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THE ECOLOGY OF MICROORGANISMS IN A SMALL CLOSED SYSTEM: POTENTIAL BENEFITS AND PROBLEMS FOR SPACE STATION

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16. ABSTRACT

The inevitble presence on the Space Station of Microorganisms associated with crew members and their environment will have the potential for both benefits and a range of problems including illness and corrosion of materials. This report reviews the literature presenting information about microorganisms pertinent to Environmental Control and Life Support (ECLS) on the Space Station. The perspective of the report is ecological, viewing the Space Station as an ecosystem in which biological relationships are affected by factors such as zero gravity and by closure of a small volume of space.

Potential sites and activities of microorganisms on the Space Station and their environmental limits, microbial standards for the Space Station, monitoring and control methods, effects of space factors on microorganisms, and extraterrestrial contamination are discussed.

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INTRODUCTION

Microorganisms will, without question, be part of the environment of the Space Station. In no environment where higher organisms (e.g., humans) are present are microorganisms absent [26]. In fact, without them, higher organisms would quickly disappear on Earth [26]. The inevitability of the presence of microorganisms leads to questions of their influence on the enclosed environment of the Space Station and on the functioning and health of the astronauts inhabiting it over long periods of time. It was recognized early that the prevention of microbial contamination of the astronauts' environment is an important part of the function of life support systems, requiring the microbiologist to supply substantial support to the engineer during the design and integration phases [79].

This report is a review of the literature dealing with microbiological studies relevant to synthetic, closed or partially closed systems designed for human travel and/or habitation in space. The types of organisms identified in closed areas, their functional relationships with other organisms, especially humans, and their environments, their response to space flight factors, and methods of control are addressed. Any of these topics could be pursued more extensively if required. This review is intended to elucidate trends in research thus far, to identify the most information-rich literature sources, and to point out gaps in present knowledge. The experimental information presented is intended for use as a basis for decisions related to environmental control and life support on the Space Station.

This report is written for a potentially diverse group of readers including engineers, physicists, chemists, and biologists. Because some of the terminology used here originated in special areas of biology, and may be unfamiliar to those disciplined in other fields, a glossary has been included as an appendix.

MICROORGANISMS

The term microorganism includes viruses, bacteria, fungi, algae, and protozoa, a very diverse group. Viruses are the smallest organisms, most being between 20 and 300 nm in diameter. They are acellular and cannot reproduce outside a suitable host cell. Bacteria are prokaryotes with a great variety of shapes, and sizes ranging from 0.1 μm to more than 50 μm in length. When described in terms of their energy requirements, bacteria are either phototrophic, utilizing light as an energy source; chemolithotrophic, utilizing inorganic compounds as an energy source; or chemoorganotrophic, depending on preformed organic carbon for energy [9]. Requirements for carbon further identify bacteria as autotrophs, utilizing CO_2 in the atmosphere, and heterotrophs, requiring organic molecules as a carbon source. The great diversity of bacteria enables them to successfully invade and persist in a tremendous variety of habitats. The fungi are eukaryotic, spore-bearing heterotrophs lacking chlorophyll and include yeasts and mushrooms. Algae, eukaryotes containing photosynthetic pigments including chlorophyll, include both uni- and multicellular forms, and are primarily associated with aquatic habitats. Protozoa are typically unicellular eukaryotes [9]. Neither algae nor protozoa are likely to be abundant on the Space Station except as controlled experimental organisms.

SOURCES, SITES, AND ACTIVITIES OF MICROORGANISMS ON SPACE STATION

The Human Body

Crew members will be the primary source of microorganisms on the Space Station. The healthy human body is the site of more than 50 species of microorganisms associated with the surfaces of the skin, upper respiratory tract, mouth, lower intestine, and genito-urinary tract [34]. These are primarily bacteria, but may also include viruses, fungi and protozoa. The digestive tract harbors the primary quantity and diversity of indigenous microbial species.

In the colon, cecum, and lower part of the ileum, there may be as many as 10^{10} bacterial cells per gram content [95]. A person voids approximately 10^{11} bacterial cells per day, as well as smaller numbers of fungi and protozoa [26]. Lists of species of microorganisms of the intestine may be found in the literature (e.g., 9, 95, 147, 153, 154, 180). The gram negative intestinal bacteria include both aerobic and anaerobic forms. Most of these are bacilli; the cocci *Neisseria* spp. (aerobic) and *Veillonella* spp. (anaerobic) occur infrequently in the lower intestinal tract [66]. Gram negative Enterobacteriaceae, *Alcaligenes* spp., *Pseudomonas* spp., and *Flavobacterium* spp. are occasionally found. Coliforms such as *Escherichia coli* (Enterobacteriaceae) are well-known intestinal inhabitants whose presence in terrestrial habitats such as streams or water supplies is often used as an indication of fecal pollution. The most common aerobic gram positive bacteria in the intestinal tract are *Staphylococcus* spp., *Streptococcus* spp., and *Lactobacillus* spp.. *Bifidobacterium* spp. are among the gram positive anaerobes found in the human intestine [66]. Mean log counts of certain viable bacteria in normal fecal flora are:

Restellae 9-11
Bifidobacteriaceae 9-11
Lactobacteriaceae 8-10
Enterobacteriaceae 8-9
Lancefield D. Streptococci 6-8
Clostridia 4-8
Staphylococcus aureus 1-3 [95].

A small group of microorganisms is self-supportive, appearing in the digestive tract for practically unlimited periods of time [37, 123, 224]. This group forms what may be called the truly indigenous flora. A second group, with the largest number of species, is comprised of microbes incapable, or only partially capable of maintaining their populations within the body. They rely upon reinoculation from an outside source, such as food, to prevent their disappearance. The third group includes microorganisms which are transient, have entered the body by chance, and will not form viable populations [125].

In a healthy individual, the intestinal flora must be adapted to a constant temperature of 37°C and neutral to aklaline pH [9]. Within the confines of the intestinal habitat, microorganisms must compete with other microorganisms and with the intestinal cells themselves for available nutrients. Their metabolic activities generally remove oxygen, creating conditions favoring the growth of obligate and facultative anaerobes [9].

Spore-forming bacteria (those that produce a resistant resting stage) normally occur in the intestine at 10^2 to 10^4 per gram or less. Among these are *Clostridium perfringens* and other species of *Clostridium*, and some species of *Bacillus* [90].

Human skin provides a favorable environment for the penetration, lodgment, and multiplication of a variety of microorganisms [137]. The outer layers of dead keratinized cells which are formed into ridges and furrows and pierced by orifices of sweat glands, contain sebum, salts, urea, and oils which together with the proteins (largely keratin) from dead epidermal cells provide all the necessary nutrients for microorganisms [45, 171]. A review of the composition and daily amount of waste products produced by humans (e.g., nails, sweat, saliva, hair, etc.) appears in Reference 21. Many of the free fatty acids on the skin surface have antimicrobial activity; however, some successful inhabitants are able to metabolize these compounds [9]. The pH of the skin normally between 3.5 and 7.0 is also suitable for microbial growth [46]. Further, in the 0-g environment, perspiration will tend to sheet on the skin where there is no free convection. Particle behavior will vary, causing skin to become an ideal environment for microbial growth [34]. In a 1-g environment, about 105kJ/h may be lost from the body surface, causing convection currents that can carry particles of up to 50 μm [34, 37]. Thus, bacteria, usually around 1 μm in diameter, can become airborne due to body-induced convection currents [34].

In healthy people, viruses, fungi, and possibly protozoa may be present on skin as either transient or resident organisms. Those that are resident, or autochthonous, occur only on the surfaces of the skin and are not invasive [9]. Areas of high and low density are found with no observed differences between sexes [216]. Each person maintains the microbial population size within a limited range despite climatic changes and only temporary reduction in numbers achieved by scrubbing, flushing, and the use of germicides [216]. Populations of microorganisms on the skin are not uniformly distributed; the populations vary both qualitatively and quantitatively at different sites (e.g., scalp, armpits, abdomen, groin, etc.). Available water is often a major limiting factor in the distribution of microbes on the skin surface; thus, they are inclined to greater growth and survival in more moist areas.

There are relatively few fungi on normal skin. Two fungal species, *Pityrosporum ovale* and *Pityrosporum orbiculara*, do occur on the scalp. The bacterial populations of the skin include both aerobic and anaerobic species. The dominant ones are gram positive and include *Staphylococcus* spp. and *Micrococcus* spp. which normally occur in relatively high numbers. Other less abundant members of the skin flora are *Corynebacterium* spp., *Brevibacterium* spp., and *Propionibacterium* spp.. The only normally occurring gram negative bacteria are *Acinetobacter* spp. [9].

Because of the highly acidic conditions of the stomach, any microorganisms found there are considered to be allochthonous [9]. Recently eliminated urine normally has 200 to 400 bacterial colonies per ml, mostly white and golden *Staphylococcus* spp., and no pathogens although there may be latent bacilli carried in some cases [123]. A list of pathogens that may be found in the urinary tract appears in Reference 154.

The human mouth harbors a great diversity of microorganisms. There is usually a high proportion of *Streptococcus* spp., and *Veillonella* spp. are often abundant. There are usually a few *Lactobacillus* spp., and *Actinomyces* spp., *Pseudomonas* spp., and *Bacterioides* spp. may be present [9]. The yeast, *Candida albicans*, is commonly found, and the protozoans, *Entamoeba* spp. and *Trichomonas* spp., may be present. Certain species of *Actinomyces*, *Bacterionema*, *Leptotrichia*, and *Rothia* are found only in the oral cavity and hence may be called autochthonous flora [9].

In 1966, a 132 page report was prepared for NASA surveying the literature on intestinal and skin flora [77]. The goal of the report was to define as completely as possible the microflora present on the integument under ordinary environmental conditions. The microflora studied were aerobic and anaerobic bacteria fungi (yeasts and molds), actinomycetes, and viruses. The report, written as a basis for detecting the influence of space flight on human flora, although written two decades ago, provides a thorough review and an extensive bibliography which is useful currently, and treats the subject much more completely than it can be treated here.

Pathogens/Interchange

Several organisms present in the environment and on the skin or within the body are potentially pathogenic, causing disease when circumstances favor their transfer to a suitable site, and their proliferation [34, 167]. Some microorganisms commonly found associated with humans can cause illness given the proper environmental conditions. For example, boils and infections of the skin can be caused by *Staphylococcus aureus*, infections of wounds by *Streptococcus* spp., *Enterobacteriaceae*, or other gram negative rods, dermatomycosis and onychomycosis by *Candida albicans*, exterior auditory canal infections by *Pseudomonas aeruginosa*, and erythrasma by *Corynebacterium minutissimum* [154]. *E. coli*, normally found in large numbers in the intestinal tract, causes infection when transferred to the urinary tract [9].

During a 3-month tropical military exercise, a number of skin infections became apparent due to the proliferation of various microorganisms such as *Pseudomonas aeruginosa*, *Corynebacterium minutissimum*, *Candida albicans*, and *Staphylococcus aureus*, among others [206]. All these organisms occur normally in the human's environment but became infectious in the tropical setting favorable to their growth.

In the respiratory tract, *S. pyogenes* can cause pharyngitis, tonsillitis, and scarlet fever. *S. aureus* can cause abscess of the larynx and pneumonia. *Streptococcus pneumoniae* can cause pneumonia. *Proteus* spp., *Pseudomonas* spp., *Kiebsiella* spp., and other coliforms can cause non-specific membranous laryngitis, chronic sinusitis, pneumonia, and abscess of the larynx. Thrush, bronchitis, and pneumonitis can be caused by *Candida albicans* [154]. *Proteus* sp. is highly pathogenic when it is found outside the gastrointestinal tract, often acting as a secondary invader in dermatitis, especially of the feet [155]. All the above microorganisms implicated in the appearance of disease are normally found in small numbers in association with healthy humans. They are usually pathogenic only when they occur in large numbers and/or when the host organism has characteristics of reduced resistance.

Many organisms, not normally associated with humans but found in their environment, can lead to disease when they are able to establish populations on or within the body. Such are the protozoans, *Trichomonas vaginalis*, *Giardia lamblia*, *Trypanosoma cruzii*, *Entamoeba histolytica*, and others [88]. The bacterium, *Legionella pneumophilia*, the source of Legionaires' disease, occurs naturally and is widely dispersed [9, 34]. Its growth is supported by terrestrial air conditioners, heat exchangers, and cooling towers, which provide a reservoir and a mechanism through which the bacteria can become airborne by means of aerosol formation [9]. Usually, only when its growth is amplified and it occurs in very large numbers, does its pathogenic potential increase dramatically [34]; however, such amplified growth is not always necessary for pathogenicity (A. K. Highsmith, personal communication). Altitude stress may reduce resistance to infection by *Klebsiella pneumoniae*, *Pasteurella tularensis*, and *Salmonella* sp. [17, 63, 191].

S. aureus and *C. albicans*, both potential pathogens, have been present on all NASA missions. In addition, enteric bacteria have frequently been recovered on these missions and are considered potentially threatening when found in large numbers at sites other than the lower gastrointestinal tract. *E. coli*, *Enterobacter aerogenes*, and *P. mirabilis* have been recovered from the upper respiratory tract as well as from other areas on some missions [154]. *Acinetobacter calcoaceticus* and four species of *Haemophilus* were isolated from the oral cavity of astronauts. These last mentioned species can lead to septicemias and upper respiratory tract infections [154].

Of concern on the Space Station will be the potential for interchange of flora between astronauts, although some claim that most interchange will occur prior to departure during prolonged ground training involving close personal contact [147]. Pathogens are normally transmitted by direct contact, by airborne (e.g., aerosols) or waterborne dispersal, ingestion with food, by fomites, or by vectors. Usually, high numbers of microorganisms must be released in order to assure successful dissemination to a host organism. However, some pathogens

exhibit an extremely low $L.D._{50}$ in experimental animals and presumably in humans (R. Zahorchak, personal communication). Where fecal contamination is not controlled, as in water systems, contamination of food and outbreaks of waterborne diseases may occur [9].

Normally, there is great variability in the indigenous flora of individuals [125, 131, 189, 226], despite the intermingling of floral communities [125]. In closed environments, this interchange of microflora among organisms may be facilitated [14, 57], leading to rapid decline of variability among individuals [131, 224]. Many studies document the preferential exchange of pathogens [e.g., 14, 57] which could lead to infection in a host organism with reduced resistance [125]. Conditions in a small confined space can reduce natural protective function, creating favorable conditions for pathogenic and saprophytic disease causing organisms [15, 85, 126, 158]. The bactericidal properties of skin are reduced in people in confined spaces where insolation is absent and hypodynamic conditions exist [218].

Ordinarily, many pathogenic organisms must outcompete the resident flora in order to produce populations large enough to produce disease. Their ability to compete is influenced by such factors as bathing habits of the host, perspiration levels, pH, clothing (e.g., fiber content, etc.), distribution of hair on the body, the level of environmental contaminants, body temperature, and individual resistance [182].

There is a limited number of routes available by which infectious microorganisms can enter a host. Skin is normally a most effective barrier, preventing entry into the body of unwanted microorganisms and foreign material [46], by both mechanical means and the secretion of specific antimicrobial substances [26]. The skin itself is a mechanical barrier, and mucous membranes provide such a barrier in the gastro-intestinal, respiratory, genital, and urinary tracts. Thus, most organisms invade the skin through pores or wounds. The ability of the skin to act as a biological barrier to microorganisms may be impaired by microscopic injuries or intermittent contact with harsh detergents or sanitizing agents [46]. Caustic substances, burns, frostbite, and other physical traumata may also allow microorganisms to enter the body (R. Zahorchak, personal communication). Unrestricted washing of the skin, removing all products of the sebaceous and sweat glands, interferes with the protective function of the skin and is thus not a rational hygienic measure [130]. Most microbes, unable to penetrate the outer skin layers, usually enter through the respiratory tract, genital tract, or breaks in the surface of the skin [9, 26].

