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## *BIOLOGICAL CONTAMINATION OF THE MOON*

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The extensive deposition of both hard- and soft-landing packages on the lunar surface seems to be imminent. There has been recent concern that terrestrial microorganisms and organic matter, deposited with the packages, may obscure detection of, or interact with, possible organisms or organic matter indigenous to the Moon.[1, 2] Such biological contamination of the Moon would represent an unparalleled scientific disaster, eliminating promising approaches to such problems as the early history of the solar system, the chemical composition of matter in the remote past, the origin of life on Earth, and the possibility of extraterrestrial life. Because of the Moon's unique situation as a large unweathered body at an intermediate distance from the Sun, scientific opportunities lost on the Moon may not be recoupable elsewhere.

There are four possible kinds of lunar biological contamination, which we discuss under the following headings:

1. *Biomixy.*—The Moon may contain no indigenous living organisms, and may be incapable of supporting terrestrial organisms. Nevertheless, there may be relics of primitive indigenous organisms and deposited cosmobiota on or beneath the surface. Especially on a low-gravity, high-vacuum body such as the Moon, a vehicle impacting at or near escape velocity will distribute its contents over most of the lunar surface. Subsequent investigations might then be unable to distinguish among primitive indigenous organisms, cosmobiota, and terrestrial microbiological contamination.

2. *Sapromixy.*—The Moon may contain no indigenous living organisms, and may be incapable of supporting terrestrial organisms. But subsurface prebiological organic matter may exist which would be indistinguishable from deposited terrestrial organic matter, either biological or abiological in origin.

3. *Phagomixy.*—The Moon may contain no indigenous living organisms, but may be capable of supporting some terrestrial organisms. This would require subsurface organic matter, moisture, and heat sources. The possibility then exists that a deposited terrestrial microorganism, in the absence of biological competitors or predators, will multiply at a geometric rate limited only by the availability of

water and metabolites. Such a biological explosion might in a short time destroy large quantities of organic matter produced in the early history of the Moon.

4. *Ecomixy.*—The Moon may contain indigenous living organisms. There is then the possibility that deposited terrestrial microorganisms, by competition with or parasitism upon even one species of lunar organism, will disrupt the autochthonous ecology completely.

The purpose of the present paper is to evaluate these possibilities.[3]

*Survival of Terrestrial Microorganisms on the Moon.*—There seem to be three major hazards for survival of terrestrial life on the Moon—temperature, corpuscular radiation, and solar electromagnetic radiation—which we discuss in turn. The probable absence of oxygen, water, and other substances from the Moon's surface is not, of course, evidence against survival, particularly of dormant anaerobic microorganisms; but it does preclude their reproduction on the surface of the Moon.

Surface temperatures range from about $+100°C$ to about $-180°C$ during a lunar day and night.[4] However, just beneath the surface the temperatures are much more moderate; at a depth of less than half a meter, microwave thermal radiation data indicate a temperature variation between $0°$ and $-70°C$.[5] It is important to note that the thermostability of microorganisms is greatly improved in the dried state and *in vacuo*, as they would be on the Moon; even at temperatures approaching 100°C, survival of a significant fraction of the total number of vegetative bacterial cells and spores may be expected.[6] Still higher temperatures are required to inactivate desoxyribonucleic acid.[7] Especially since it is likely that many of the deposited microorganisms will find themselves lodged just beneath the surface, as we discuss below, the debilitating effects of the high lunar surface temperatures can be neglected.

The work of Biermann on the acceleration of comet tails indicates a flux of solar protons in the vicinity of the Moon of about $5 \times 10^{10}$ protons $cm^{-2}$ $sec^{-1}$, and a mean particle kinetic energy of about 1 KeV.[8] Charged particles will be excluded from regions where the magnetic energy density exceeds the particle kinetic energy density. For the surface of the Moon, the lunar magnetic field strength must exceed about $10^{-2}$ gauss for the solar proton flux to be deflected.

