are listed in Table 17 along with some details of their characteristics. The present and projected estimates of the sensitivity, speed, multiagent capability, effect of interferences, logistic considerations and the present stage of development of each of the detection methods applicable to this problem is shown in the table. From the list, however, it is expected that the esterase and the cytochemical staining method would be most applicable for detection of bacteria and fungi and that the hemagglutination

TABLE 17

DEVICES AND CONCEPTS FOR MICROBIOLOGICAL DETECTION

Concept	Sensitivity	Speed (Min)	M.A.C.	Interferences	Logistics	Stage of Development
Partichrome (Reference)	30-60 org. or 1 particle/l	8	Viruses, Bacteria, Toxic proteins	Generally nonspecific	Generally good	A*
Esterase	Now: 10^4 org.		Living bacteria Viruses?	No known interferences	Equivalent to Partichrome	B
	Proj: 10^2 org.	<5				
Hemagglutination	Now: 10^4 part.	15-20	Viruses Bacteria	Agglutins Suspected	Blood	B
	Proj: 10^2 part.	5-10				
Cytochemical Staining (FITC-Stain)	Now: 30 org.	8	Living Bacteria	Generally nonspecific	Good all around	B
	Proj: 1 org.	1	Bacteria Virus	Not fully explored		
Polarization of Fluorescence	Now: 10^5 org.	2	All nucleic acids Bacteria Viruses	None known	Moderately high power	C
	Proj: 10^4 org.	Instantaneous				
VPC Lipids	Now: 10^4 org.		Bacteria Viruses-Injected Tissues	None known	Little more complex but generally good	C
	Proj: 10^3 org.	5				
FAST	Now: 50-100 org.	15	For specific bacteria	Fluorescence Particles	Specific Antibody	A
	Proj: 1 org./ liter of air	5	Virus Protein			

*
A Advanced development
B Has been prototyped
C In research

method would be most applicable for detection of viruses in the spacecraft microbial control system. These devices which have been prototyped are automatic and have the potential of being put into a small light weight package.

The portion of the future work will be aimed at determining whether an automatic detector or a manual detector would be most suitable for the spacecraft.

Some of the considerations which will go into the design of a small compact automatic detector would consist of the following functional components: (1) a method of collection, (2) automatic sample preparation, (3) automatic sample processing, (4) automatic detection and (5) logic and readout. With a manual detector steps 2, 3, and 4 would be manual.

Performance Criteria Analysis: In this discusssion a detection system is considered to consist of the following functional components: (1) a method of collection, (2) automatic sample preparation, (3) automatic sample processing, (4) automatic detection, and (5) logic and readout.

In any given system all of these broad categories of components are interdependent. Specific design and development must be controlled so that a given component is compatible with the needs of the problem and with its associated components. Thus, the collection system should really be viewed as an air or water sampling system. The kind of sample obtained is controlled as to size and frequency by the capacity and sensitivity of the succeeding components.

Though mediated by the process of automatic sample preparation, it must be emphasized that the sensitivity of the given system must be viewed from a standpoint of the combined capacity of the collection and detection portions of the system. For example, when a detector whose

maxiumum sensitivity is 10^3 organisms is coupled with a collector that samples 1000 liters/minute, the resulting system can be considered to have a sensitivity of one organism/liter/minute. Similarly, a detector which detects individual organisms can be considered to have the same sensitivity when coupled with a collector that samples a volume of 1 liter per minute. Equally important, the automatic sample preparation must submit to the sample processor and detector a sample that has been prepared so as to provide optimum detection conditions. In addition, the state of the sample that the collector produces must be fully compatible with the needs of all further processing. Finally, the output of the detector must be subjected to whatever logical processing is required to make an instrumental decision as to whether or not an alarm should be given or any other action is to be taken. The basis for such an instrumental decision is developed from the results of laboratory and field trials of the system.

Because of the characteristics of specific systems differ widely the requisite knowledge must be developed with the particular candidate system being used as the means for this development. Hence, the readout must be more than a simple visual one which simply indicates the presence or absence of organisms of interest. Within its limitations of biological specificity, a given system should provide information on dose and dosage. These numerical values should be derived from carefully controlled experiments conducted with a complete system and should be considered the means for calibrating the system. The final numerical values should be arrived at by comparison with appropriate conventional techniques for determining the counts of the organisms of interest that are present in

the course of a given experiment. When satisfactory calibration of a given system has been completed, then additional logic can be incorporated into the equipment so that it functions not only as a detector but as an alarm of known capabilities. When this stage of system development has been obtained, it should be possible to confidently assign a proper place to a given system in the overall microbial control system in the spacecraft.