The production of toxic substances which affect invading microorganisms or which alter nutritional and physiological aspects of the host organisms so that the microbe cannot grow and establish itself are examples of physiological and biochemical mechanisms by which pathogens are resisted [26]. Antibacterial products such as lysozyme probably play an important role in defense versus invasion, especially in the eye, despite the fact that many bacteria are lysozyme tolerant [26]. Some dermatophytic fungi are able to produce keratinases which break down skin keratins and promote invasion [26].

The spread of disease is an ecological process, dependent upon the biological properties of the causative organism, the biological properties of the host, and the abiotic and biotic factors that affect the transmission of the pathogen between hosts [9]. Cooperation in the population appears to play an important role in the success of disease-causing microorganisms. Generally, many cells of a pathogen are required to establish a disease. Each pathogen has an optimal population density for maximal growth rate, low densities of pathogens usually, but not always, being ineffective at establishing growth within a host [9, 234]. Although this is sometimes due to the presence of virulent mutants in the larger population [236], it may also be due to physiological cooperation, as in production of sufficient toxin to neutralize the host's defenses or the creation of a favorable redox potential [234].

Most of the microorganisms that cause disease in man or animals are obligate parasites. Although many of them sur[illegible]n the inanimate environment, very few can multiply there [234]. On Earth, pathogens dispersed by man are ra[illegible]y diluted to levels at which new infections do not occur, especially outdoors. The airborne spread of

animal pathogens is usually confined to indoor spaces where dilution by air is reduced. Fungal diseases are an exception to this rule, showing effective dispersal even outdoors on Earth [234]. Air movement and velocity are important in the dispersal of airborne pathogens; temperature and humdity are important in their survival [9]. The severe limitation of air volume on the Space Station will essentially eliminate the mechanism of dilution of pathogens for preventing disease.

Even when pathogens can establish a population on or within a suitable host organism, they can generally grow for only a limited period. Following a period of growth by the pathogen, the host animal either dies or develops an effective immune response, ridding itself of the disease. When either of these phenomena occurs, populations of the pathogens must be transmitted to new susceptible hosts in order to survive [9].

Few sources in the literature have dealt with the potential for problems with viruses on the Space Station, although viruses do exist in fairly high concentrations in microenvironments in nature [26], and do not reproduce outside a suitable host. The importance of viral contamination of the Space Station should not be considered secondary to concern with contamination by other microbial forms. If research animals or plants are on the Space Station, it must be realized that some viruses can multiply in unnatural hosts to cause diseases different from those caused in their normal hosts [16]. There is no basis for assuming that the viruses of animal origin cannot enter human cells, regardless of whether these viruses are serologically related to viruses of human origin [16]. For example, some plant arboviruses have the ability to multiply in their arthropod vectors [16]. The possibilities for and consequences of intrusion of microorganisms between the links of an ecosystem need to be known [85].

A controlling factor in a host-parasite relation is adsorption. In many host-virus systems, a productive infection occurs every time a virus particle adsorbs to a cell [205]. The length of time required before a viral particle will adsorb to a cell is determined by the concentrations of both the virus and the host cell. If both concentrations are low, infection will likely not occur [206]. At least one experiment demonstrated a relationship between susceptibility to viral infection and atmospheric pressure. Mice were more susceptible to Mengo virus in an altitude chamber, whether the pressure was increased or decreased by a factor of 380 mm Hg [84].

Examples of interchange (or its absence) of microorganisms in confined spaces abound. Data from submarines provide some of the earliest information. On one cruise, an outbreak of *Mycoplasma pneumoniae* occurred after a latent period of 26 days. It was assumed that the organism had been brought onto the submarine by one of the crew members [189]. There was, in general, wide variation among crew members in numbers of associated microorganisms [155]. Because counts on the forehead were higher than those of the groin or axilla, it was postulated that treated clothing reduced microbial growth at those sites. *Pseudomonas* sp. was isolated from the water supply, the men, and the environment of the Ben Franklin with great frequency and spread throughout the crew during the mission [155]. On the same cruise, *Proteus* sp., originally isolated from a crew member's feet, spread to the shower floor and the other crewmen [155]. The transmission and fate of these kinds of organisms are of great importance in a small enclosed area because of their potential as pathogens.

On eight cruises conducted by the Naval Biological Laboratory there was widespread interchange of respiratory infection during the first week, followed by group immunity by the fourth week [226]. However, during a 56-day experiment isolating 4 men in a cabin, there was only one instance of interchange of *Staphylococcus* sp. unaccompanied by adverse clinical signs [149]. When eight males were confined 34 days, potentially pathogenic *Shigella* Poly B, Bethesda-Ballerup and coagulase-positive phage-typable staphylococci were isolated but did not cause illness and were not readily transferred from one subject to another [78].

In 14- to 30-day experiments with two men at a time in a space simulator, there was evidence for both transference and non-transference of microbial flora [147]. Transfer of enteric organisms was clearly established. When interchange occurred, it was usually transitory [147]. On Apollo 17, the opportunistic pathogen, *Pseudomonas aeruginosa*, spread from the toes of one crew member to the toes of all other crew members, without

resulting in secondary infections. On Apollo 7 and 12, *Staphylococcus aureus*, B-hemolytic streptococci, and *Aspergillus fumigatus* were transferred between crew members [72]. In the Apollo-Soyuz Test Project, intra-crew, but not inter-crew transfer of pathogens occurred [165, 210].

Although not an example of confinement, a middle-aged man, and his wife who was subject to intermittent diarrhea, did not exchange enteric microorganisms even after 451 days [191]. In a survey of 75 married couples whose mouth flora were cultured to isolate the frequently found species, *Hemophilis influenzae* and *H. haemolyticus*, there was only a 55% occurrence of interchange [101]. Regarding hemolytic streptococci, only 14% of the couples simultaneously carried the organism [101]. In another experiment, attempts to replace resident strains of E. coli in dogs, with new strains failed [191].

The data in the literature, derived primarily from studies of confined spaces, do not present unified support of either inevitble interchange or its absence among a group of people in a closed space. One primary reason for this is that the data came from very different sources, some experimental, others not adequately designed to elucidate the problem of interchange, and all subject to very different factors affecting the interchange phenomenon. In a comparison of five studies of interchange of human flora, there was noted a wide range of test conditions, including atmospheric conditions of normal gas mixtures and 100% oxygen, and pressures of 14.7 to 3.5 psi [230]. The number of test subjects ranged from 2 to 6, and the duration of the tests ranged from a few days to 56 days [230]. Interchange of microflora occurred in three of the five tests [147, 148, 149, 153, 230]. In one test, microfloral interchange resulted in the replacement of the normal mixed flora of the nose with a pure culture of the transferred bacteria [153, 232]. These kinds of results lead to difficulty in comparing them and drawing conclusions pertinent to the Space Station.

Benefits of Microorganisms

If microorganisms offered no advantage to people and their environment, it would be safe to attempt to eliminate them all on the Space Station. However, certain microorganisms and groups of microorganisms are beneficial and their elimination (although practically impossible) would be disadvantageous. The fact that many mammals are dependent on microorganisms for their livelihood suggests that they have evolved mechanisms for discriminating between beneficial microorganisms encountered early in evolutionary history, and harmful microorganisms encountered later [26].

Microorganisms have been shown to have beneficial effects on an animal in several ways. Specific microorganisms synthesize specific vitamins and growth factors needed by an animal. For example, E. coli has been shown to synthesize vitamin K in rats [26] and fat soluble vitamin K is produced by the flora of the human mouth [95]. The total flora of an animal may collectively create a favorable biochemical environment for the host. For example, the redox potential of the cecum of a normal guinea pig is much lower than that of a germ-free guinea pig [128].

The flora of the skin possesses immunizing, enzyme-forming and vitamin producing action as well as properties that are antagonistic to certain external microorganisms [128, 130]. Substances released by skin microflora increase the amount of free higher and lower fatty acids, ketones and aldehydes [129]. Results of one study lead to the suggestion that personal hygiene materials should possess selective action against only pathogenic and potentially pathogenic microorganisms causing external contamination [130]. Stabilization of the bacterial population of the skin arises primarily as a result of the accumulation of sebum cutaneum in the underclothing and on the skin surface [130].

On the Ben Franklin submersible, numerous skin rashes occurred, presumably as a result of reducing the diversity of the skin flora [155]. Other factors that could also have supported the development of the rashes were humidity, pH, temperature, etc. (A. K. Highsmith, personal communication). It was suggested that although the

reduction in the microbial population of the skin may decrease body odor, the overall effect on the balanced skin ecology may outweigh any advantages of diminishing odor, and that the maintenance of a balance of the diverse microflora may be the key to healthy skin. For longer missions, the deliberate introduction of gram positive microbial organisms was suggested should the balance of the flora become seriously upset. [155].

In a study with isolated (but not completely sealed off) subjects conducted in the mid-1960's there was the suggestion that the corynebacteria of the skin partially controlled the growth of staphylococci. During the 60 day test, the microbial flora never showed uncontrolled growth characteristics [174]. The conclusion was drawn that the natural control of potentially harmful microbes may be a desirable solution to microbial problems on very long space missions since natural control would reduce the danger of "microbic shock" [175], to be discussed later.

Numerous benefits are derived from the assemblage of microorganisms found in the human intestine. The intestinal flora may aid in the digestion process [222]. In a study of 125 fecal samples from 25 healthy young men ranging in age from 18 to 35, type cultures representing the most frequently occurring strict anaerobes were isolated. The ability of these anaerobes to metabolize simple and more complex carbohydrates, certain proteins and fats was tested. Several of the cultures were capable of metabolizing all classes of substrate tested and many of the cultures produced the vitamins B2, B12 [120], pantothenic acid, and folic acid. Others confirm the ability of the intestinal flora to produce growth factors [e.g., 9]. A few of the cultures used one or more of these vitamins [77]. The intestinal flora may influence the metabolism of cancers, serum proteins, cholesterol, hormones, and vitamins, and affect the incidence of caries [97]. Thus, metabolic links between humans and their flora are numerous and important and may involve many metabolic reactions of the host [97].

The normal intestinal flora, by its preemptive colonization and competitive inhibition, constitutes an important barrier to attack by intestinal pathogens [9, 184, 154]. This is illustrated by the high incidence of severe intestinal infection that occurs when germ-free animals are exposed to normal non-sterilized food [9]. Aerobic spore formers such as *Bacillus subtilis* occur in large numbers in the gut tract of conventional guinea pigs without harm. However, when pure cultures of aerobic spore formers are introduced to the environment of germ free guinea pigs, they usually kill the animal within 48 hours [214].

Intestinal microorganisms may challenge their host's defense mechanisms and thereby effect a relatively non-specific increase in resistance to disease [61, 134]. When resistance of the host is lowered or the predominant microflora are eliminated, this mechanism fails [147, 183]. Intestinal morphology may be influenced by the resident flora. Studies with germ-free mammals have resulted in grossly distorted ceca whose size could be reduced by the restoration of the flora or the inclusion of sterile fiber in the diet [134]. Alterations in the human flora can lead to changes in the immune response, difficulty in re-establishing the original flora, and the emergence of a previously controlled pathogen [154].

Humans aboard the Space Station will certainly not be gnotobiotes (germ free organisms). An important consideration will be the determination of what biological associates should be taken along [214]. One would assume that all pathogens, parasites, and pests should be excluded. However, as outlined above, whether or not an organism is pathogenic is determined not only by its species but also by its numerical density, resistance of the host(s), its site of growth, etc. Both *E. coli* and *Staphylococcus* spp. have caused many deaths of humans but are among the normally occurring flora [215].

Air

Bacteria, algae, yeasts, fungal spores, viruses, and protozoa can all be airborne [26]. On Earth, fungal spores, large numbers of which can be carried by air currents, appear to predominate over the other forms in the atmosphere [26]. Densities of 550 to 4000/m^3 have been collected 25 m above the ground in Texas [27]. On a

submarine cruise, air samples taken two times per week revealed fungal spore densities of less than or equal to 690/m^3, not high considering the presence of slime growths near the sample sites [56]. The recirculated air had few microbial particles. The highest fungal spore counts were 200 to 240/m^3; most were a factor of 10 lower than this [56]. About 98 percent of the spores were *Penicillium* spp., *Cladosporium* spp., *Aspergillus* spp., yeasts, *Cephalosporium* spp., and *Phoma* spp. each comprised 1 percent or less of the total number [56]. *Aspergillus* spp., known to cause human illness, were represented by eleven strains, of which two were *A. niger*, and nine were *A. versicolor* [56].

Microorganisms present in the atmosphere of the Space Station are likely to be primarily of human origin. For example, on Tektite I, there were 21.3 staphylococci/m^3 and 284.1 *Pseudomonas* sp./m^3 (presumably *P. aeruginosa* in the atmosphere [41]. The level of microbial contamination of the air in a closed space depends upon the number of crew members, the duration of their stay, the conditions of their work, and the filtering capability and cycling of the mechanical system, among other factors [159].

When experimental plants and/or animals are on board they will contribute both metabolic and bacteriological contaminants to the air [2]. When experimental animals are taken on board, the number of microorganisms can increase five times [226]. Experimental plants can also lead to an increase in airborne microbes. In a Russian semi-closed experimental system, there were always more mold spores in the air than on surfaces. During one segment of the experiment, there was a connection between the crew module and module for plant growth. With the introduction of plants and fungal spores *Penicillium* spp. and *Aspergillus* spp. became dominant in the atmosphere [85]. In a 90-day Space Station simulator test, fungi in the atmosphere were mostly hardy, non-fastidious types, ubiquitous in soil and air. *Aspergillus* spp., *Penicillium* spp., *Pullularia* spp., *Rhodotorula* spp., and various yeasts were dominant. No instances of fungal proliferation were observed [198].

Although airborne microorganisms may occur individually, they are more likely to be carried in clusters, in aerosols [26, 85, 114]. Early studies indicated the unlikelyhood of growth of microorganisms while airborne [26]. Later evidence showed the capacity of microbes to propagate at least two generations of viable cells while airborne in aerosols of 1 to 5 μm [55].

There is a continuous distribution in the sizes of airborne particles carrying bacteria. One investigation concluded that most airborne particles range between 8 and 16 μm [223]. The smaller particles that penetrate to the alveoli of the lungs are relatively rare among the particles discharged from the upper respiratory tract during talking, coughing, and sneezing. Most diseases can be transferred by the deposition in the upper respiratory tract of the very small proportion of 2 to 3 μm particles liberated during coughing and other expiratory activities [233]. The mean airborne bacterial particle size on Tektite I (1969) was 4.6 μm over a range of 1.5 to 7.0 μm. Particles less than 5.0 μm are capable of penetrating the alveolar spaces of the lungs. Particles between 0.8 and 1.9 μm are retained in the alveoli in greater numbers than are particles larger or smaller [21]. One of the mechanisms by which inhaled airborne particles are deposited on the walls of air passages is by sedimentation by gravity [151]. In the absence of gravity, it is anticipated that particles normally depossited (most being 1 to 8 μm diameter) will be exhaled [151].

