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THE MICROBIOLOGICAL CHALLENGE IN SPACE

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It is the purpose of this paper to crystallize, within the allotted time, the possible potential position of the microbiologist on that team of scientists and engineers who are currently applying their several disciplines in meeting the challenges represented by today's astronautical frontier. As nautical became prefixed with astro-, so biology, zoology, botony, and microbiology can be expected to grow into the new experimental and applied areas which are connoted in the prefix astro-. Astromicrobiology, like any of the other new areas so created, will seem a little uncomfortable at first. This concept in microbiology may be subject to criticism, but evaluated it must be. The evaluation best comes from the microbiologist himself. It may be recalled that at the symposium of May 14-17, 1958, on "Possible Uses of Earth Satellites for Life Sciences Experiments," the biologist considering satellite uses was cautioned. My own notes on this read, "Biologists not yet ready to go into orbit. Keep your feet on the ground." From this memo we can abstract and build on the idea "not yet ready." What the state of readiness is, for the microbiologist in particular, represents an early challenge. What he does with his own state of readiness once he has determined it represents a portion of the opportunities associated with the expanding effort and must be his own decision. Opportunities here, however, do father a continuing host of additional challenges.

The nonbiological use of satellites and rockets, reflecting the engineering capabilities of contemporary missiles, has been reviewed and published in the proceedings of the symposium "Scientific Uses of Earth Satellites" (Van Allen, 1958). The 34 papers contained therein all concern themselves with the geophysical aspects of space probing and illustrate well the fundamental instrumentation, telemetry, and other factors involved in sensing and transmitting back to earth the data collected.

Approximately two years later, Dr. Pickering, presenting Dr. Froelich's paper, "The Army Rocket, Satellite, and Space Flight Program," at the "Biological Uses .... Symposium," indicated the following five factors as governing our ability to use successfully earth satellites in scientific exploration. These concern the adequate development of:

1. A rocket cluster of adequate size
2. Guidance and control systems
3. Environmental control
4. Communication
5. Production and operational capacity

The 15 intervening months have seen some marked advances in many of these areas. A summary of this progress has been reported by Medaris (1959). This list of space probe achievements, conducted for the National Aeronautics and Space Administration, can well be supplemented by achievements of the other services. In 1959 it is expected that, under the auspices of the Advanced Research Projects Agency, a cluster of booster rockets will be developed for Project Mercury which will produce a thrust of 1,500,000 lb. This can indicate for us what payload potentials exist. A good rule of thumb indicates that the launch to weight ratio may be estimated as 1000:1 and proved valid for example, in the Vanguard. This represents an advance in capabilities particularly since pre-Vanguard ratios were more nearly 2000:1. Assuming however that a 1,500,000-lb thrust is achieved, a payload of only 1500 lb would represent our capability in Project Mercury, where we expect to carry a man and all his supporting gear. The significance of biological probing, therefore, where microorganisms are proposed to act as environmental sensors looms high, in relationship to available payload. Properly miniaturized experiments can utilize with maximum efficiency any space on the payload dedicated to biological research. There is no contention here implied that data obtained with microorganisms as probes have a high transfer value in terms of studying spatial environmental effects on man. On the other hand, it is generally contended that man's sensitivity to various types of radiation is higher than that of any other organisms, including other primates and therefore he would actually be his own best test animal. Microbiological space probes must be evaluated with the same reservations that exist for all bio-assays. Beyond this, the academic "need to know," what extraterrestrial environmental effects will be on biological systems is basic to the growth of biology.

The effect of environmental factors on bacteria and fungi is a study of fairly long standing and suggests parameters may well be used as a basis for comparative studies of limiting environments. The limiting environments would involve such factors as temperature, including high and low values, moisture, pressure, as in vacuums producing sundry outgassing phenomena. Outer space explorations would take us through various types of atmospheres. These may be enumerated as:

1. Chemical
2. Radiations
    a. nuclear, alpha, beta, gamma, x-rays
    b. solar
    c. cosmic
    d. magnetic
    e. auroral
    f. electrostatic
3. Particulate
    a. space dust, meteoritic grit
4. Gravity-associated
    a. zero and near zero
    b. accelerated, shock
5. Planetary (various combinations)

Table 1, for which I am indebted to Erik Linden of the Signal Corps, is an abridgement of an excellent compilation made by him in his studies on the effects of high altitude environments on materials. The biological implications in the column "Remarks" reflect areas of biological significance which we can profitably consider. In passing, it may be well for us as microbiologists to recognize that here in the geophysical arena a nomenclature problem exists too, in relation to the naming of the various zones enveloping the earth. The system advanced by the International Union of Geodesy and Geophysics has been invoked here.