The proposed studies to be performed with the microbial detectors will consider design modifications which could be made on present detection instruments to make them applicable to spacecraft use.

8. A FUTURE WORK PLAN

The research performed to date indicates that microbial control in water supplies and on surfaces may be accomplished most effectively in Apollo by the use of silver. It is felt that future studies should deal both with methods for controlling silver ion concentration in aqueous solution and with the essential chemical mechanism by which silver produces a bactericidal effect.

The specifications of the Ag-resin-system proposed for Apollo use would be defined further in terms of contaminant effects and loading capacity. The range of bactericidal and vericidal effectiveness of such resin systems would be determined using a variety of microorganisms.

Attention would be given to the design of systems in which microbial detection is coupled to microbial control devices.

8.1 The Application of Metal Buffer Systems to Microbial Control

Future work will consists of a theoretical and experimental analysis of Melpar's concept of using metal buffer systems to control microbial growth in water and on surfaces. The function of metal ion exchange systems in controlling microbial growth is explained to a large extent by the ability of the system to function as metal buffers.

A metal buffer system tends to keep the free metal concentration of a solution constant by means of reactions such as

$$M^{+n} + HA^{-m} \rightleftharpoons MA^{m-n} + H^{+}$$

The concentration of reactants are related by the stability constant

$$K_{MA} = \frac{(MA^{m-n})\ (H^{+})}{(M^{-n})\ (HA^{-m})}$$

From this equation a quantity can be derived expressing free metal concentration as a function of total metal, total ligand, K_{MA}, K_{HA}, and hydrogen ion concentration:

$$pM = \log K_{MA}K_{HA} + \log \frac{(A^{-m})}{(M^{m-n})} + pH$$

The free metal concentration, the derived quantity, is expressed as pM and it characterizes the system with respect to free metal in a manner analogous to the characterization of hydrogen ion by pH. It is apparent from this equation that theoretically a system can be devised capable of maintaining a desired metal concentration at different concentrations of hydrogen ion. If the metal concerned had bactericidal properties its concentration could be maintained within narrow limits in a given system even if the microorganisms undergoing inactivation, or other material, take up free metal from solution. On the other hand, if a larger or smaller concentration of free metal were required in such a metal buffer system to control microbial growth it could be obtained simply by changing the pH of the system.

The anticipated research in this study will be concerned with finding ligands (MA or HA) with K_{MA} and K_{HA} values appropriate for the concentration of metal required for sterilization and for the maintenance of the system in a hydrogen ion concentration range from pH 6 to pH 8.

The ligands sought will be immobile macromolecular material with charged sites of appropriate avidity for the bactericidal metal. In the present study this metal would probably be silver, and the immobile ligands tested would be the many available cation exchange resins.

Attention would also be given to the use of metal buffer systems to maintain exceedingly low metal concentration where water is desired with an exceptionally high pM or low metal concentration.

The application of the concept of metal buffers to the control of microbial growth by metals permits a rigorous analysis of the potentiality of such metal resin systems in terms of classical chemical parameters[14].

8.2 Research on Bactericidal Compounds of Silver/Metals other Than Silver and Organic Compounds.

From the literature it is not altogether certain that silver ion is the chemical species responsible for the bactericidal activity of solutions of silver salts. Bacteriostatics has been observed in solutions which undoubtedly contained both inorganic silver compounds and silver complexes of amino acids. A minor research effort should be directed to a systematic study of these compounds.

The bactericidal and viricidal activity of mercury is well established but its possible uses as a biocide in the sterilizing resin column system proposed by Melpar should be investigated. The use of compounds of arsenic, perchlorate ion, and selenium, and rare elements should be tested in column arrangement also.

Studies of silver bacteriostatics will be concerned with biocidal compounds most appropriate for application to fabrics. The effect of contaminants of the bactericidal activity of impregnated fabrics will be studied.

The bactericidal resin column system will be tested also with ionizable organic compounds such as phenols, and salycylic acid.

If column resin systems can be devised in which the amounts of bactericidal material removed from certain modules of the system are proportional to the level of organisms present then the detection of this agent can serve as a means of monitoring the efficiency of the control methods used, and the level of organisms present.