From lunar occultations of cosmic radio sources, it can be estimated that the lunar atmosphere contains less than $10^{14}$ molecules above each square centimeter of surface.[9] Ultraviolet absorption cross sections for all molecules likely to be in the lunar atmosphere are generally less than $10^{-16}$ $cm^2$ at all wavelengths. Hence the optical depth in the ultraviolet is less than $10^{-2}$, and there is no attenuation of incident solar ultraviolet radiation by the lunar atmosphere. For the solar proton wind, a 1 KeV proton has a range of about $10^{-2}$ cm-atm, or roughly $3 \times 10^{17}$ molecules $cm^{-2}$. Consequently, if the lunar magnetic field strength is less than about $10^{-2}$ gauss, the solar proton stream strikes the Moon's surface with negligible loss of energy due to its passage through the tenuous lunar atmosphere. A similar conclusion applies to the more energetic cosmic rays.

We now consider the effect of these radiations on terrestrial microorganisms deposited on the lunar surface. Expressions can be derived for the time, $t$, in seconds, in which a population of $N_0$ organisms, having a mean lethal dose $D$ for a given radiation, and characteristic diameter, $a$, in cm, is reduced to N organisms by radi-

ation of intensity I ergs $cm^{-2}$ $sec^{-1}$. For ionizing radiation with $D$ in reps, one finds[3] with an exponential survival law,

$$t = 214a\rho(D/I)\ [1 - e^{-(\mu/\rho)\rho a}]^{-1} \log_{10} (N_0/N),$$

where $\mu/\rho$ is the mass absorption coefficient of the organism in $cm^2$ $gm^{-1}$, and $\rho$ is its density in gm $cm^{-3}$. When the mean lethal dose is given directly in units of ergs $cm^{-2}$, as is the case for nonionizing radiation, the expression for $t$ is the same if the coefficient $214a\rho$ is replaced by 2.30.

The intensities to be used in this equation are those appropriate to the lunar surface for negligible atmosphere and magnetic field strength, and so are equally appropriate to interplanetary space in the vicinity of the Earth-Moon system. Consequently, the derived lifetimes are also those of an unprotected microorganism in free space, and so have a bearing on the panspermia or cosmobiota hypothesis.

For ionizing radiation, the high value of $D = 10^7$ rep was chosen.[3, 10] For ultraviolet radiation, a mean value $D = 10^7$ erg $cm^{-2}$ was selected for 2000 Å $\leq \lambda \leq$ 3000 Å; for $\lambda \leq$ 2000 Å, $D < 10^6$ erg $cm^{-2}$.[3, 10] It should be emphasized that these mean lethal doses are purposely high to allow for anaerobiosis and drying. The resulting lifetimes should be upper limits, except, perhaps, where $\rho/\mu \ll \rho a$ for ionizing radiation.

A 1 kg instrumented lunar package may easily contain $10^{10}$ microorganisms;[1] it is very unlikely that any packages for the immediate future will contain as many as $10^{20}$ microorganisms. With these limits on $N_0/N$, we compute that all microorganisms deposited and exposed to the Sun will be killed by ultraviolet radiation in a few hours. Similarly, fully illuminated microorganisms in cislunar space will also survive only a few hours. Hence the panspermia hypothesis is untenable for unprotected microorganisms of comparable radiosensitivity to terrestrial microorganisms. On the other hand, suppose some microorganisms are deposited in a lunar crevasse or other depression, always shielded from solar radiation. Then, radiation killing will be effected only by cosmic rays and by natural radioactivity. Microorganisms shielded from the Sun, but just beneath the lunar surface, need, at the assumed high $D$, more than a few hundred million years to be killed by cosmic radiation; organisms at greater depths will have even longer survival times. Similarly, cosmobiota imbedded in, for example, a meteorite would have lifetimes comparable to the age of the solar system, and under these circumstances the panspermia hypothesis remains tenable.