Table 1. Altitude Characteristics (after Linden)

Miles (Approx.)	Remarks	Sphere
10	O_2 respiration limit	Strato-
10	Temperature is -80F	Strato-
12	Water in biosystem boils	Strato-
15	Ambient air insufficient for pressurization	Strato-
13-26	Ozone filters dangerous ultraviolet light	Strato- & Chemo-
20-50	Ultraviolet light intense, energy and chemical activities high O_2, N_2 — monatomic, corrosive	Chemo-
23-27	Biologically effective ultraviolet light	Chemo-
30	Temperature is +30F	Meso-
25-72*	Meteor-safe zone ends	Meso-
50-240	Gases ionized by ultraviolet of sun, reflects radio waves (Layers) D — 50 miles E — 100 miles F — 180 miles F_2 — Variable; seasonal, sun spots, flares	Iono-
55	Temperature is - 95F	Thermo-
80-100*	Vision changes from atmospheric to space optics	Hetero-*
100*	Zone of complete silence	Hetero-*
120-140*	Aerodynamic support ends. Centrifugal force required.	Hetero-*
120*	Aerodynamic heating ends. Heat transfer by radiation only.	Hetero-
600*	Outer space: molecules can escape earth's gravity	Exo-
1,400-40,000	Van Allen Layer (Two belts of electrons or protons, possible solar origin, producing x - rays on striking satellites. Layer 1 starts at 1400 mi, 200 mi thick. Layer 2 starts at 8000 mi, 4000 mi thick. Between them lies a region of no radiation. Beyond 40,000 mi manifestations are absent. There appear to be voids over the poles.)	Exo-

* — and beyond

A closer look at the altitude characteristics should encourage the microbiologist. The hazards indicated here in terms of temperature, anaerobic conditions, chemical effects, desiccation, solar, and other radiations are not ones with which he is unfamiliar. Under laboratory conditions these influences have been thoroughly surveyed in several areas. Data of this kind represent an excellent information resource. In other areas, for example, the new environmental reference frame represented by zero or near-zero gravity, will have to be evaluated by the microbiologist *even as it must be evaluated by every other discipline* represented on the astronautical team. In the compound appraisal of gravity, particularly in the applied areas no discipline has a long history of cognizance, much less a history of achievement. Theoretical support according to Surosky, Hill, and di Rende (1959) is lacking, and the environmentalist seeking help with his applied problems must continue to be empirical until significant breakthroughs occur. Biological history is rife with empiricism. Biology with its innumerable coexisting and interacting systems has used empiricism well and will continue to do so.

Experiments in gravitational effects involving greater and lesser values than *g* can well be considered by the microbiologist in conjunction with his allied engineer. The technology and energy resources required to study plus and minus gravity values as affecting biological systems may be considered more feasible in the microcosm of the microbiologist than in a representative of the macrocosm. Time factors such as generation times favor the microbiologist and as our ability to extend experimental periods of both high and, particularly, low *g* values increases, the effect of gravity on microbial life cycles should be contributory to biological advances. The free fall experiments, simulating weightlessness, again may be more easily achieved with microorganisms. Although experiments of this kind are difficult to imagine, they have been done, in high *g* values with other animals (Surosky *et al*., 1959), and would seem to be less difficult with the smaller living systems.

In anticipation of that time when planetary visits will be practical—and the astronauts are optimistic—the environment and atmospheres of the explored planets will need biological evaluation which must be made by biologists. Here it is expected that physical traits again will be a mixture of environmental hazards differing specifically from the terrestrial one but involving chemical atmospheres, temperature variations, desiccation, radiation, and other characteristics. The introduction of terrestrial life forms into these atmospheres, and their success or failure, is not only a challenge in adaptiveness to the organism, but an evaluation of the capability of microorganisms to meet this challenge is, in turn, a challenge to the microbiologist, and well within his proper cognizance.

With the above introduction let us leave the consideration of physical properties and continue further into the microbiological challenges. The latter can be divided arbitrarily into the open space ecological system as we have considered above, and the closed system which we will consider later. Apart from these two, but supplementary to both, I would like to mention an area of microclimatology and microecology.