Water

Water systems on the Space Station will be an ideal habitat for microorganisms, providing nutrients and a large surface to volume ratio, both ideal for growth. Waste water on Earth is purified in large part by the natural processes of predation of microbes by other organisms, the biocidal effect of sunlight, aeration, adsorption of microbes to soils, and dilution by great volumes of clean water [83]. These mechanisms, as they occur in nature, will not be available on the Space Station. Although bioregenerative systems utilizing microbial and higher organisms to biologically break down pathogenic microbes may be part of future space station designs, current

designs do not include them. Filters and the possible use of UV light will simulate the natural effects of soils and sunlight. However, the dilution factor, of great importance in natural systems, cannot be simulated on the Space Station.

The nature of water recycling is such that microbes originating on the human body or in the air may find many suitable niches [136]. The source of terrestrial drinking water is primarily rain and runoff from soil [79]. Some investigators suggest that a larger number of microbes from these sources can be tolerated than from human origin [79], but this is questionable (R. Zhorchak, personal communication). Recycled water is more likely to harbor organisms of human origin, few of which can be tolerated [79].

In a Russian experiment with a semi-closed system, the main bacteria present were indigenous to humans. Pseudomonas was present, and died within 5 to 6 hr on the surfaces of the sealed cabin, but was viable and reproductive in the water system [83]. In the MESA experiment, a community of microorganisms became established on the ion-exchange resins and charcoal filters of the water recycling system. The populations on the water system cartridges were not destroyed by UV light and by the end of the test, the potable water tank had visible slime growth of bacteria and actinomycetes on the bottom and sides of the tank [153]. In the Ben Franklin submersible, the changes in the nature of bacterial contamination in the water system increasingly reflected human-associated organisms. After thirty days, the character of the contaminants had reverted mainly to *Pseudomonas* spp. which had been the persistent pre-mission problem. There was noted a need for eliminating outside-to-inside contamination [155].

Surfaces

All surfaces on the Space Station, whether enclosed within a subsystem or exposed to the cabin atmosphere, will be potential sites for microbial growth because both microorganisms and their nutrients adsorb to surfaces [26]. Any direct contact by crew members will readily contaminate surfaces [34]. Wherever there are sufficient adsorbed nutrients and water, there is the potential for bacterial, fungal, and possibly algal growth in a spacecraft [227]. *Legionella pneumophila* is an example of an organism whose growth is amplified by the concentration of water and nutrients on the surfaces of heat exchangers, cooling towers, etc.

The type of surface can influence the survivability of microbes. A study of a variety of surfaces using aerobic, mesophilic, and heterotrophic microorganisms, resulted in a greater number of viable microbes on non-metallic surfaces. The highest die off rates were on stainless steel; the lowest die off rates were on copper and non-metallic materials like epoxy, laminate, Lucite, and Teflon [220]. When the microorganisms were examined after 12 to 20 weeks, those surviving were mainly spore-forming species [220].

Some authors claim that the natural decay rate of bacteria on surfaces will greatly reduce the population size of microbial species in the environment [134]. The experience on the Ben Franklin submersible, however, was that of cleaning of surfaces resulted in only a transient decrease of contamination levels followed by a rapid resurgence, especially on the floors [155]. Thus, a reduction in bacterial populations as a result of natural decay rate will not occur where there are sufficient nutrients and water.

Clothing

Many microorganisms can find favorable conditions for growth in contaminated clothing, especially when it is wet. *Staphylococcus* spp., including some pathogenic forms is particularly inclined to grow in clothing [122]. Potentially pathogenic forms appear after only two or three weeks of isolation in clothing [21]. In a study to isolate bacteria from a laundry wash and rinse cycle, 30 species in 13 genera were found, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Paracolbactrum* sp., *Escherichia* sp., *Aerobacter* sp., *Proteus* sp., and *Alcaligenes* sp. [143]. These were found both in the rinse water and on the clothing after washing [143]. Some contamination in a

closed area like the Space Station may arise from dust from clothing and footwear [220]. One author stated that cotton/synthetic underwear leads to less contamination than cotton/viscose underwear [17].

Despite the fact that clothing may harbor microbes, it may not be a major source of contamination in an enclosed space, according to one experiment. In small disinfected enclosed rooms, in which there was almost no influx of dust with the air system, contamination of skin and clothing was only minimally affected by dust from clothing and footwear [39]. More likely sources of contamination were food residues, untrapped urine and feces, and bacterial aerosols [39]. When 20 men were isolated six weeks during the Aerospace Medical Research Laboratories Life Support System Evaluation while wearing space suits, the assumption was not substantiated that the more constant temperature, high relative humidity and reduced air flow maintained on skin surfaces would be favorable to certain microbial strains. Higher bacterial counts were observed only on the axilla and groin [183].

Food

Undoubtedly, there have been many studies addressing the potential for foods, both stored and spilled, to harbor microorganisms. This report does not survey those studies except to cite one reference stating that unsterilized, sublimated foods with undamaged standard packing are frequently contaminated with coccal flora, mold spores, bacilli, and yeasts [85] and another to a reference stating that *Staphylococcus aureus*, staphylococcal enterotoxins, *Clostridium botulinum* toxins, atypical strains of *Mycobacterium tuberculosis*, aflatoxins of *Aspergillus flavus*, and viruses, can all occur in dairy products [24].

Instead, the relationship between human nutrition and indigenous microbial flora, of great interest in planning for food on the Space Station, will be touched on here. This relationship is considered an important factor in the ability of the host to remain in nutrient balance. Radical shifts in the proportions of carbohydrate, protein, and fat have been shown to alter the relative prevalence of the human floral populations [182]. Diets rich in carbohydrates favor increase in the numbers of gram positive bacteria, especially lactobacilli and bifidobacteria [66]. For example, the number of lactobacilli increase as a result of large quantities of lactose in the diet. Diets high in meat protein, gluten, or casein can cause a decrease in bacteroides or a simultaneous increase in gram negative forms such as coliforms and enterococci [182].

Sterile food can reduce the numbers of intestinal flora significantly. The intestinal flora of guinea pigs was reduced to two predominant species, *E. coli* and *B. subtilis*, as a result of sterile food [177]. When placed in an environment with sterile air and water as well as sterile food, guinea pigs harbored only two fungal (one external) and one bacterial species after two months [161].

In a confinement experiment utilizing freeze-dried foods with four men for six weeks, the obligately anaerobic character of the fecal flora remained constant [76, 182]. There was, however, a shift in the types of anaerobes present and the frequent presence of *Shigella* sp. and enteropathogenic types of *E. coli* was noted (see also Reference 78). Most of the new microbial types were gas-forming, black slime producing forms [183]. The aerobic flora isolated were significantly different from that reported in the literature [103, 182]. It was considered improbable that these changes would not affect the host in beneficial, harmful, or unknown ways [76]. These data can only suggest trends, as only two men were given the experimental diet which were not enough to assure statistical validity.

In a 56 day test with 6 males confined in an atmosphere of 70 percent oxygen, 30 percent helium, and a total atmospheric pressure of 258 mm Hg, a diet of compressed, nutritionally adequate, freeze-dried foods was indicated as the cause of decrease in the numbers of enterococci [43]. Upon return to a normal diet, the numbers of

enterococci regained their original levels. There was no evidence at any time of the emergence or presence of enteric pathogens such as *Salmonella* sp. or *Shigella* sp. [43]. The influence of diet on intestinal flora has also been shown to be changed by antibiotics. This could be an important consideration for people in space placed on antibiotic therapy [182].

Corrosion

By means of one of their fundamental metabolic processes, the production of organic acids, microorganisms can be effective corroders and degraders of a wide variety of materials.. For example, fungi produce oxalic, citric, succinic, fumaric, shatokonic, gluconic and 14 amino acids which diffuse from their cells [204]. Minute quantities of these metabolic materials become more continues or as evaporation takes place [204]. When the corrosiveness of microbial enzymes was compared with that of the organic acids, the acids proved to be more corrosive [204]. In a Russian experimental system designed to support dense growths of algae and bacteria for use on a space station, the structural materials were damaged by the fungi *Aspergillus niger* and *Spicaria* sp. [85, 166].

Microbial corrosion of metals has been extensively studied. A roster of microorganisms associated with biogenic corrosion studies, including a list of 104 references, was published as early as 1964 [13]. In iron and steel corrosion, microbes intervene directly in the corrosion process by acting as cathodic depolarizing agents. In this anaerobic process, they utilize the hydrogen available at the cathodic areas to reduce sulfates and produce hydrogen sulfide [20, 102, 211]. In the aerobic corrosion of aluminum alloys in Bushnell-Haas medium [35], certain biologically essential ions in the medium are inhibitors while others are stimulants of corrosion [18]. Microbes remove phosphate and nitrate more rapidly than calcium or iron from the medium in which they grow, making the medium progressively more corrosive [18]. Thus, the composition of the medium overlying the material of concern determines to a large extent the effectiveness of the corrosion process. The presence of calcium sulfate or ferric hydroxide in a medium was shown to cause aluminum corrosion. Nitrate and phosphate inhibited this corrosion [18]. However, aluminum alloy 7075 corroded in a medium with low nitrate concentration as well as in a medium with high nitrate concentration [18], suggesting complex mechanisms.

An experiment was conducted to identify the water soluble and insoluble corrosion products resulting from the interaction of 21 organic acids with 14 metals [150]. The effects of these products on the growth of *Bacillus megaterium*, *Pseudomonas aeruginosa*, and *Cladosporium resinae* were noted. Bacteria were exposed 24 hr; fungi were exposed 72 hr. Eight of 15 aluminum products inhibited bacterial growth, but few were effective versus fungi. Of 31 lead corrosion products, 22 inhibited neither bacteria nor fungi. Copper produced effective bacterial but not fungal inhibitors [150].

There is a large literature dealing with degradation of fuels, including rocket propellants [89]. The hydrocarbons of some fuels can serve as the carbon source for microbes [65]. Also required are the presence of water, a biologically available nitrogen source, and trace elements [65]. One study reported the persistence of microbes for at least 6 months in essentially anhydrous diesel fuel, however [51]. The utilization of certain metals in aluminum alloys, especially magnesium, by fuel contaminating microorganisms, was found to be one of the possible factors most influential in the corrosion mechanism [102]. Also, the type of surfactant used, which may provide nutrients for growth, plays a role in microbial corrosion of fuels [47]. Among the microorganisms viable in hydrocarbon fuels over a long period are the bacteria *Enterobacter aerogenes*, *Bacillus cereus*, *B. subtilis*, *Pseudomonas aeruginosa*, and *P. fluorescens*, and the fungi *Alternaria tenuis*, *Aspergillus niger*, and *Cladosporium resinae* [124, 178].

If microorganisms were present in rocket propellants and were able to survive rocket motor firings, they would be able to directly contaminate sterile space vehicles or extraterrestrial environments [93]. Microorganisms have been recovered from inoculated and uninoculated samples of aluminized polybutadiene [93].

Fungal degradation of electronic parts has been documented. After 150 days, epoxy-phenolic capacitors, vinyl-covered wire, and vinyl-jacketed coaxial cable innoculated with *Streptomyces* sp. showed deterioration. *Penicillium* sp., *Trichoderma* sp, and *Alternaria tenuis* are other species implicated in electronics degradation [80, 81]. Synthetic chemicals such as pesticides and herbicides are known to be degraded by several microbial genera, especially species of *Arthrobacter, Bacillus, Nocardia,* and *Pseudomonas* [40]. However, in nature, complete biodegradation may be due to the activities of a mixed population rather than a single species [40]. This ability of microbes to degrade certain synthetic chemicals is noted here to suggest the potential for degradation of a large variety of chemicals.

Paint films can be degraded by microorganisms, especially *Pullularia pullulans, Cladosporium* sp., *Pseudomonas* sp., and *Flavobacterium* sp. [185]. Each of these species is capable of hydrolytic and oxidative deterioration of polymers containing the ester linkage, and derivatives of these polymers released by UV attack [185].

The ability of many microorganisms to produce polysaccharides protects them from adverse environmental conditions. The chemical content of the polysaccharides produced is related to the substrate upon which growth occurs. *Bacillus, Arthrobacter, Alcaligenes,* and *Azotobacter* are bacterial species capable of producing polysaccharides on certain substrates [113]. Fungi are also capable of producing polysaccharides. The high moisture-holding capacity of polysaccharides enables microbes to maintain at least a minimum of moisture in their immediate environment, even after exposure to low humidity [113].

Microbes can be released from solids if they have been inoculated there, whether inadvertently or not. When *B. subtilis* var. *niger* spores were inoculated experimentally in Eccobond and methyl methacrylate which were machined into projectiles and fired from guns into stainless steel plates, viable microorganisms were released [207]. Although only a very small percentage of those inoculated were released, the difference between recovery rate in the two materials was significant, suggesting that materials need to be evaluated for this potential [207]. Some authors concluded that it is essential that the microbial populations within solid materials be accurately known in order to assess their contributions to the total contamination load of a spacecraft, especially when there is concern with contamination of other planets [142].

Release of Toxins

Wherever there is significant accumulation of living microbial biomass on the Space Station, there will be potential for release of volatile gaseous and/or toxic metabolic by-products. Biological growths in the water system may add toxic chemicals or undesirable taste and odor [136]. It is probable that the study of the extremely varied biochemical activity of organisms living over a long period of time in a closed ecological system will disclose new and especially toxicological problems [192].

MICROBIAL ENERGETICS

Understanding the mechanisms that microorganisms use to obtain energy for growth and reproduction is important for understanding their sites and rates of growth. Energy is derived either from light or oxidation of certain organic or inorganic compounds. Bacteria can synthesize ATP by a variety of routes including fermentation, oxidative phosphorylation photophosphorylation, and possibly by the excretion of metabolic end products [96]. The efficiency of oxidative phosphorylation can vary not only between different bacteria that have adapted to particular ecological niches but also within a bacterial population grown under different conditions or modified genetically by mutation [96]. Thus, bacteria do not always grow with maximum thermodynamic efficiency. The amount of protoplasm which an organism can synthesize with a given substrate is roughly proportional to the amount of

energy available to it from its substrate [94]. Most organisms oxidizing inorganic substrates are obligate aerobes and must have oxygen as an electron acceptor. Organic substrates are relatively rich in energy and can support heavier growth than can inorganic substrates [26].

The growth rate of a population of microorganisms is subject to Liebig's law of the limiting factor which states that the rate of growth or activity of some processes in an organism is controlled by the environmental factor which is limiting. If the limiting factor is changed so that it is no longer limiting, then another factor becomes limiting, and so forth [164]. The growth of *E. coli* is limited by the exhaustion of a nutrient under anaerobic conditions [74].

Limits to population growth have been explained by physical crowding, exhaustion of an essential nutrient, and production of toxic substances [26]. In *Streptococcus faecalis*, the exhaustion of an amino acid needed for protein synthesis but not for cell wall synthesis, resulted in a population of cells which ceased dividing and making protein but continued making cell wall material [213]. An example of the production of toxic substances occurs in lactic acid bacteria which cease to grow when the production of lactic acid lowers the pH [26,74].

A simple mathematical model of the growth rate of a population of organisms is:

$$\frac{dN}{dt} = kN - \frac{k}{G} N^2$$

where

N = numbers of organisms

G = the maximum population attainable as controlled by environmental factors
k = growth rate constant

kN = intrinsic growth rate, or the most rapid rate at which the population can grow under the conditions used.