8.3 Studies on Simulated or Actual Environmental Control Systems with the Objective of Enhancing their Ability to Retain or Inactivate Microorganisms

Development of the most promising microbial control measures recommended by this study will require extensive simulated tests. In most instances these tests should be performed with actual spacecraft subsystems and/or components. Developing the otpimum microbial filter for the Apollo Air Recirculation System will require as a minimum a CO_2 and Odor Absorber Unit with additional filter cartridges. It will be necessary to add biocidal materials to actual components of spacecraft water systems to adequately study problems of chemical interactions as well as mechanical interference. Melpar can complete the detailed design of tests following the acquisition of appropriate ECS components.

It is also highly desirable to obtain typical samples of materials containing microbial contaminants found in the spacecraft environment. Actual samples of fuel cell water and aged potable water, water samples from condensate areas, and concentrated collections of airborne particulate matter from filters, etc. would be useful in assessing the specificity and overall performance of applied control techniques. Current and planned environmental around tests of manned capsule systems are obvious sources of the samples needed.

8.4 The Design of a Microbial Control System Using Coupled Microbial Detection and Bactericidal Components

Consideration will be given to two types of detectors for monitoring the efficiency of the bactericidal device. One type would be manual, which could be used periodically by the astronaut, which would permit him to make manual adjustment of the control measures. The other type could be an automatic system for sampling, processing and correcting system levels of microbial contamination. Conceptual designs of the most appropriate systems for microbial control will be made.

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Patent Numbers 2,500,113; 2,562,488; 2,595,290.

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Detrick: Safety Regulations

Detrick TM2: Practical Procedures for Microbial Decontamination

USAD School of Aerospace Medicine: numerous reports and papers

NASA: Aerospace Medicine; Bibliography

NASA (JPL): Literature Searches on Sterilization

DDC Bibliographies: Water Purification, Microorganisms in Manned Spacecraft

OTS Bibliography: Space Environmental Conditions.

APPENDIX

TABLE A. ROUGHING FILTERS

Particle Retention* 10 to 60 Percent

Nomenclature	Manufacturer	Media	Capacity cfm/ft^2 of face A	Face velocity ft./min.	Press. drop In of H_2O	Max. oprn. temp.
AAF type HV 2	American Air Filter Corp. Louisville, Kentucky	Adhesive-coated V-crimped wire screen mesh	250 to 430	300 to 500	0.04 to 0.1C	110°F
AAF PL24 w/type G media	American Air Filter Corp.	Glass filament	up to 250	250	0.06	250°F
Drico puff-glass	Drico Industrial Corp. Passaic, N.J.	Spun glass fiber	32 to 1000	300	0.08 to 0.11	175°F
Far-Air HP-2	Farr Filter Co. Los Angeles, Calif.	Pleated cotton fabric	250 to 435	250 to 435	0.045 to 0.115	225°F
Farr 44-68	Farr Filter Co.	Crimped screen and wire mesh	250 to 435	250 to 435	0.040	275°F

*One to five microns.

TABLE B. HIGH-EFFICIENCY FILTERS

Particle Retention* 90 to 99 Percent

Nomenclature	Manufacturer	Media	Capacity cfm/ft^2 of face A	Face velocity ft./min.	Press. drop In.of H_2O	Max. oprn. temp.
Multi-Pak w/ 50 FG	American Air Filter Corp. Louisville, Ky.	Glass fiber	125 to 250	250	0.42	400°F
Deep bed w/ 50 FG	American Air Filter Corp.	Glass fiber	40 to 200	200	0.42	400°F
Microtain	Cambridge Filter Corp. Syracuse, N.Y.	Glass-asbestos pleated	50 to 250	Up to 250	0.4	220°F to 800°F
Aerosolve 85	Cambridge Filter Corp.	Glass fibers pleated	125 to 500	250 to 500	0.22 to 0.32	400°F
Aerosolve 95	Cambridge Filter Corp.	Glass fiber pleated	125 to 500	250 to 500	0.35 to 0.45	400°F
Hp-100	Farr Filter Co. Los Angeles, Calif.	Glass fiber pleated	250	250	0.20	275°F
HP-200	Farr Filter Co.	Glass fiber	250	250	0.38	275°F

*One to five microns.