The behavior of organisms, and this can well include the microbial forms, in relation to their microclimate, has been considered by Geiger (1957) as micrometeorology. For certain reasons which we cannot discuss here in full, this may be an unacceptable designation, particularly since, in some of the Dubos (1959) studies, considerations allied to those of Geiger have been discussed under the concept of bioclimatology. Both Geiger and Dubos summarize well the responsiveness of organisms to meteorological and geophysical influences. From his own experiences the microbiologist knows too that the organisms with which he is familiar have wide sensitivity ranges and specific tolerances that are often governed by easily recognized influences. Not infrequently, however, the causes for specific responses remain elusive. This challenge, namely, the need to mobilize and expand the knowledge of the influence of environmental factors on microorganisms would yield parameters of value in both terrestrial and extraterrestrial probing.

Microbiological challenges in closed ecological systems lie in the following areas:

1. Gas exchange microbiology
2. Waste disposal or modification
3. Food production, waste utilization
4. Microbiology in psychobiological support

An excellent source document in the energy requirements in waste handling in a closed system has been prepared by Slate (1957). Palevsky (1957), in a recent progress report on handling air contaminants in a biosatellite, sets out very well the optimum atmosphere necessary for maintaining life and sedentary work in confined space. Parameters in terms of temperature, humidity, air motion, foreign matter, odors, bacterial population, and sanitation are indicated. Ingram (1957) deals with skin excretion and sweat biochemistry in terms of specific chemical compounds. The microbiological implications here in terms of odor and possible utilization of excreted compounds in a balanced independent ecology seem obvious. In the over-all problem, Ingram points out that in considering today's needs in terms of sustaining man in a space capsule, investigations progressively reveal that questions and needed answers multiply. No longer is the man in a box only the clean-cut model which stimulated Einstein in his thinking, but it is also a model for one of today's important challenges to all of biology—microbiology not excepted. Apparently, where man goes his microbes go also.

Masson (1957) in his review covers methods of obtaining oxygen from carbon dioxide. Applied biology in terms of the algologist receives its fair share of attention. Whether oxygen recovery must be mediated through chlorophyll-containing organisms is a question the microbiologist should explore. Miller (1959), in reporting on the Space Logistics Conference of 1958, points out that Roadman indicates the weakest link in space travel will be man. Requiring ideally 283 lb to maintain himself in space for 7 days the present equipment for this purpose is actually a 707-lb requirement. Reduction of this weight penalty must surely be considered by the closed-space ecologist. Perforce the microbiologist and others have become ecologists.

Within this framework it seems readily apparent that industrial microbiology in its fermentative, synthesizing, and organic material utilization should be carefully reviewed for applicability. Even the psychobiological support value of microbiology as an entertaining, diversionary device should not be dismissed lightly. One calls to mind the history of Woronin (Large, 1940) with his fungus gardens and laboratory in his bedroom where he lived, almost a recluse, studying and enjoying his rusts, nitrogen fixing organisms, and club root-causing fungi.

The challenges associated with astrobiological exploration are as follows:

1. Extent of biosphere, limiting factors, duric forms;
2. Origin of life, comparative planetary bioevolution, life precursor biochemistry, sampling procedures, contamination prevention.

The extent of the biosphere has long intrigued microbiologists and aerobiologists. Distribution patterns, dissemination studies, epidemiology, and etiology have long speculated upon and in part explored, higher and higher regions of our atmosphere. Lindbergh, among others, you will recall, exposed slides for microbiologists over northern areas of the globe. Have these studies indicated just where the biosphere ends? Are some of the factors listed in our earlier environmental considerations limiting in nature? Probably, but how, where, and why, have not all yet been answered completely. With the coming of the space vehicle, satellite, and ultimately fixed space stations, operational bases will exist for pursuing duric forms into areas never before seriously considered. If the terrestrial biosphere is traversed and new planets are approached, where will evidences of the Martian or Venusian biosphere be detected? When they are detected how will they compare with each other and with the terrestrial biosphere? We tend to think in anthropomorphic terms, but microbes came before man, and it seems that precursor biochemistry, in the framework or our knowledge of organic evolution on the earth, went from molecules to microorganisms, probably plant forms. It would be a reasonable preliminary assumption to anticipate a similar pathway on other habitable planets. Doesn't the microbiologist belong on the front line of these explorations? Tikhov (1955) answers this question in the affirmative.

Before we dwell any longer in outer space, however, I would like to return, for a moment, to some rather mundane but important aspects of space microbiology as it is manifest here on earth and now. This has to deal with the application and use of existing microbiological knowledge. Those who participated in the symposium sponsored by our society last Christmas at the AAAS meeting in Washington will recall that Dr. Ezekiel reviewed the equipment hazards of microbiological origin which he had witnessed in Navy installations. He indicated at that time that this hazard should be evaluated in terms of our space-exploring vehicles. Fortunately, this is being done. Unfortunately, some of this evaluation work being done at the Engineers Research and Development Laboratories at Belvoir, and Army Ordnance Corps at Detroit Arsenal, indicates that materials susceptible to microbial degradation are still being incorporated into missiles and components, and failures from this cause can be anticipated. Before the microbiologist shoves off into outer space he should stop long enough to address the design engineers. I would suggest the title "Applied Microbiology in Design Engineering."