The second term accounts for self-crowding effects.

A more complex model was derived experimentally using a completely mixed body of water with nutrients and water continuously flowing in and out at a fixed rate and concentration [139]. The growth rate of a given species of bacteria growing on a single limiting nutrient was described by:

$$\mu = Y_m k_m \frac{S}{K_s + S} - b$$

where

μ = fractional increase in bacterial mass/unit time

Y_m = maximum yield of organisms/unit substrate consumed for energy

k_m = rate at which substrate is consumed for energy per unit mass of bacteria

S = concentration of growth rate-limiting substrate (usually the electron donor for the energy reaction)

K_s = coefficient representing the substrate concentration at which the growth rate is half the maximum

b = coefficient expressing all decay rate.

The actual mechanisms of organic material turnover are more complex than these simple formulae may indicate [139]. However, when the steady state anaerobic treatment of sewage sludge consisting of a multitude of different organisms operating in series and in parallel in a complex chain of reactions, was evaluated, the data indicated that the kinetics of the overall reaction were determined by studying individual substrates [164].

ENVIRONMENTAL LIMITS OF MICROORGANISMS

A large number of physical, chemical, and biological factors affect the ability of a microorganism to survive and perpetuate itself. Physical factors include temperature, hydrostatic pressure, osmotic pressure, surface tension, visible radiation, UV radiation, ionizing radiation, gravity, adsorption phenomena, and viscosity. Chemical factors include water structure and activity, pH, inorganic nutrients, quality and quantity of gases, organic nutrients, hormones, growth regulators, metabolic control substances, poisons, inhibitors, nutrient analogues, redox potential, etc. [26]. Biological factors include the duration and type of life cycle of the organism, the presence or absence of other organisms of the same species or of other species and their interactions, the genotype and phenotype of the organism, etc. Adding even greater complexity is the fact that the effect of any one of these factors alone may be changed with a change in any other factor.

Microbes have been found to survive at temperatures from -269 to greater than 100°C [176, 196]. Most are metabolically active between 5 and 70°C and achieve optimum growth at 20 to 40°C [195] or 35 to 40°C [25, 122]. Some grow optimally over a fairly wide range of temperatures. E. coli may grow optimally between 37 and 45°C [122]. On the Ben Franklin submersible, on board bacterial counts rose on the eleventh day of the mission when temperatures rose to 83°F (30°C) [155], suggesting that the higher temperature was closer to optimal growth temperature.

Certain terrestrial habitats in which microbes can usually be found represent the lower temperature extreme (frozen foods, snow fields, glaciers), and the upper (hot springs, volcanoes, laundry wastes) [26]. Microbes may survive low temperatures by greatly reducing metabolic activity, but temperatures above the normal growth range usually cause a rapid drop in growth rate [122] and are often lethal.

In experiments designed to study the survivability of microbial aerosols, temperature changes over a range of 24 to 30°C had no effect on survival of *E. Coli, Staphylococcus aureus,* or *Streptococcus salivarius* [234]. In other experiments, the death rate of *Salmonella pullorum* and pneumococci increased with rising temperature over a range of 28 to 37°C [52]. *Serratia marcescens* mortality increased as temperature rose from -40 to 32°C and humidity increased from 20 to 80 percent [100]. These limited studies suggested that in general the death rate of microbial aerosols increases with temperature.

Some microorganisms produce resistant resting stages such as bacterial spores [89]. Resting structures are able to withstand high temperatures, UV radiation, and toxic chemicals, resist drying, and survive nutrient or other deficiencies [26]. Spores of *Bacillus* sp. ATCC 27380, isolated from surface soil have been found to be extremely

resistant to dry heat, surviving 139 hr at 125°C and 13 to 17 hr at 138°C to 90 percent mortality [238]. Most spores survive only 5 to 100 min under these conditions. This marked resistance to heat occurs only in bacterial spores [26].

Low temperature by itself will not kill fungi; in fact, quick freeze-drying is one method of preserving fungi [176]. *Aspergillus niger* can withstand temperature of -6°C in a vacuum of 1×10^{-4} alternately with ambient conditions for 4 days without apparent damage to viability [29]. *Schizophyllum commune* has been exposed to -190°C without obvious damage [33]. Dry spores of *Neurospora crassa* germinated after exposure to temperatures of -170 to -190°C for 1 hr [69].

The resistance of organisms to elevated temperatures is sometimes expressed as the thermal death time (TDT), the time required at a particular temperature to kill a specified number of organisms [9]. Some TDT values of interest — *Escherichia coli*, 20 to 30 min at 57°C, *Staphylococcus aureus*, 19 min at 60°C, *Bacillus subtilis*, 20 to 50 min at 100°C, and *Clostridium botulinum* (spores), 100 to 300 min at 100°C [9].

The effects of temperature on microorganisms are often interactive with other factors. For example, moist heat is usually more effectively lethal than dry heat. Sterilization of materials is usually adequately achieved with steam heat for 15 to 20 min at 121°C, whereas dry heat for 3 to 4 hr at 160 to 180°C would be necessary [9]. A pH of 7 tends to render microorganisms more resistant to high temperature than either acidic or alkaline pH [9].

Humidity is important in the germination of fungi. In an experiment designed to demonstrate this, 21 species of fungi were tested at 95, 85, 80, 75, 70, 65, 55, and 50% relative humidity, and at temperatures of 100, 80, 70, 60, and 50°F. Numbers of germinated spores were counted periodically over a period of 32 days. No spores germinated below 70% relative humidity. At relative humidity between 70 and 80%, overall germination was low. Above 80% relative humidity, germination increased rapidly as the humidity increased [19]. Reduced temperature retarded germination without preventing it [19].

Salinity may increase tolerance to low temperatures. In an unfrozen saline pond in Antarctica, water temperature ranged from -24 to -5°C and salinity was 14 times that of sea water. Although the organic content was low, bacteria, including *Bacillus megaterium*, a *Micrococcus*, and a *Corynebacterium*, all grew well in the pond. These bacteria all would thrive as well in laboratory media of low salinity and high organic matter content at 20°C [144].

The optimal growth temperature of a sulfate reducing bacterium was shown to increase with increasing hydrostatic pressure. At 1000 atmospheres, the organism grew at 104°C [241]. Microorganisms may exhibit new nutritional requirements as the growth temperature is raised, so that the nature of the medium and other factors will determine the optimum or maximum temperature for growth [26].

Environments with osmotic pressures ranging from 0 to 20,000 psi are known to support microbial growth. However, 15 to 200 psi is the range of optima for most species [118]. Extremes of salinity in terrestrial habitats are found in oligotrophic lakes and saline lakes [26]. Some organisms (e.g., *E. coli*) can show physiological, but not genetic adaptation to increased salt concentration [109]. Some common fungi of the *Penicillium-Aspergillus* group can be grown in a variety of brines or on moist salt crystals even in the absence of reduced organic nutrients other than glucose [195]. Microorganisms unable to regulate osmotic pressure are restricted to specific habitats such as the inside of animal cells. Few microorganisms can tolerate high osmotic pressures of saline brines, or high sugar concentrations, but many can tolerate distilled water [9].

Specific microorganisms cannot generally tolerate wide ranges in pH, although within the diverse taxonomic group, there are representatives that can tolerate pH as low as 0.5, and others that can tolerate pH of 10.5 [196]. Terrestrial representatives of microbes that tolerate low pH may be found in acid mine waters and acidic

springs. Those that tolerate high pH may be found in alkaline lakes and certain industrial waters [26]. The optimal ranges for most species is 6 to 8. The pH ranges of some microbes that might be on the Space Station are listed below [9]:

	min	opt	max
E. coli	4.4	6.0–7.0	9.0
Proteus vulgaris	4.4	6.0–7.0	8.4
Aerobacter aerogenes	4.4	6.0–7.0	8.4
Pseudomonas aeruginosa	5.6	6.6–7.6	9.0
Erwinia cartovora	5.6	7.1	9.3
Clostridium sporogenes	5.0–5.8	6.0–7.6	8.5–9.0
Nitrosomonas spp.	7.0–7.6	8.0–8.8	9.4
Nitrobacter spp.	6.6	7.6–8.6	10.0
Thiobacillus thiooxidans	1.0	2.0–2.8	4.0–6.0
Lactobacillus acidophilus	4.0–4.6	5.8–6.6	6.8

Optimal values may depend upon other factors such as salinity and temperature [9].

The resting structures of many microorganisms are resistant to drying [26]. However, metabolically active cells vary in their resistance. The flexible spirochaetes, such as the organism that causes syphilis, are very sensitive to drying. This explains why syphilis is a veneral disease. Free-living spirochaetes are found only in aquatic environments [26]. The gram positive microbes are usually those with the thickest cell walls and usually resist drying more than the gram negative organisms. Asporogenous organisms isolated from the air are usually gram positive yeasts or cocci [26]. The resistance of the gram positive staphylococci to drying is notorious [26].

Relative humidity influences the survival of viral and bacterial aerosols, although most studies suggest the absence of narrow relative humidity tolerance zones [6]. However, when the survival of E. coli was measured in small increments of relative humidity, there were detected narrow zones of instability in atmospheres of air and nitrogen [5, 6]. This phenomenon was not detected by some other investigators [e.g., 229]. Microbial decay rates tend to be highest at the extremes of the humidity range (e.g., 20 percent, 100 percent) [6]. The polysaccharide capsule of *Cryptococcus* sp., an asporogenic yeast, delays the drying process by collecting moisture even at low relative humidity [3].

In general, microorganisms are not affected by changes in atmospheric pressure. At extremely low atmospheric pressures (e.g., in the upper atmosphere or in an artificial vacuum), water may evaporate, limiting oxygen and curtailing metabolic activity [9]. The limits of hydrostatic pressure for survival of microorganisms are wide, 0 to greater than 10^5 psi; 15 psi is optimal for most species [196]. Marine bacteria inhabiting the deep sea survive pressures equal to 1000 atmospheres [12, 26].

Stationary phase cells of *E. coli* B/r were inactivated when experimentally exposed to high vacuum (10^{-6} torr). About 5 percent of the cells were able to form a colony after 45 min exposure. Vacuum dried cells (0.5 torr, 120 min) had 30 percent colony forming ability. Cells in a vacuum were shown to have increased sensitivity to UV radiation [32].

Pressure can interact with temperature in its effects on microorganisms. Approximately 500 atmospheres pressure was shown to retard the killing of bacteria at temperatures above their optimum for growth [110].

The presence or absence of oxygen does not in itself determine whether microorganisms can grow in a particular habitat. Aerobic microbes utilize oxygen in their metabolic processes; anaerobic microbes do not. Some microorganisms can be active only in oxidizing environments; others can exist only in reducing environments [9].

Some are facultatively anaerobic, being capable of using biochemical systems which are metabolically active in the absence of oxygen, and also survive in an oxygenated environment. *E. coli* is facultatively anaerobic, having the ability to generate energy without using oxygen, by means of electron transport using fumarate or nitrate rather than oxygen as a terminal electron acceptor [96] or by substrate level phosphorylation (Zahorchak, personal communication). The sulfur bacteria lived without oxygen for 800 million years [12].

The germination of spores of *Bacillus subtilis* (va. *niger*) collected from aerosols was found to be dependent on the oxygen content of the atmosphere by one investigator [6]. When air was replaced by nitrogen, the survival of *Micrococcus* spp., *Candida* spp., *E. coli*, and *Serratia marcescens* was enhanced in stored aerosols [73]. This effect was attributed to the toxicity of oxygen on these facultatively anaerobic organisms.

Carbon dioxide is necessary for growth of some microbes. Bacillus circulans and *B. stearothermophilus*, both thermophilic bacteria, require CO^2 for the initiation of growth of germinated spores, but not for germination itself [42].

Atmospheric composition may interact with humidity to effect microbial survival. A test to demonstrate this interaction resulted in dependence of the survival of *S. marcescens* and *E. coli* on the partial pressure of O^2 in oxygen-nitrogen systems for low, but not high relative humidities [105]. The survival of three strains of *E. coli* was enhanced at low, but not high, relative humidities when nitrogen replaced air [44].

Some strains of *E. coli* showed almost no change in rate of survival or in colonial morphology when exposed to liquid nitrous oxide, carbon tetrafluoride, or perfluoropropane [75]. Sulfur dioxide was completely inhibitory. Trimethylamine, monomethylamine, and methyl mercaptan were bactericidal. Carbonyl sulfide inhibited one strain and was lethal for 20 others [75].

Terrestrial microorganisms live at widely varying intensities and wavelengths of visible light. Those living in caves and on the ocean floor receive almost no light, whereas those living at high altitudes receive a great deal of light [26]. Ultraviolet light, well known for its ability to kill microorganisms, is critical between 2,000 and 3,000 Å for most species. It is highly bactericidal between 2,500 and 2,800 Å [195]. Visible light between 4,000 and 8,000 Å is essential for photosynthetic species. Infrared radiation, greater than 8,000 Å, is utilized by some species such as purple sulfur bacteria [196]. Artificial sunlight is lethal to some organisms. The decay rate of *Pasteurella tularensis* in artificial sunlight was found to be directly proportional to the applied light intensity in an experimental system [10], and decreased linearly with increasing humidity [10]. *P. pestis* behaved similarly, except that increase in decay rate was not directly proportional to light intensity [10]. *Serratia marcescens* in microbial aerosols exposed to artificial sunlight was found to be less stable at low than at high (>65%) relative humidity [229].

Surface tension of about 25 to 100 dynes/cm is necessary for microbial survival; about 75 dynes/cm is optimal for most species [195]. Less than 50 dynes/cm is lethal for many microorganisms [195].

Microbial requirements for nutrients and growth factors are diverse and complex. *E. coli* grows in nature in the nutritionally complex environment of the intestinal tract, whereas in the laboratory it will grow on a simple medium containing only glucose and salts [26]. Thus, the nutritional requirements of an organism may vary with the physical environment. The concentration of nutrients is important. Many substances which can be utilized for growth in small concentrations (e.g., phenol and pyridine), may be toxic in high concentrations [26]. In some cases, even though an organism may be able to synthesize certain growth factors internally, it will cease synthesis when the growth factors are available in the medium. For example, *E. coli* will stop synthesizing arginine if it is available in the medium [26]. Of 300 strains of marine yeasts tested, the majority required an exogenous source of at least one growth factor [1]. *Candida* spp. needed biotin, and *Rhodotorula* and *Cryptococcus* needed thiamin [1]. With continued maintenance of the cultures, deficiencies, and inhibition were found to diminish and be overcome [1].

The electrolytic products of gaseous halogens, existing as impurities in water can be toxic in concentrations of 71 percent [118]. Halogens and certain ions are highly lethal to most microbial species [118]. Mercury is highly toxic to most species; silver, copper, and cadmium are toxic to most vegetative cells [118]. Copper ions were shown to inhibit the anaerobic growth of *E. coli* from small inocula but not from large inocula. Removal of the copper from the small inocula allowed continued growth. It was hypothesized that with many cells present, each one may adsorb a small amount of copper, but not enough to cause inhibition [106]. Organic toxins include formaldehyde which is lethal for most species. Phenolic compounds and detergents are toxic under various conditions. Most organic toxins (e.g., antibiotics), are highly specific for individual species [195].