TABLE C. ULTRA-HIGH-EFFICIENCY FILTERS

Particle Retention* More than 99.99 Percent

Nomenclature	Manufacturer	Media	Capacity cfm/ft^2 of face A	Face velocity ft./min.	Max. drop In.of H_2O	Max. opm. temp.
AAF Type F (glass)	American Air Corp. Louisville. Ky.	Glass fiber and draft paper or alum sep.	30 to 400	68 to 325	1.0	$250^{\circ}F$ to $1000^{\circ}F$
AAF Type F (ceramic)	American Air Filter Corp.	Ceramic asbestos fiber and alum sep.	30 to 250	250	1.0	$1600^{\circ}F$ to $2300^{\circ}F$
Cambridge Absolute	Cambridge Filter Corp. Syracuse, N.Y.	Glass fiber asbestos paper sep.	30 to 345	Up to 275	1.0	$800^{\circ}F$
Magnamedia	Farr Filter Co. Los Angeles, Calif.	Glass fiber	30 to 400	Up to 250	1.0	Up to $1000^{\circ}F$
Airpure absolute glass F 600	Flanders Filters Riverhead, N.Y.	Glass fiber (F600)	30 to 400	Up to 320	1.0	$850^{\circ}F$
Airpure absolute ceramic abestos	Flanders Filters	Ceramic-asbestos	50 to 250	Up to 250	1.0	$1600^{\circ}F$
Ultra-Aire	Mine Safety App. Co. Pittsburgh, Pa.	Glass fiber	35 to 250	Up to 250	0.9	$500^{\circ}F$

Note: 1. Capacities are in cfm per sq. ft. of face area, not total area of filter.
2. Face velocities are fpm for 1 sq. ft. of face area, not media velocity.

*One to five microns.

SUMMAR
MICROBI

Methods	Microbial Control Medium	Basic Control Technique	Human Toxicity Factors	Outgassing Factors
Filtration (Non-impregnated)	Air	Mechanical collection/ retainment of microbial particles	None	None
Filtration (Impregnated)		Mechanical and chemical inactivation	not defined	coating dependent
Electrostatic Precipitator		Electro-mechanical collection/retainment of microbial particles	None	Ozone
Ultraviolet Light		Chemo/physical photo-dynamic inactivation	wavelength exposure conc. dependent	wavelength dependent
Impaction & Impingement		Inertial collection/ retainment of microbial particles	None	None
Silver Coatings & Cation exchange Resins	Water	Bacteriocidal & Virucidal	None	
Photodynamic Activated Dyes		Photochemical inactivation	None	
Filtration (Non-impregnated)		Mechanical collection/ retainment of microbial particles	None	
Filtration (Impregnated)		Mechanical & chemical inactivation	Coating dependent, controll limits	
Chemical Additives		Chemical reactions with essential physiological components	Additive dependent (f)	
Ultraviolet Light		Chemo/physical/photo dynamic inactivation	Dose & wavelength depende	

TABLE D

Y - PERFORMANCE CHARACTERISTICS OF
AL CONTROL METHODS FOR SPACECRAFT

Human Palatibility Factors	Mutagenic Factor	Est. Power, Weight, Size	Spacecraft Subsystem Affected	Comments
———	None	< 1 watt < .03 oz (a) < 20 in^3	Pressure-suit supply system	(a) power equivalent for clean filter, Δ_p = 1.0 in. H_2O 35 cfm over 0.5 ft^2 area
None	None	10% Non-impreg filter	Pressure-suit supply system	
None	None	(b)	Capsule Air recirculation system	(b) power, weight & size dependent on integration with existing spacecraft components (blower, collection plate, etc.)
None	Yes, wave-length depend.	0-15 watts (c)	Separate instrumenration	(c) power range represents use of sunlight to utilization of mercury arc lamp
———	None	>150 watts for 0.5 cmf	Pressure suit supply system	
———	None	neglibiele power 7 oz. 10 in^3	Water subsystem Reservoirs	
d) ———	Probably None	< 5 watts continuous 0.1 oz. 0.25 in^3	Water subsystem reservoirs	(d) Dye removal will eliminate problems of toxicity and palatibility
———	None	0.2 watts (e) 5 oz. 10 in^3	Water subsystem Reservoirs	(e) power equivalent for clean filter with Δ_p = 0.1 psi 0.5 gal/min.
ble within	None	10% non-impreg filter	Water subsystem circuit lines	
———	possible but controllable	no power 1 gm 0.25 in^3	Water Subsystem Reservoirs	(f) Tablet with controlled leaching properties
t (g) ———	Yes, wave-length depend.	<15 watts 2 oz. 5 in^3	Water subsystem Reservoirs	(g) biocidal effects may be attenuated by particle concentration

During the course of this study Melpar representatives visited several laboratories with the purpose of conferring on problems in the microbiology of closed systems.

Some of the places visited and people contacted are the following:

Wright-Patterson Air Force Base
Dayton, Ohio
Dr. Sheldon London

Brooks Air Force Base
Aerospace Medical Division
San Antonio, Texas
Major James Moyer

Chloramine Company
New York City
I. B. Romans, A.B.