In discussing microbiological potentials and contemporary experimental design requirements here we can illustrate, by drawing from microbiological studies, the existent environmental parameters that have already been explored by the microbiologist. Long before Sputnik, studies on the biology of existing earth forms were producing data that can well serve us for comparative purposes now. Through the years some interesting data have evolved. Some instances of extreme tolerances have been listed for us by James (1955).

Lichens: (algae and fungi) survive as adults down to −80 C; at −30 C still photosynthesize slowly.
Rotifers: in antarctic lakes tolerate alternate freezing and thawing without damage, endure −78 C for hours. Desiccated, tolerate electric ovens at 170-200 C for 5 minutes, 151 C for 35 minutes, liquid helium (−272 C) nearly 8 hr.
Tardigrades: found all over world up to 10,000 ft; under dry conditions, ball up and float around for long periods.
Protozoans: tolerate temperatures down to −30 C; others are found in hot springs at 64 C.
Tumor cells: −253 C.
Normal Skin: −150 C.
Mice: live and breed at −21 C.
Arctic mammals: −50 C for long periods.
Bacteria, marine: pressures equal to 1000 atm.
Ascaris eggs: pressures equal to 800 atm, centrifugation for 1 hr at 400,000 atm.
Snails: indefinitely at an oxygen partial pressure of 2 mm.
Bacteria: sulfur, learned to live without oxygen 800 million years ago and have not yet found it necessary to convert.

To these instances we can add many additional parameters particularly from the field of industrial microbiology, using the organisms with which we are most familiar. Contemporary data on the effect of high vacuum on spores of *Aspergillus niger* and several organisms have been provided by our sumposium chairman, Dr. Prince. He, too, is able to report high tolerance values in *A. niger*. By using the excellent bibliography by Sparrow *et al*., (1958) we can isolate some representative radiation studies on *Aspergillus* and *Chaetomium* spp. The parameters revealed here can be of great use in space probing. Joan Munro Ford (1948) alone, and with Kirwan (1949), in her ultraviolet and x-ray irradiation of fungal spores has characterized mutation and lethal effects based on thousands of spore irradiations. These data from controlled laboratory conditions can provide a comparative base for irradiations made in outer space.

The work by Berk (1952, 1953) on irradiation of deteriorative fungus spores represents good quantitative data, and his review (1952) of the work in this field allied to his own strengthens the position of the microbiologist. The biochemical fundamentals of ionizing radiations on biological systems is adequately introduced by Kuzin (1956) and affords an excellent base for comparative radiobiochemistry. Buchwald and Weldon (1939) have established stimulative dosages for *A. niger*, and Weldon in 1938 and 1940 has catalogued effects of cathode radiation in a vacuum. Dickson (1932) reports the effects of x-radiations on ten fungi initially and later (1933), concentrating on *Chaetomium*, has quantitatively evaluated saltant production in relation to accessory factors such as UV light, heat, and substrate biochemistry. Kresling and Shtern (1936) also establish radiobiochemical criteria which may well be utilized in studying comparative radiation effects: terrestrial versus outer space exposures. Stapleton and Martin (1949) compare lethal and mutagenic effects of alpha particles, fast neutrons, gamma rays, and x-rays, thus contributing good physical biological values for comparative purposes. Additional studies (Swanson, 1952; Swanson *et al.*, 1948) concern themselves with mediation of radiation effects and interaction of radiation with other influences.

From this brief review just on the two genera *Aspergillus* and *Chaetomium* it can reasonably be expected that by mobilization of the data on the similar test organisms, for example those used in specifications testing, an excellent lattice of parameters, traits, response criteria can be made available and will make the microbiologist and his organisms a welcome asset on the astronautical team. The geophysicist and others will continue to map out the radiation zones and other environmental characteristics of space, but they await the biologist, for an evaluation of these regions, in terms of effects on living systems.