MICROBIAL STANDARDS FOR SPACE STATION

Standards for microbial control on the Space Station will be touched on only briefly here, since there were few theoretical or empirical discussions of such standards found in the literature. In 1967, the National Academy of Sciences Space Board suggested that there is no justification for the establishment of standards (microbial density) based on individual species [136]. Essential sterility of water supplies (with a maximum of 10 microbes/ml) was the suggested goal, as determined by total counts of aerobic, facultative, and anaerobic organisms [136]. Another early (1966) reference concluded that bacterial numbers must be at least 10^6/ml or 10^6/gm before it can be concluded that an organism is making any significant contribution to the ecosystem [26]. Current standards for the Space Station specify zero microorganisms per 100 ml for the potable water system [145].

Current standards for Space Station respirable atmosphere are (in colony forming units/m^3) 500 operational, 750 90d degraded, and 100 emergency. These values reflect a limited base and must be further evaluated [34].

Some early workers suggested that astronauts be germ free to exclude the possibility of cross contamination between food, feces, etc. [95]. Were it possible, this would obviously alleviate many problems of personal hygiene, food storage, and waste disposal. But a germ free human would not be able to metabolize bile acids, cholesterol, and many other constituents [85]. The necessary minimal microbial flora might be the best to establish although the decreased diversity might lead to greater fluctuations in density [95].

MONITORING METHODS

Methods for sampling and monitoring microbial populations on the Space Station have not been well developed in terms of achieving rapid, accurate analyses that will predict unusual growth of undesirable microbes. It will be very important to develop methods for monitoring microbial activities, not only to identify the presence of pathogens, but to ascertain the health of the whole ecosystem of the Space Station. Changes in microbial communities will be one of the most sensitive signs of change in the condition of the biocoenosis as a whole [85].

Many authors point to the need for improvement of methods for sampling and identification of microorganisms on the Space Station [99, 156]. Methods for recovery of microbes from surfaces of space hardware were outlined by Vesley in 1966 [221]. The use of agar as a medium for microbial growth is a basic standard technique in microbiological laboratories. However, cultural methods using semi-solid media (e.g., agar) are selective for organisms which are unicellular, produce many propagules, or which spread rapidly on the surface of the medium, and are selective against organisms which can not grow well against the resistance of the solid surface [26]. Also, an organism which was dormant in the ecosystem may produce a colony on the medium, leading to misinformation about the metabolically active microbial load in the environment [26].

Counts of total coliform bacteria are often used as a measure of efficiency of water treatment, signaling the possible presence of fecal contamination. Coliforms are usually not pathogenic themselves, but their presence indicates the likely presence of pathogens such as Salmonella and Shigella [71]. There are several drawbacks to using coliforms alone as indicators of microbial contamination: (1) coliforms are often found in the absence of fecal contamination, (2) pathogens and outbreaks of disease may occur in places where coliforms were not detected, (3) coliforms are inadequate for predicting the presence of pathogens not associated with fecal contamination (e.g., *Pseudomonas* spp., *Legionella* spp. and algal toxins), and (4) coliforms may not adequately predict the presence of enteric viruses, *Giardia* spp., and some other organisms, because they are isolated on different media (A. K. Highsmith, personal communication) and may be less resistant to disinfection than these organisms [71]. Furthermore, there is an absence of empirical data supporting the use of a particular coliform density as a criterion for water purity [71]. Pathogens and outbreaks of disease have been associated with coliform densities ranging from 0 to very high levels [71].

Few literature sources related to space travel were found that dealt with monitoring the intestinal flora. One author did propose that the metabolic products of the intestinal flora, rather than the bacteria themselves, be characterized [95].

A NASA publication emphasized the need for a computerized monitoring system, augmented by sensitive biological indicator species. It was suggested that the development of genetic strains that are sensitive to specific toxic conditions should provide efficient early warning of potentially hazardous environmental changes [203]. Experiments with Aspergillaceae showed the importance of using eukaryotes in demonstrating tolerance or adaptibility to harsh and extreme physical-chemical environments [193].

The suggestion of analyzing for ATP (adenosine triphosphate) to detect the presence of living organisms has been put forth by some [136, 237]. ATP, used to store and transmit chemical energy in all living organisms, rapidly degrades in dead organisms. Firefly enzymes can be used to produce light using ATP from living organisms as an energy source. It is a rapid method, working equally well with all types of living organisms and giving quantitative results in about 20 min [237]. The time required may be considerably underestimated according to A. K. Highsmith (personal communication). Since the amount of ATP in microorganisms is proportional to the mass of the cells, its measurement defines total living biomass in a particular sample. A photometer is used to detect and record the amount of light produced, eliminating the necessity for plating, culturing, and the resulting time lag associated with using growth media [169].

CONTROL METHODS

References to control of microbial contamination range from general recommendations for good housekeeping to measures specific for site of contamination and/or organism. Sterilization, the destruction of all life, will be impossible on the Space Station. Even if sterility of flight hardware were possible, its proof would be nearly impossible [111, 141]. But microbial population levels can be stabilized by sanitary and hygienic procedures [85], and the quantification of these levels is amenable to statistical analysis [111]. Contamination control is achieved if the microbial load does not exceed the level established as the lowest acceptable limit. However, the maintenance of this level is complicated by the fact that the microorganisms in a population may be going through simultaneous processes of multiplication and death [188].

The surface on which microorganisms are situated was shown to be important to their control in an 18 day experiment using 5 strains of *Bacillus subtilis* spores growing on sterilized smooth metal, porous plastic, multilayer composition material, and limonite ground in powder. Four types of sterilization procedures were employed: (1) 160°C dry heat for 60 min and flowing steam for 180 min, (2) 6 percent hydrogen peroxide for 180 to 250 min,

(3) radiosterilization (D_{10} index = 270 krad), and (4) gas mixture "OB" at 100 mg/l and 35°C for 120 min [219]. The greatest survival occurred on the ground limonite and the highest antimicrobial activity was caused by UV radiation. Thus, spores exposed to UV radiation on smooth surfaces suffered the greatest mortality.

Microorganisms on the Space Station will exist in three main ways in and on spacecraft hardware; they will be encapsulated within materials, on surfaces (including human surfaces) and in mated surface areas [168]. Possible locations are the interiors of many electronic piece parts, on coated surfaces, or embedded in potting compounds and solid propellant [168]. A D-value of 125°C for 5 hr is suggested for encapsulated microorganisms by one author [168]. The D-value is the reciprocal of the slope of the death rate curve and is equal to the time for a 90 percent kill or a 1-logarithm reduction in count [28]. In this process, water movement from the spore will be a function of the vapor pressure difference across the spore wall. If microbial spores are encapsulated in a solid without transmission or absorption of water vapor, there will be no gain or loss of water during heating. When spores on surfaces are dry-heat sterilized, they will lose water until they are in equilibrium with the surrounding atmosphere. Thus, the D-value of encapsulated microbes is high [29] and humidity has a greater effect than temperature [168]. Temperature and moisture are the major factors that determine the D-value. Reduction of D-values of microorganisms on a spacecraft can be achieved by using low moisture assembly conditions, a post-assembly drying cycle, and very dry gas during the final sterilization cycle [168].

There must be thorough pre-loading screening for potential pathogens and pre-flight sterilization of any experimental animal bedding, cages, food, water and equipment [34]. However, some diseases are so common and the carriers so difficult to identify, that their exclusion may not be possible [203]. Some think that experimental animals should be gnotobiotic [34]. Any soils used in experiments must be assayed for potential pathogens and laboratory facilities should be designed to isolate the crew from experimental systems [34]. The growth of microorganisms on and in plants is discussed in Reference 26.

The physical design of the Space Station will be extremely important in terms of microbial control. The design must eliminate areas that catch dust or are hard to clean, and must provide for easy access to filters [79]. Pipes and ducts must be designed with as few bends, loops, or dips as possible [79]. Any places in which condensate forms must be accessible for cleaning. Space Station habitat design may involve problems like those of hospital design with the major difference that on the Space Station there will not be the ability to bring in outside air as a "sink" or diluent for contaminants [203].

The conclusion reached by the authors of the 1985 report for NASA by Batelle [34] was that control of airborne microorganisms on the Space Station will best be achieved by filtration of bacteria, viruses, and fungi, by chlorination in moist areas (e.g., for *Pseudomonas* spp. and *Legionella* sp.), and by UV radiation. In the MESA test, two aerosols were separately injected into the system, *Serratia marcescens* and *Bacillus globigii*, in an attempt to obtain information on the effect of subsystem components on bacterial longevity [153]. Superoxide and Hopcalite appeared to remove *Serratia* spp. from the air. It was suggested that the obsrved low recovery of *Serratia* spp. from surfaces indicated that either the organisms died rapidly or were adsorbed on surfaces of the air conditioning system [153]. Superoxide was not as efficient as Hopcalite in the removal of spores of *Bacillus* spp. and the recovery of *Bacillus* spp. from surfaces was extremely high [153].

Maintenance of the water supplies on the Space Station will be especially difficult because of the affinity of most microbes to moisture, and the ease with which they can reproduce in water. Common methods of microbial control in water systems are the use of chlorine, bactericides, fungicides, algicides, and pesticides, etc. [203]. Experience thus far (1985) indicates that microbial filters, UV radiation, chlorination, and silver ions are deficient in terms of microbial control for the Space Station [34]. The authors who made this statement maintained that the only effective method of microbial control in space water reclamation systems involves heating at pasteurization

temperatures in conjunction with an iodine system [34]. On the Ben Franklin submersible, however, during the 30 day Gulf Stream Mission in 1969, 75 ppm tincture of iodine alone was successfully used for sterilization of unpasteurized potable water [155]. In waste tanks, the germicides Wellodyne (1.75% I_2 and 15.95% H_3PO_4) and Microgard (Alkyl benzylammonium Cl -D) were routinely used [155]. The effectiveness of pasteurization of shower water has been shown to be reduced by the presence of soap [[153]. The hazards associated with any use of toxic substances must be completely understood before their use can be seriously considered in the closed system of the Space Station [203].

UV light was shown to be effective in the water system in the MESA test, when distilled water was inoculated with suspensions of *E. coli* and run past a UV lamp at rates from 3 to 6 times the rate at which water would flow in the system [153]. In general, a low flow rate of recycled water will allow a high UV light dosage rate [153]. However, organisms may become resistant to UV light over time (A. K. Highsmith, personal communication).

Pseudomonads tend to be resistant to many types of disinfectants and sanitizers [104, 155]. *Pseudomonas* sp. has been shown to be the dominant microbial constituent of swimming pools treated with iodine, because of its resistance to that disinfectant [70]. *Pseudomonas aeruginosa* has an exceptional ability to survive and multiply in the hospital environment, often being cultured from hand creams, mop buckets, sinks, sterile solutions, water baths, humidifiers, and similar ecological niches [68].

In general, it is common for anti-microbial chemicals to act more strongly and rapidly on gram positive organisms [155]. The selective inhibition of one segment of the microbial community can confer a survival advantage to those less susceptible (i.e., gram negative organisms), leading to their enhanced multiplication and overgrowth [155].

Theoretically, UV light can only approach 100 percent control of most airborne bacteria, viruses, and yeasts. About 90 percent control may be achieved at dosages between 1,000 and 20,000 microwatt sec/cm^2 exposure [153]. In a recycling air system like that of the Boeing Manned Environmental System Assessment (MESA), a 4,000 microwatt sec/cm^2 exposure was considered adequate. This exposure was expected to result in equilibrium in the airborne population approximately 20 percent above the average hourly rate of production [153]. Microbial aerosols generated from the dry state were shown to be less sensitive to radiation than those generated from liquid suspensions [11, 91]. Only dry disseminated aerosols showed a spontaneous reactivation when held in the dark after UV irradiation [54].

Organisms which may be found in the upper boundary of the biosphere (at 49 to 77 km from Earth) tend to be very resistant to high vacuum (10^{-9} mm Hg) and UV radiation. Microorganisms which have been isolated from these altitudes are *Aspergillus niger*, *Penicillium notatum*, *Circinella muscae*, *Papulaspora anomala*, *Mycobacterium luteum*, and *Micrococcus albus* [107]. Only the pigmentless *M. albus* was not resistant to these conditions, thus suggesting the resistance to UV light imparted to microbes by pigments. In an experiment with simulated Martian dust clouds using the bacteria *B. cereus*, *B. subtilis*, *E. coli*, *S. marcescens*, and *S. aureus*, daily UV irradiation of $2^{-9} \times 10^7$ erg/cm^2 was not sufficient to sterilize the dust clouds. Soil particles protected the organisms from the UV light [98].

A survey of most dry heat resistant microbes in nature indicated that a cycle of 125°C for 24 hr is inadequate for sterilization of some specimens [28]. It was assumed that the microbial death rate in dry heat was a logarithmic function from which a D-value could be calculated. Some factors in the soil from which the microbes were collected were responsible for the observed increased resistance to heat [28]. Several strains of *B. subtilis* and one strain of *B. coagulans* were shown to be highly resistant to destruction by dry heat at temperatures of 120°C and 125° [29]. Earlier studies had indicated that a vacuum would decrease the sterilization time [29].

Thermal sterilization requires temperatures that may degrade certain heat-sensitive materials. The simultaneous application of lower temperature and low levels of gamma radiation yields synergistic effects which can sterilize with fewer damaging side effects. In an experiment with *B. subtilis*, the time to death with 105°C alone was 4.5 hr, whereas at the same temperature, with 7.5 krad/hr gamma radiation, the time to death was 1.5 hr [196].

Attempts to disinfect the skin can be complex. Pneumococci, streptococci, and vegetative cells of gram positive rods are highly susceptible to soap; however, staphylococci, the colon-typhoid group, spores of gram positive rods, *Mycobacterium tuberculosis*, and related acid-fast organisms are known for their resistance to soap [140]. Anti-bacterial additives to soap can supplement, but not replace, the mechanical removal of bacteria and dirt by good cleaning practices [46]. Hexachlorophene may be inadequate in washing hands for handling food because of its erratic control of gram negative microbes [173]. Only iodine appears to have a broad enough spectrum of activity to control the great variety of contaminents present on skin, according to some investigators [45].

Antibiotics, organic compounds synthesized by living organisms which inhibit or kill other organisms, may be employed to a certain extent both prior to and following launch of the Space Station. There are no non-toxic antibiotics which are completely effective against all fungi and protozoa [26]. Also, because they may remain latent for a long time and are vulnerable to antibiotics only when metabolically active, other microbial forms (e.g., viruses and other intracellular organisms), will probably not all be eliminated by antibiotics [26]. *Bacterium antitratum* is generally known to be resistant to antibiotics [155].

Examples of antifungal antibiotics are cyclohexamide and nystatin [23]. Cyclohexamide inhibits protozoans and green algae at 1 μgm/ml. Simultaneously, bacterial and blue green algal populations increase markedly. Nystatin was shown to suppress germination of seeds of zinnia, popcorn, and cantaloupe, etc. at 20 μg/ml [23]. Added to aquaria, nystatin was found to harm several species of green algae and to be deleterious for fish and snails. Ten μg/ml killed all snails and increased guppy mortality [23]. The finding of greatest significance to the Space Station was that both the continuous presence of apparently subinhibitory concentrations of an antibiotic drug and its transitory presence in biocidal concentrations can lead to persistent changes in the composition of the flora and fauna of a community [23]. This phenomenon would be as applicable to the floral community of the human body as it would to a terrestrial pond community of algae, snails, and fish.

The metabolic activities of microorganisms are sometimes capable of gradually eroding the effectiveness of a biocide. For example, when a 0.2 percent solution of methyl p-hydroxybenzoate was applied to hydrolized *Cladosporium resinae*, growth of the mold was suppressed until the level of the fungicide was reduced to less than 0.15 percent by esterase activity of the mold [199]. Following this suppression, the mold again grew rapidly at 25°C [199]. Examples of non-antibiotic fungicides tested for terrestrial use are sodium pentachlorophenate, copper-8-quinolinolate, 2,2'-methylene-bis(4-chloropenol), N-trichloromethylthi-4-cyclohexene-1,2-dicarboximide, phenylmercury-8-quinolinolate, copper pentachlorophenate, 2,2'-dithiopyridine-1,1'-dioxide, and 1-fluoro-2,4-dinitrobenzene [186].

In an experiment designed to show whether methyl bromide could increase the effectiveness of the sporicidal activity of ethylene oxide [172], the two compounds were tested separately and together against *Bacillus subtilis niger* spores on cloth patches either unsealed or sealed in polyethylene or PVC bags [172]. The indication was that methylene bromide has no synergistic effect on the rate of ethylene oxide sterilization, but that it promotes the rate of ethylene oxide penetration, at least through some plastics [172].

EFFECTS OF SPACE FACTORS ON MICROORGANISMS

Of concern on the Space Station will be not only the effects of microorganisms on the environment and its inhabitants, but the effects of the space environment on the microorganisms themselves. The main factors of concern in space are confinement, weightlessness, and radiation [99]. Over time, microbes exposed to factors to which their original populations were not accustomed, can lead to mortality or physiological or genetic adaptation, thus causing changes in the initial properties of the organisms [50, 99], which in turn could affect humans. Of interest are how great a change can be achieved in an organism's tolerance to extreme conditions, whether acquired capacities cause an equivalent loss of other characteristics, and the identity of the physiological mechanisms that enable adaptive changes [50]. These questions are of importance not only for maintaining the health of the environment, but also for deciding what organisms should be used for experimental purposes, and perhaps be used as monitors of environmental change.

There have been numerous experiments designed to study the effects of space flight factors on microorganisms. Many of these experiments were not designed to elucidate the effects of a particular factor of space flight, such as vibration, apart from other factors; instead they showed effects of combined factors. In observing six dogs which flew in 1960-1961 in either a geophysical rocket or in satellites, Soviets found that the bactericidal activity of dogs can change after being subjected to a single excessive treatment with vibration. A moderate wave-like change in the bactericidal activity occurred which, during the first weeks, was accompanied by a disturbance of the state of the autoflora, skin, and oral cavity [4]. The changes in bactericidal activity appeared to be an adaptation to the effects of the physical factors that accompany space flight. Moderate immunological changes persisted in all the dogs for months and sometimes years [4].

Mutation, or change in the genetic material, of microorganisms may be of particular concern for the Space Station. The mutability of microorganisms will be increased due to increased radiation of space [126, 223], significantly increasing the mutational frequency of microbes [59, 87, 126]. Although the mutation rate is roughly constant throughout the microbial world, in a given environment a mutant will more likely occur among bacteria than among other microorganisms simply because of the tremendous population size of bacteria [26]. Thus, attention should be paid to the possible appearance of mutant strains which possess altered properties to which humans are not accustomed [126]. It is even possible that new human pathogens may appear, producing infections not normally encountered [126].

Two bacterial species, *Pseudomonas* sp. and *Staphylococcus* sp., were used in experiments to determine whether mutations in common and ubiquitous microorganisms can affect the organism's virulence for humans. These two genera contain both pathogenic and normally non-pathogenic species [50]. *Pseudomonas fluorescens* normally cannot grow at temperatures above 37°C. By spontaneous mutation, heat tolerant forms were obtained whose optimum, minimum, and maximum growth temperatures were 49, 20, and 54°C, respectively [162], temperatures much higher than those of the wild type. The virulence of *P. fluorescens* increased with increase in the upper temperature limits [50] and the mutants were more resistant to streptomycin and more sensitve to erythromycin [163]. It was thus indicated that a non-pathogenic organism may increase its virulence through mutation, or a pathogenic strain may become less virulent [50].

Ionizing radiation is well known for its mutagenicity. Mutagenic doses of radiation vary from species to species. Microorganisms tend to be more tolerant of ionizing radiation than macroorganisms [9]. The life stage of an organism also determines mutagenic radiation dose. Bacterial endospores have a high resistance to gamma radiation. It takes 0.3 to 0.4 mrads to achieve a 90 percent kill and only 10 percent of this amount to reach the same mortality in vegetative bacteria [9]. Anaerobic spores lose half their DNA in sporulation creating redundant DNA and diluting mutagenic radiation effects [160]. Dry spores are said to have a high RBE, the ratio of dose from the standard radiation (X-ray, 250 kV peak), to the radiation dose for a given effect [160].

The relative dehydration, the dipicolonic acid content, or other metabolic factors may be as important as the possible redundancy of DNA in sporulated bacteria in determining RBE levels [161]. Also, low levels of radiation may be mutagenic while high levels may inactivate both nucleic acids and enzymes and kill the microorganisms [9].

An unexpected result of experiments with *Neurospora* spp. on Biosatellite II and Gemini XI was that rapidly metabolizing cells showed greater effects of radiation in the control samples on the ground than did those from the space flights [53]. In non-dividing, inactive spores there was no difference in genetic effects between control and flight samples [53]. For bacteria and actinomycetes, it has been found that maximum mutability of a population occurs only when the cells are synchronized [169].

Experiments with lysogenic bacteria have been numerous. Lysogeny is the hereditary ability to produce viral particles without infection from the outside. The lysogenic cell possesses the genome of the virus (the prophage) integrated with its own genome [240], but is immune to the virus it produces and transmits to all progeny [26]. The virus is usually active against closely related bacterial strains [26]. Bacterial viruses are obligate parasites which can increase in number only when their specific hosts are available and are possible agents in the lysis and elimination of pathogenic bacteria in intestinal infections [205].

During four short-duration Soviet space flights, including Vostok-1 and Vostok-2, the level of phage production by E. coli K-12 (lambda) in flight did not exceed that of controls, despite the fact that phage formation is induced by ionizing radiation in this lysogenic bacterial culture [240]. However, in one experiment, there was a trend toward increased phage production in the treated population [237] and with longer space flights, phage producing cells increased significantly [240], although there was a lack of clear dependence between level of induced phage production and length of flight [7]. Vibration appeared to increase the sensitivity of lysogenic bacteria to subsequent gamma irradiation [240]. There was thus the indication that spaceflight factors have cumulative nonlinear effect [240].

In other experiments with E. coli, data suggest that heavy ions up to 0^2 ions are no more effective than gamma rays for killing or induction of mutation in bacteria [8]. For yeasts, there was shown a higher effectiveness of heavy ions. The experiments were restricted to reverse mutations. Thus, the results do not exclude the possibility of greater effectiveness of heavy ions for induction of forward mutations due to chromosomal deletions [8]. *Bacillus subtilis* spores were used in Biostack I and II on Apollo 16 and 17 to study the effects of HZE particles. The hit area of an HZE particle is of the same order of magnitude as the spore length. There was observed reduced outgrowth of spores [31]. In the Apollo-Soyuz Test Project, *Bacillus subtilis* was again tested with HZE particles. Spores were killed by the particles at distances of up to 4 μm [67]. Dose values at these distances were of the order of 0.1 Gray (= 10 rad), whereas the D 37 value (the dose necessary to reduce survival to 37 percent) for electron radiation is about 800 Gray. It was concluded that the biological hazard of the cosmic HZE particles, especially its high Z component, has been much underestimated [67].

In experiments using protons with energies of 630 and 100 MeV, and accelerated C ions with energies of 36 MeV, the accelerated C ions had more pronounced effect on phage production of lysogenic *E. coli* [92]. There was no significant effect of proton radiation does rate (from 0.3 to 35.0 rad/sec) on the radiation sensitivity of the bacteria which produced induced bacteriophages [92].

On Biosatellite II, the lysogenic bacterium *Salmonella typhimurium* BS-5 (P-22)/P-22 was used to test the hypothesis that weightlessness both with and without gamma irradiation would not affect bacterial cell growth or induction of bacterial prophage P-22. The hypothesis was rejected when significant effects on growth rate and induction of prophage resulted [53, 138].

The effects of spaceflight factors on actinomycetes have been investigated in several space related experiments. Two strains of *Actinomyces erythreus* were sent up on the second Russian space ship. The first was strain

2577 with large nucleated cells, known to be resistant to UV light, and the second was strain 8594 with small nucleated cells, known to be sensitive to UV light [117]. The viability of strain 2577 as measured by the number of spores of 8594 increased 6X [117]. The survival of *Actinomyces aureofaciens* LS-B-2201, sent up on the fourth and fifth Russian satellites, was reduced to 24 percent of the control. It was noted that different species and strains have different degrees of sensitivity to spaceflight factors [87, 117].

On the Apollo-Soyuz test project, biorhythms of most *Streptomyces levoris* populations decreased although a few accelerated [2]. Ring deformation occurred in response to landing only in cultures on Soyuz [2]. The rhythmical disturbances were possibly caused by change in temperature, absence of geophysical periodicities, local radiation, etc. [2, 165]. There was no visible evidence of naturally occurring or radiation-induced mutagenic alteration during spaceflight [184]. *Chlamydomonas reinhardi* Dang on Soyuz 19 had decreased survival of cells [60]. Twenty-two tests were conducted with *Proteus vulgaris* upon return to the laboratory from flight on Soyuz 19. The experimental populations differed from the controls in average cell size biomass distribution, character of haemotaxis, rate of cell migration over the substratum surface, dehydrogenase activity, ribosomal aggregation, and ultrastructural peculiarities of the cells [60]. The experimental cells were smaller, with inhibited biomass accumulation [60].

The combined effects of weightlessness and light were studied on Salyut 5 (17 days) and Salyut 6 (20 days), using the basidiomycete, *Polyporus brumalis* [116]. Cultures put on board before primordia were formed, did not form them in the dark. Primordia formed on Earth continued their development in the absence of light. Stems and caps in space were oriented toward the light [116]. For these mushrooms, light played a greater role than gravity in their orientation during growth and development.

Experiments simulating certain spaceflight factors were conducted to elucidate the influence of these factors singly and in combination. *E. coli* B/r was subjected to a vacuum of 10^{-6} torr, temperatures of 2, 20, and 37°, and UV radiation [30]. Sensitivity of the bacteria to UV radiation increased in the vacuum, whereas temperature had little effect [30]. It was concluded that the chance of survival for living matter in space is less than could be estimated from adding the effects of single spaceflight factors [30]. Another experiment with microbial aerosols resulted in a sharp decrease in sensitivity of *E. coli* to irradiation by 250 kX.S-rays when the relative humidity fell below 70 percent. Maximum damage occurred at relative humidities between 70 and 80 percent [228].

When aerosols of *Serratia marcescens* were exposed to visible light (3,400 to 4,500 and 5,200 to 5,800 Å) and UV light (2,800 to 3,200 Å), the two visible light wave bands were found to photo-reactivate organisms which had been irradiated with the UV band [228]. Simultaneous irradiation with the visible and UV bands had an additive lethal effect [228]. In another test, UV light in the solar range was lethal to *Pasteurella tularensis* in an aerosol. Mortality was proportional to radiation intensity and moisture was protective [11]. Pigments also play a role in protecting microbes from UV radiation as evidenced by the dominance of pigmented microorganisms in the upper layers of the atmosphere [108].

The probability of survival of the initial bacterial burden of a spacecraft when exposed to the Jovian trapped electron radiation belt and the solar wind proton radiation, was investigated. The lowest electron energy (2 MeV) tested was the most effective in reducing initial bacterial populations [208]. Although temperature was insignificant with vegetative cells, spores survived better at -20°C than at 20°C [208]. Protons at energy similar to those in solar wind were effective in reducing initial populations of both spore forming and non-spore forming spacecraft isolates. The results indicated that the probability of bacterial burden of a spacecraft surviving the Jovian trapped electron belt will be a function of the location and time in the belt, and the temperature of spacecraft surfaces [208].

Several investigators have discussed the use of microorganisms as indicators of radiation in space. Because of the potential for hereditary changes in the sex and somatic cells of humans caused by radiation, monitoring of

radiation on the Space Station will be necessary. An early study using a streptomycin-dependent strain of the bacterium, *E. coli*, and an adenineless mutant of the mold, *Neurospora crassa*, in a balloon spaceflight, suggested that relatively high energies were required to cause genetic mutation at a given locus [48]. It was concluded that use of a microbial genetic system to detect the biological effects of space radiations would not be practical because of insufficient sensitivity of the genetic marker [48]. However, although others reiterated the fact of the high degree of resistance of microbes to ionizing radiation, they suggested that highly sensitive microbes or well studied sensitized strains be used for space studies [121]. It would be important to select biological indicators which would be reliable controls for physical methods of measuring radiation [39]. For example, the production of lysogenic bacteria, induced by X-rays, gamma rays, protons, or neutrons, and dependent on the radiation dose, is a good model because it is resistant to other flight factors such as vibration [39]. Thus, these bacteria are good detectors of cosmic radiation.

Research with *Bacillus subtilis* on Apollo 16 later lead to stronger confirmation that microbes might be used as monitors of lethal and mutagenic effects in space environments. The experiments indicated that filtered solar radiation at 254 nm and 280 nm appeared to be less lethal to spores at these doses and induced lower mutation frequencies than did control ground studies [200]. The T_7 bacteriophage was inactivated to a greater degree by 254 nm solar UV than in ground studies, although the shapes of the dose-response curves were comparable [204]. Space vacuum appeared to enhance the lethal effect of solar UV at both 254 and 280 nm with the wild-type strain but not with the repair-defective strain of *B. subtilis* [200].

EXTRATERRESTRIAL CONTAMINATION

During the 1960's, there was a great deal of concern about the possibility of cross contamination by microorganisms between Earth and other planets [28, 214, 238]. In 1966, NASA, the Jet Propulsion Laboratory, and the American Institute of Biological Sciences, sponsored a symposium concerned with the probability of contamination of extraterrestrial sites [156]. Experience with intercontinental contamination by organisms of many levels of development suggested the potential danger; three centuries of sickness and death on both sides of the Atlantic Ocean followed the discovery of the new world [214]. Also, if terrestrial microorganisms were taken to other planets, there would be lost opportunity for study of the origin of life on those planets and on Earth [240]. One author suggested the possible need to establish satellite quarantine stations [214].

A more recent opinion suggests the unlikelihood of extraterrestrial contamination. Evolution in terrestrial organisms, accompanied by changes in the sequence of bases in DNA molecules, results in descendants which show little or no molecular resemblance in their nucleic acid and protein sequences to their common ancestor [112]. In this opinion, the recombination of "Martian DNA" with the DNA of terrestrial organisms was considered unlikely because of rejection [112]. Many pathogenic organisms and all viruses are dependent on a biochemical relationship with their hosts. Thus, their Martian counterparts would not infect terrestrial organisms. The author states that "if it is hazardous to return a surface sample from Mars, then it would be even more dangerous to return a sample of soil from Antarctica" [12].