Contemporary experimental design requirements, then, should consider simulation of known environments. Dr. Prince, for example, uses a high-altitude environment chamber in studying low-pressure effects. Similar single and combined environments must be created or mobilized and where no data exist, experimental work conducted. Various atmospheres in simulation chambers can be produced and evaluated in terms of tolerances and adaptability of microorganisms. Many of these systems need not be very elaborate. Tikhov (1955) makes reference to the presence of microbial life on the barren and waterless soils of the Sahara. Whatever water was present there to support this activity was undetectable even with special instruments. How xerophilic or xerofacultative are our desert organisms? Doesn't this warrant some further laboratory studies? Some of our microbiologists at Randolph Field are doing this but apparently under conditions not quite as rigorous as the Sahara. The dry desert and other type environments may very well lend themselves to simulation.

Much of the needed knowledge can be mobilized from past studies; additional and new parameters that can be simulated (and let us not be deceived into thinking this is an easy matter) will govern new experimental designs on earth-bound laboratories. In designing microbiological experiments in space vehicles, miniaturization and small cubage and weight provisions should be kept constantly in mind. As more cubage becomes available, new phases can be introduced, providing the general rule of miniaturization and microminiaturization is kept constantly in mind. This requirement should not embarrass the microbiologist—his field lies in the microcosm. In satellite experiments telemetering is of prime importance. Biological experiments will have to express data in terms of what may be transmitted by radio, video, and voice signals.

In reviewing the material for this paper it was interesting to me, and I believe it will be interesting to you, to note in a historical sense and for other purposes, those documents that have come to hand indicating in an approximate way, how America is dealing with the biology-in-space problem. This, of course, is supplementary to space medicine, flight physiology, and similar areas characteristic of man in space.

1. March 26, 1958. "Introduction to Outer Space," a statement by the President and introduction to outer space. An explanatory statement prepared by the President's Science Advisory Committee.

2. May 14-17, 1958. Symposium "Possible Uses of Earth Satellites for Life Sciences Experiments," National Institute of Biological Sciences, National Academy of Sciences and National Science Foundation.
3. August 3, 1958. "National Academy of Science Establishes Space Science Board," "including both the physical and the life sciences."
4. December, 1958. "Microbiology in Outer Space Research," A symposium sponsored by the Society for Industrial Microbiology and its Washington Section, and the American Astronautical Society under the auspices of the AAAS and AIBS.
5. February 9, 1959. "Academy Research Council, Armed Forces Join in Study of Biological Effects of Space Flight, Announcement of Organization of Armed Forces—National Research Council Committee of Bio-Astronautics.
6. April 29, 1959. "Symposium on Problems in Space Exploration," 96th Annual Meeting of the National Academy of Sciences.
7. August 22, 1959. "NASA Names Advisers on Health Issues," The National Aeronautics and Space Administration [announces a] new bio-science advisory committee, on problems associated with manned space flight.

In conclusion, I offer "Enter the Space Doctor, Dr. James Absolum Whalebottom."

Dr. Whalebottom in reading this manuscript has graciously summarized for us in highly characteristic style—using free verse—his conclusions. This piece is entitled "Microbiologia Mutabilis," and follows.

Microbiologica Mutabilis

Herr Microbiologicus, I said to him one day
The end is here; no more we cope alone with these
Thy fungicides and vats and carbon links,
Thy enzymes one and all, thy rets and brews
Thy copper eights and quinolates, thy phenols
Yellow, white, and gray, and blue.
Thy cheeses, yes, and yeasts we need, and probably
Thy algae too, but all in all, the greatest
Need is this, that thou through thy menagerie,
Go far aloft, where apogee and perigee
Have much to tell, if we but can
Convince thee that thou must, at last,
Disjoint thy scope, and lo, in spite of many decades past
Which have conditioned thine objectives toward the bowels of earth,
Assembly redesign, so now she skyward pokes—
To tell us this through your comrades dear
Penicillum, Aspergillus, Rhizopus, Chaetomium,
Lord how these names of thine, strain my simple runic rhyme!
Yet these I say must go aloft for thee;
Harnessed then to our telemetry, in such a way
Through thy shrewd design that soon we little information lack

Of dangers, dreams, hypotheses, deep spread throughout the perigees.
Now fear not friend, they go not but to die
But rather go as the canary goes—down into the mine
To probe ahead the miner's foes, of dank and gas and goodness knows.
But tools these are thy microbes fine,
Which for the future we must design
To serve us well in space confined.
No whale, no cow, no man or dog
Are we yet prepared to send aloft
But well it seems, the vanguard might be
Your unpronounceably labeled coterie.
I've heard thee speak of duric forms
Of xerophiles and thermophiles and, if thou search a bit
Could not thou turn up too some cryophiles
Which, sent aloft could tell us things
By tape and otherwise, what cares we need to exercise
Before, we ourselves, cope with destiny and impending zero gravity?

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