MICROBIAL POPULATION DENSITY

Another major concern for the Space Station is the potential for increase in numbers of microorganisms associated with the crew members and their environment. An increase in the absolute numbers of microorganisms per unit area may occur as a result of closure [14, 21, 156]. A "microbiotank" was designed to quantify the rate at which microorganisms are shed by the human body [183]. Subjects in street clothes, confined to the tank 10 to 40

min, shed 3,000 to 62,000 viable microbial particles per minute [183]. Even given this great variation, it is evident that in a confined space occupied by a human, an enormous number of microorganisms will be present which will have the potential for increase in suitable environments. As indicated earlier, such shedding may diminish in the 0-g environment [34].

Studies of subjects in confinement cite instances of increase, decrease, and relative stability of microbial populations. Information from Apollo and Soyuz missions had resulted in the idea that only a few aerobic microbial species would survive spaceflight and that these species would produce large populations [165]. The Ben Franklin submersible conducted a 30 day mission during which the presence of microorganisms was monitored. Statistical analyses were not reported but evident trends indicated lower counts of microorganisms on board than control counts in the laboratory. The lower counts may have been attributed to low incubation temperatures of the on-board samples because, when temperatures rose about 10°F on the eleventh day of the mission, on-board counts approached the level of the base laboratory counts [155].

The opportunitistic pathogen, *Bacterium anitratum*, was isolated from the air just 5 days into the cruise. Its most widespread recovery occurred on day 14. Studies have indicated that this organism is a part of the normal skin flora, but is implicated as a potential pathogen in burn, wound, and urinary tract infections, and pneumonias. *Staphylococcus aureus*, another opportunistic pathogen, showed a general trend of increase in numbers but led to no instances of evident illness [155].

In a confinement study with 8 males confined 34 days, 2 in a control area and 6 in a chamber at 5 psi and 100 percent oxygen, the total number of colonies on aerobic blood plates from all body areas and from the environment increased as the experiment progressed. The floral buildup on the axilla, groin, glans penis, and buccal area reached a plateau by the mid-point of the experiment and then remained constant or decreased. Buildup of throat flora was more variable. Increase of microbes in the environment of the isolation chamber was greater and fluctuated more than in the control area [78].

The bacteria of the axilla, groin, and glans penis were staphylococci, micrococci, and corynebacteria. Streptococci increased in the throat and buccal areas. In the chamber environment, staphylococci or micrococci, gram negative rods, and to a lesser extent, streptococci were present [78]. Minimal hygiene, rather than atmospheric conditions, were seen to be the cause of bacterial increase. Twenty men confined to the Aerospace Medical Research Laboratories Life Support System Evaluation showed buildup of numbers of bacteria present in various cutaneous areas after about 23 days of confinement, at which it remained the next few days [183]. The numbers on the axilla and groin did show buildup, and in all but one experiment, corynebacteria built up to significant levels and were recovered to a greater extent than staphylococci. Bacterial levels in the personal hygiene and eating areas also rose to "dangerous" levels [183].

In Soviet experiments with isolation for 23 to 56 days, the test subjects were allowed to clean only their faces and hands and did not change their clothing. The chemical composition of lipids on the skin surface changed, shifting the pH toward acidity (5.2 to 5.68) [171]. During the first two to 3 weeks, the numbers of microorganisms on most parts of the skin increased to levels exceeding those of freshly washed skin (4 to 5 colonies/cm^2) by 3.0 to 3.5 times, after which stabilization occurred. The change in pH of the mixture of substances on the skin surface and the cleansing action of the clothing evidently restrained the development of microorganisms and contributed to their stabilization. Microflora on the skin surface and underwear were mainly saprophytic species (*Staphylococcus epidermidis, S. albus*, diphtheroids, and Sarcina). On the skin of the plantar surfaces of feet, the perineum and buttocks, the number of colonies exceeded those of the same freshly washed areas 7 to 12 times. After 30 days, the number of colonies per cm^2 was 68 on the buttocks and 82 on the feet [171]. Along with these changes, the bactericidal properties of the skin showed gradual decrease. The lack of good hygiene undoubtedly played an important part in these results.

ECOLOGICAL SUCCESSION

Many of the phenomena observed in spaceflights, in terrestrial experiments simulating spaceflight, and to be expected on the Space Station, may be termed ecological succession. Every animate and inanimate component of the Space Station will provide a substrate for microbial growth. Initially, the inanimate components will be essentially uncolonized by microbes. However, with the inevitable presence of moisture and nutrients on and in them, these components will be the sites of growth of the first microorganisms to colonize there. The growth of these inocula will produce changes in the environment such as changes in nutrient supply, alterations in pH or redox potential, or the appearance of toxic metabolic products. In the altered environment, the organism or group of organisms which initially colonized and were well-adapted, may be less well-adapted, or may be out-competed by another organism or group of organisms. The successful organism may in turn give way to yet another species or combination of species.

Competition occurs when two species require some common resource which is present in limited amounts. It occurs most frequently between closely related species with similar environmental requirements. The outcome will depend on the relative rates of growth, the faster growing organism eventually replacing the other if a sufficient number of generations occur. Environmental requirements of the two organisms may be identical, but if growth rates differ, the one with the faster growth rate will prevail, at least for a time [9].

For example, obligate aerobes may be inhibited by the growth of facultative aerobes which consume all the oxygen [26]. Or, the production of oxygen by photosynthetic organisms may hinder the action of anaerobes [26]. These kinds of interactions will ultimately lead to changes in the species composition in a particular habitat (e.g., water system of the Space Station, or skin of humans in closed spaces). Many bacteria are known to produce antibiotics which act on strains closely related to the producing organism [26]. There is an obvious competitive advantage to having this ability. Pohunek [170] postulated that streptococci which produce antibiotics attacking vaginal lactobacilli may be able to invade the vagina and replace the resident bacteria [26]. Another example is the production of alcohol by one yeast which can limit the growth of another [82]. The pH of the medium may be lowered by the excretion of H ions or production of organic acids [26]. An analysis of the connection between increased acidity of contaminated skin and simultaneous change in the growth of microorganisms appears in Reference 129. *Chlorella* spp. cultures are known to produce antibacterial substances [201]. A possible problem on the Space Station with competitive interactions between microorganisms might be the suppression of the growth of necessary microbes [85].

With time, a relative equilibrium which is a function of both the environment and of the dominant organisms is usually reached [26]. The regulatory processes that take place in heterogeneous microbial populations are extremely complex [25]. Chance often determines which organism will be the first to colonize a new substrate. If a pioneer obtains a sufficient head start, it may be able to adapt itself to the habitat and develop a strong enough population that it may be able to maintain itself in the face of potentially stronger competitors which arrive later [26].

Substrates which have been perturbed sufficiently may respond as uncolonized substrates. The internal and external surfaces of the human body may behave as perturbed substrates in spaceflight, particularly in spaceflight of long duration. When a substrate for microbial growth is changed by physical, chemical, or biological phenomena, new niches will be created, providing opportunity for growth of colonizing microflora. The succession of microbes on the tooth surface begins 10 to 15 min after cleaning and leads to a climax community in 1 to 2 weeks [9].

Microorganisms occur in enormous numbers in nature and will occur in enormous numbers on the Space Station. Any given strain or species has numerous opportunities for extending itself into new territories and each individual has the potential for colonizing a niche [26]. The rapid growth and great adaptability of a microbe permits

it to become established in a new suitable niche within hours or days [26]. After 24 hr, the number of microbes in 1cc of urine may reach many million [123]. It is the environment which selects which organism will be successful. There will be ample opportunity for overgrowth of microorganisms which may overwhelm the normal tolerance to small numbers of these organisms [136].

Although the microbial community on the Space Station will not approach the diversity of such a community on Earth, complex microbial interrelationships will be assured by the presence of subsystems such as the water and air regeneration systems. There will likely be interrelationships between pathogenic, symbiotic, and commensal microbes, humans, and any research organisms on board [203]. Also, because the environment will be synthetic and previously uncolonized, new relations between organisms are likely to develop [203].

The stability of a biological community is generally dependent upon the diversity of living organisms in the community. With succession, the number of kinds of organisms in a community usually increases and energy flow between biological components becomes more evenly dispersed. It is presumed that on the Space Station species diversity will decrease [85], although the mean number of microbial units will go practically unchanged [133]. It is for this reason that it will be impossible to evaluate, from numbers alone, the ecological significance of an organism in a given habitat [26]. The mere presence of microorganisms may not reveal anything about potential for pathogenicity [34]. Data are needed on the organism's mass, its metabolic activities, and its possible ability to produce and react to substances which have unique biological activities [26].

Several studies, both in space and on Earth, have documented the fluctuations that have occurred upon partial or complete closure of an ecosystem. Although some find relatively small deviations from normal microflora in a closed space [85], available data suggest that the prolonged stay of animals and people in closed environments leads to simplification of indigenous microflora [125, 127]. The simplification of microflora in closed systems is due partly to the limited number of persons there, the microbial coenosis of the areas surrounding people being determined to a significant degree by its contamination by indigenous human flora [167]. And, conversely, although the microbial communities of humans and their environment each have their own regulatory mechanisms, the formation of and changes in the human microflora are determined to a large extent by the microflora of the environment [125]. While this is true on Earth, in the closed environment of the Space Station, it will be of paramount importance. "In small enclosed systems, extinction becomes increasingly probable with time because of the small numbers of organisms involved, the accumulation of metabolic waste products, and the general decrease in free energy of the system with time" [217].

In a small group of people, a composite mixture of the various species of microorganisms will be reduced in comparison with the microflora of large communities on Earth [125]. The limited volume of the closed environment will reduce the number of different reservoirs for the storage and reproduction of microorganisms. Such reservoirs on Earth are soil and natural water [125]. On Earth, people are surrounded by numerous different microbial coenoses, whereas in space, they are part of only one very limited coenosis [125].

If not continuously inoculated, animals placed in isolation for two months are found to have only one or two species of microbes in the intestinal tract, usually one yeast and one bacterial species [134]. The authors who provided these data stated that this change may or may not be harmful, depending on the dominant organism [134]. It is probable that the number of microbial species in the intestine will decrease although the total number of organisms will not diminish markedly [134].

Simplification of human microflora was observed among 20 men confined for six weeks to the Aerospace Medical Research Labortories Life Support System Evaluation in which humidity, temperature, and the partial pressure of gases were controlled in a space of 1,100 ft^3 [182]. There was a buildup of numbers of bacteria on various cutaneous areas. At about 3 to 4 weeks, a numerical level was reached and maintained for a few days,

after which it underwent a small decrease [182]. The axilla and groin showed increased microbial numbers also. In all but one experiment, corynebacteria built up to significant levels and were recovered to a greater extent than staphylococci [182], indicating shifts in relative numbers of species.

On the Ben Franklin submersible, a major obsrvation was of a general simplification of the flora, a decrease in the number of genera isolated as the mission progressed [155]. A cause of concern was the lack of recovery of *Neisseria* sp. in the throat, and of the anaerobes and corynebacteria, which are important segments of the major true or indigenous skin flora [155]. The authors indicated that some of the lack of recovery may have been due to culturing constraints such as transportation, storage, and inoculation temperatures, etc. [155]. However, their complete absence during the mission implies some effect other than simply culture techniques [155].

In a Russian experiment with isolation, the absolute number of microbes increased during the first 2 to 3 weeks followed by a period of normalization [85]. A three-month test with one human subject showed increased numbers until the end of the experiment [85]. At the same time, the inoculation of the skin with *Staphylococcus* spp. increased, and diptheroids were present in very small numbers [85].

During the 6- to 9-day Apollo-Soyuz test project (ASTP), when two crews were joined in space, reduction of the number of microbial species was not observed, at least within the community of medically important microorganisms [165, 210]. In another study of human microflora in spaceflight, there was the indication of periodic increases in the number of microflora, each accompanied by a change in the qualitative composition, primarily showing an increase in the proportion of microflora with pathogenic properties or increased resistance to antibiotics of the penicillin and tetracycline groups [63, 226]. In 30- to 90-day confinement tests, there was a reduction of intestinal *E. coli* and there were changes that indicated developing dysbiosis or flooding of the intestines with microorganisms from another site [85, 134]. During the tests, stability of the intestinal flora was not achieved [85].

Although aerobic flora comprise only 1 to 10 percent of the total number of the intestinal flora, they play a significant protective role against such pathogens as *Salmonella enteritides* and *Aerobacter aerogenes* [85]. Thus, a decrease in populations of these species could affect these protective functions. With simplification of the human indigenous flora, one may expect disruption of intestinal peristalsis and absorption of liquid in the large intestine, reduced vitamin formation in the intestine, reduced digestion of food, increase in dental caries, accumulation of toxic substances, and intensification of gas formation [125]. Morphological changes seen in mammals with reduced indigenous flora are decrease in size of the lymph nodes, decrease in plasma cell production, atrophied thymus, and decreased tonus of the intestinal tract [134]. Further, in a closed system such as the Space Station, if the astronauts lose one symbiotic bacterial species, they will not get it back [95].

Limited data on airborne microbial communities in confinement suggest that they also tend to decrease in species diversity. There were high diversity and low numbers of microbes in the air during the first few days of 59 days submerged on Tektite I [41]. Following this, diversity decreased while the number of colonies increased. The reduction in variety was assumed to indicate the emergence of a single species of bacteria [41]. The most common organism collected from day 35 through 57 was *Acinetobacter phenon* [41].

The simplification of microflora which has been almost universally observed in closed systems, provides conditions which are favorable to colonization by new species or variants that are frequently pathogenic [14, 38, 57, 152]. Numerous data indicate that when there is a change in the composition of the indigenous flora and in the state of the macroorganism, as there often is in small confined spaces, there is a relative intensification of the pathogenic significance of the microflora [49, 62, 119, 133, 215]. Data supporting this observation showing the increase in the number of pathogenic or conditionally pathogenic strains of indigenous flora in humans who have spent prolonged periods in closed environments may be found in References 134 and 176. Along with the increase of general

bacterial contamination of the air, there are shifts in the yeasts and other specific microflora present, such as *Candida*, saprophytic white staphylococci, diphtheroids, bacilli, and sarcinae [226].

On the Ben Franklin submersible, a major observation was of the general simplification of the flora, a decrease in the number of genera isolated as the mission progressed (although no statistical analyses were presented) [155]. The final limits of simplification were not reached in the 30 days of the mission [155]. Along with the simplification noted, was a shift in the bacterial populations from the more usual gram positive organisms toward gram negative bacteria, particularly *Psuedomonas* spp. and *Enterobacter* spp. [155], despite the fact that earlier studies using chambers do not indicate this phenomenon [155].

The shifts noted were attributed to the fact that antimicrobials are typically more effective on gram positive bacteria, as well as to the low incubation temperatures and long holding time in the transport media [155]. Culturing methods favored the hardier gram negative rods, especially those such as *Pseudomonas* spp. which have lower optimum growth temperatures than most indigenous human flora [155]. Also, only absolute rather than relative counts were made, indicating trends only [155].

On the Apollo flights, only one potentially pathogenic species (*Pseudomonas maltophilia*) was recovered from the command module sites before lift off; four pathogens (*Herellea vaginicola, Klebsiella pneumoniae, Proteus mirabilis,* and *Streptococcus faecalis*) were recovered after flight. This pattern was generally noted in all flights for which these samples were collected [72]. Urine samples which were generally free of microganisms before launch, contained *Klebsiella pneumoniae, Proteus Mirabilis, Herelle vaginicola, Pseudomonas aeruginosa,* and *Haemophilus* sp. upon return to Earth [72]. *Haemophilus* sp. is likely to cause recurrent urethritis.

There was also found an increase in the number of species of pathogens recovered from the skin surfaces of crew members of Apollo missions. Three species (*Staphylococcus aureus, E. coli,* and *Herellea vaginicola* were recovered the morning of the launch; seven medically important species were recovered immediately following splashdown [72]. This increase in number of species was a reflection of numerical increase within the four additional species during the flight great enough to allow their detection. These trends were not consistent among the Apollo flights, however. For example, an average of 175 percent more medically important species were recovered from the seven Apollo 13 postflight skin swabs as compared with an average increase of only 33 percent for Apollo 14 [72].

It was not unusual to find at least one crew member from each Apollo team harboring *Candida albicans* in the mouth. Although the presence of *Candida* sp. in the mouth does not in itself pose a threat, it was an indication that the other fungi that normally exert competitive control over *Candida* sp. populations decreased dramatically during space flight [72].

The Soviets also observed major microbial changes in enclosed ecosystems intended to provide human life support. They found that species composition and population numbers changed throughout the time and possibly degraded the performance of some components of the system [85]. Other studies with isolation showed shifts in both anaerobic and aerobic fecal flora, with the frequent appearance of *Shigella* sp., enteropathogenic types of *E. coli*, and *Candida* spp. [22, 197]. Thus, there has been found to be a trend toward increase in the proportion of pathogenic and conditionally pathogenic organisms in isolated areas [e.g., 72, 218].

Another possible consequence of the simplification of the microbial coenosis on the Space Station is "microbic shock," a term for the health problems that can arise upon the introduction of a different group of microbes, as might occur on the Space Station with exchange of crew members or new supplies [155]. This can occur in an isolated crew which has become adapted to a group of microorganisms with changed composition and reduced diversity [155]. The simplification of flora observed in closed systems may lead to reduction of the

immunocompetent system [125] and the development of infectious complications [125, 134]. This phenomenon may occur not only in the indigenous flora of humans, but also to the microbial community of the Space Station as a whole. Even in certian terrestrial ecosystems, when there is a large input of foreign microorganisms, the native population becomes unstable [146].

A major factor in the simplification of human microflora in closed spaces can be the diet of the inhabitants [14, 21, 159]. Animals given sterile food for a certain time have only 2 to 3 species of microorganisms remaining in the intestine [38, 134]. The number of microbes in 1 gm of human feces decreased 100X as a result of hypodynamia and various food rations [36, 63, 230]. In experiments combining the effects of sterile food and sterile air, it was found that simplification of the microflora occurred more quickly with both sterile food and air [134]. The occurrence of some microbial species within the community of the mouth are influenced by diet [9]. The need to establish balanced, innocuous, non-invasive microflora maintained by continuous re-inoculation with a pill or diet supplement has been proposed [8]. It is possible that if all the necessary human endosymbionts are known, freeze-dried cultures of them could be kept to feed to the astronauts [95].

SUMMARY AND CONCLUSIONS

— Microorganisms will, without question, be part of the environment of the Space Station.

— Microorganisms include viruses, bacteria, fungi, algae, and protozoa. Bacteria and fungi are likely to be the only abundant forms on the Space Station.

— Crew members will be the primary source of microorganisms, the healthy human body being the site of at least 50 microbial species. Any experimental animals or plants will also be a major source.

— Several organisms present in the human environment and on the skin or within the body are potentially pathogenic, causing disease when circumstances favor their transfer to a suitable site, and their proliferation. Other organisms, not normally associated with humans but found in their environment, can lead to disease when they are able to establish populations on or within the body. *Legionella pneumophila*, the source of Legionaire's disease, is an example of an organism which is widely dispersed on Earth and whose growth is supported by terrestrial air conditioners, heat exchangers, and cooling towers.

— Potential pathogens have been present on all NASA missions, and will be present on the Space Station. Under normal circumstances, these organisms are not harmful to humans. Their pathogenicity can become effective when they are transferred to sites of the body on which they do not normally occur, when their numbers increase dramatically, or when human resistance is lowered by stress, by breaks in the skin, etc., or when all of these phenomena occur.

— Conditions in a small, confined space like the Space Station can reduce normal human resistance to disease-causing organisms.

— On Earth, pathogens dispersed by humans are rapidly diluted to levels at which new infections do not occur. Both atmospheric and aquatic dilution will be absent on the Space Station.

— Interpersonal exchange of microorganisms is often facilitated by enclosure in confined spaces; examples of such exchange on NASA missions, submarine cruises, etc., are numerous.

— If microorganisms offered no advantage to people and their environment, it would be safe to attempt to completely eliminate them on the Space Station. However, certain microorganisms are beneficial and their elimination (although practically impossible) would be disadvantgeous.

— Among the benefits of microorganisms are their aid in the digestive process, their resistance to infection by pathogenic organisms, and their ability to synthesize certain vitamins and growth factors.

— Sites in which microorganisms can proliferate will be numerous on the Space Station. Viable microbes can occur and multiply in the atmosphere, in water, on surfaces, and within certain substances and materials.

— Microorganisms at any site on the Space Station will very likely be of human origin, unlike those in outside areas on Earth which arise primarily from the soil and natural waters.

— Any surfaces which are moist or wet will be the most favorable sites for microbial growth because water is essential for biological growth, and nutrients adsorb to surfaces. The cleaning of surfaces usually results in only a transient decrease in levels of microbial contamination.

— Dirty clothing can be a suitable habitat for the growth of many microorganisms. Clothing with synthetic fiber content is less supportive of microbial growth than that with natural fibers.

— Microbes can be effective corroders and degraders of a wide variety of materials, by means of their ability to produce organic acids as metabolic by-products. Microbes facilitate the corrosion of iron, steel, aluminum, fuels, propellants, paint films, and electronic parts, etc.

— Where microorganisms are inadvertantly allowed to produce large populations, there will be the potential for release of volatile gaseous and/or toxic metabolic by-products. This phenomenon could impose hazards on both the atmospheric and aquatic recirculation systems.

— All organisms must have suitable amounts of heat, light, water, nutrients, etc. to carry on growth and reproduction, but the limits of tolerance to these and other factors vary widely between species. Thus, the manipulation of certain factors, such as temperature and oxygen concentration, to control the growth of a particular microbe, could promote the growth of another.

— Methods for monitoring the presence and growth of microorganisms on Space Station are currently in a primitive stage. Because of the increased ability of pathogenic and potentially pathogenic microbes, and microbial toxins to effect human illness in a small, closed system, rapid identification and enumeration of microbial populations will be necessary. Many authors point to the need for such methods for the Space Station.

— Controlling microbial growth on the Space Station must begin with the design of all subsystems to limit surface area as much as possible, to limit the accumulation of moisture on surfaces, to allow maximum access to surfaces for cleaning, and to utilize materials least likely to support microbial growth and corrosion.

— Various methods are being considered for microbial control during missions. These methods include UV radiation, atmospheric and aquatic filtration, iodination, chlorination, and pasteurization. The methods to be used must be chosen with the knowledge that most microorganisms are very plastic genetically; that is, they have a remarkable ability to adapt to adverse environmental factors such as chemicals, either by mutation or by shifts in genetic dominance. The use of a variety of methods in concert should significantly reduce this ability.

— The effects of space flight factors such as confinement, weightlessness, and radiation on microbial populations may be of concern on the Space Station. Over time, microbes exposed to factors to which their original populations were not accustomed, can lead to mortality or physiological or genetic adaptation, causing changes in the initial properties of the organisms.

— The rate of mutation, the biochemical change in the genetic material of microorganisms, may be increased by space flight factors. It has been shown that mutation can increase the virulence of a potentially pathogenic organism. It is conceivable that new human pathogens may appear, producing infections not normally encountered.

— Microorganisms may, with further research, become useful as monitors of lethal and mutagenic effects in space environments. It has been suggested that highly sensitive microbes or well studied sensitized strains be used, particularly in detecting ionizing radiation.

— There is the potential for increase in numbers of microorganisms associated with the crew and the environment on the Space Station. However, efficient filtering, disinfecting, sterilizing, and cleaning procedures should stabilize population levels.

— Microorganisms on the Space Station may be expected to undergo ecological succession, resulting in sharp reduction or extinction of some species and the proliferation and numerical dominance of others.

— These successional changes will be most likely to occur in areas inaccessible for frequent cleaning, such as the air and water regeneration systems.

— Because the Space Station environment will be synthetic and previously uncolonized, new relationships between organisms, including humans, are likely to develop.

— It is expected that, on the Space Station, microbial species diversity (the number of species present) will decrease, although the mean number of microbial units will be essentially unchanged. In general, a decrease in species diversity in a biological community leads to reduced stability and results in unpredictable fluctuations of dominant species.

— For crew members, reduced species diversity, or extinction, of indigenous microflora over time may lead to the necessity for inoculation (e.g., in food) of beneficial microorganisms that have been lost.

— The reduced microbial diversity which has been almost universally observed in closed systems, provides conditions which are favorable to colonization by less abundant species or variants that are frequently pathogenic. Numerous data support the observation of the increase in number of pathogenic or conditionally pathogenic strains of indigenous flora in humans who have spent prolonged periods in closed environments.

— Major differences between terrestrial and Space Station ecosystems will be the vastly decreased diversity of living organisms on board, the decreased space, the increased surface to volume ratio, and the decreased free energy of the system caused by enclosure. These are differences of great importance to the functioning of biological systems which will probably lead over the long term to some relationships that have not been observed on Earth. In such a "drastically altered environment, biological change is likely to be abrupt, dramatic and unpredictable" [203].

These summary statements emphasize microbial functions and relationships which will be pertinent to the Space Station and must be recognized in its design. Many of the statements are applicable to current subsystem designs. NASA ECLSS administrative and contractor personnel working on life support subsystems should be aware of the ways in which microbes can cause problems and should encourage engineering design based on this awareness.

Many of the summary statements lead to necessary areas of research for the Space Station. Problems needing solutions include the following:

1) Undesirable microorganisms must be eliminated or controlled. Much progress has been made in doing this, but there still remains the problem of pockets of microbial growth in areas not reached by filtering, disinfection, or sterilization techniques.

2) The number of anti-microbial treatment methods necessary to eliminate the possibility of adaptation or mutation must be determined. Whether these treatments should occur linearly through space and time or be concurrent must also be determined.

3) Some level of microbial species diversity needs to be maintained on the Space Station in order to maintain human health. If certain beneficial microbes must be introduced periodically to the environment, it must be determined which microbes should be introduced and how to maintain them (e.g., cultures maintained on board or other means).

4) Methods should be developed to rapidly identify and quantitate microbial populations continuously or periodically. Until these methods are developed, the preceding questions will be academic. Without rapid monitoring techniques, there can be no assurance that microbial population densities and species diversity are being maintained at levels healthy to humans. As stated in the text of this report, many authors point to the need for improvement of methods for sampling and identification of microorganisms on the Space Station. Therefore, microbial analysis techniques should be the focus of near-term research for Space Station technology.

APPENDIX

Glossary

accellular — not composed of living cells

aldehyde — organic compound of the general formula RCHO containing the carbonyl group C = 0.

arbovirus — a virus carried by an arthropod vector which multiplies in the arthropod and in a vertebrate host (e.g., encephalitis and yellow fever)

arthropod — invertebrate animal with jointed appendages (e.g., insects and arachnids)

asporogenous — not producing spores

bacteriophage — a bacterial virus, often referred to as a phage

basidiomycete — a class of fungi that produces sexual spores (basidiospores)

biosphere — the portion of the Earth in which ecosystems function

chemolithotrophic — deriving energy from inorganic chemicals such as ferrous iron, hydrogen sulfide, or ammonium

chemoorganotrophic — deriving energy from organic chemicals

chlorophyll — a green pigment found primarily in plants which aids in trapping energy from sunlight

coagulase — enzyme that clots plasma –coagulase activity is used by microbiologists to distinguish between pathogenic and non-pathogenic staphylococci

coenosis — cenosis - ecological community which includes all the populations occupying a given space

corynebacteria — gram positive, rod-shaped, nonmotile, aerobic or facultatively anaerobic bacteria

diphtheroids — gram positive bacterial rods which are common components of human normal floral assemblages

endospore — spore produced within the bacterial cell that is extremely resistant to high temperatures and desiccation - produced by a small number of soil bacteria

endosymbiont — an organism that lives within another in a symbiotic relationship

enteropathogen — a pathogen that causes gastrointestinal problems

enzyme — protein which catalyzes biochemical reactions

eukaryote — a cell that has one or more nuclei and certain other intracellular structures such as mitochondria, etc.

exogenous — introduced from or produced outside the organism

genera — plural of genus

genus — a group of closely related biological species

germination — the emergence of a single vegetative cell from an endospore

ketone — organic compound of the general formula RR'CO containing the carbonyl group C = O

flora — the sum total of the non-animal organisms in any given area or environment

gnotobiote — a germ-free organism

heterotroph — utilizing organic molecules as a carbon source

lactobacilli — lactic acid bacteria – gram positive, fermentative, spherical and rod-shaped bacteria that carry out lactic acid fermentation (e.g., Streptococcus and Lactobacillus)

lactose — a disaccharide found in mammalian milk

locus — a gene which has been located on a chromosome

lysogen — a cell that carries a provirus, the viral DNA that is integrated into the host's hereditary material

metabolism — the chemical reactions of the living organism including those that break down materials (e.g., respiration) and those that synthesize complex molecules

microflora — the sum total of the microscopic non-animal organisms in any given area of environment

niche, ecological — the position or status of an organism within its community and ecosystem resulting from the organism's structural adaptations, physiological responses and specific behavior (Odum, 1959)

opportunistic pathogen — an organism which causes disease only when its hosts are weakened

pathogen — an organism having the genetic ability to cause disease

phage — a bacterial virus – also called a bacteriophage

phosphorylation — the addition of phosphate to adenosine molecules to yield the energy-rich adenosine triphosphate (ATP) found in all living organisms

photosynthesis — the transformation of light energy into chemical bond energy by living organisms

phototrophic — obtaining energy from light

primodia — plural of primordium — the earliest stage at which the differentiation of an organ can be perceived

prokaryote — an organism whose cells lack the usual structures of eukaryotes (e.g., nuclear membrane, Golgi apparatus, etc.)

prophage — the phage DNA that is integrated into the host's hereditary material

protozoa — single-celled organisms

saprophyte — an organism obtaining its nutrients from the decomposition of dead organic matter (e.g., fungi)

somatic cells — those cells of an organism which are not related to sexual reproduction

species — a group of organisms sharing certain biologically important attributes which is usually genetically separated from other such groups

spirochaete — a group of helical bacteria that have axial filaments rather than flagella

spore — asexual reproductive cells

urethritis — inflamation of the urethra

vector — an organism that carries and transmits a disease-causing organism

vegetative cells — somatic cells; cells that are unrelated to sexual reproduction

virus — protein-covered nucleic acids that infect cells

yeast — fungi that are usually single-celled organisms

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APPROVAL

THE ECOLOGY OF MICROORGANISMS IN A SMALL CLOSED SYSTEM: POTENTIAL BENEFITS AND PROBLEMS FOR SPACE STATION

By Elizabeth Rodgers

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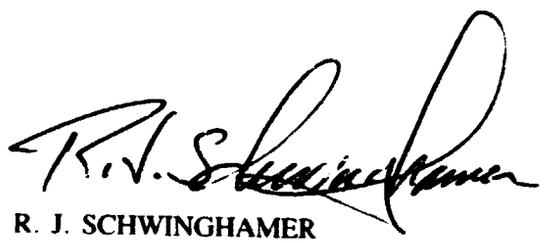

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