From eclipse temperature measurements, and polarimetric and radio observations, it is known that a dust covering exists on the Moon, but estimates of its depth range from millimeters to miles. However, Whipple[11] has called attention to the experimental fact that dust, irradiated in a vacuum, will congeal, forming a low-density, semiporous matrix. If the lunar surface material has a similar structure, microorganisms can then be lodged in the interstices of the matrix, in such positions as to be shielded from the Sun's rays at all angles of incidence. Thus we may anticipate the survival for very great periods of time of perhaps a few per cent of those dormant anaerobic microorganisms deposited at the lunar surface. A determination of the microstructure of the Moon's surface is of great importance to corroborate this conclusion.

*Survival of Deposited Nonliving Organic Matter on the Moon.*—The killing of an organism, of course, need not involve a great deal of chemical dissociation, and long after death occurs, in an anhydrous aseptic environment, many aspects of the organism's characteristic biochemical structure will be maintained. Potential activity of cellular DNA may outlast the survival of the cell itself. After long periods of continued irradiation, enough bonds would be broken to destroy most of the long-chain biological polymers. The problem is complicated by the existence of radiation-protection devices (catalase, cytochromes, sulfhydryl compounds, photoreactivation mechanisms) in most contemporary organisms.

Because of the Franck–Rabinowitch cage effect, the collection of dissociated molecules arising from the original organism would tend to remain in close physical contact. Ionizing radiation is very much more efficient than nonionizing radiation in depolymerizing and dissociating organic molecules. Breaking of all hydrogen molecular bonds and charring occurs at about $10^{10}$ rep.[4] Charring by the solar proton wind of all but $10^{-15}$ of the exposed molecular aggregates occurs in from months to years, depending on the size of the dissociated organism. If, however, the lunar surface magnetic field exceeds $10^{-2}$ gauss and the proton wind does not penetrate to the surface, it may take as long as several hundred thousand years for charring to be induced by soft solar x-rays in the 50 Å region. Thus the value of the lunar magnetic field strength has great relevance for the question of possible biomictic and sapromictic contamination of the Moon. Preliminary data from Lunik II indicate a surface field of about $3 \times 10^{-4}$ gauss;[12] in this case, solar protons will effect charring in the shorter time scale.

As dissociation advances, lunar temperature effects would become more important, small molecules being readily dissociated near 100°C. For example, the most thermostable amino acid, alanine, has a thermostability half-life at 100°C of approximately $10^3$ years,[13] with most other amino acids having half-lives not less than ten years. Molecules shielded from radiative dissociation would be relatively unaffected by lunar temperatures and, if lodged beneath a few centimeters of insulating lunar surface material, would have lifetimes determined by the cosmic ray flux and natural radioactivity.

*Conclusion.*—We are now in a position to evaluate the four possible circumstances of lunar biological contamination described in the beginning of this paper. Assume that a $10^4$ kg vehicle (the order of magnitude of existing unfueled final stage carrier rocket masses) with a microorganism population of $10^{10}$ per kg impacts the Moon at escape velocity. If half the impact energy is diverted to particle kinetic energy of the explosion products, about sixteen per cent of the microorganisms will be moving in an upward direction with velocity between the circular and the escape velocities after impact. Impact forces and temperatures are non-lethal because of the short deceleration time-scale ($\sim 10^{-3}$ secs).[3] Thus, about $1.6 \times 10^{13}$ microorganisms will be deposited approximately uniformly over the lunar surface, the mean surface density being about 0.4 microorganisms per square meter. Near the impact area, the surface density of microorganisms will be considerably greater. We have calculated that all but the small fraction of deposited microorganisms which is shielded from solar illumination will be killed by ultraviolet radiation in hours. Therefore the mean surface density of viable microorganisms deposited by such a vehicle should be less than 0.01 $m^{-2}$.

This surface density of viable microorganisms is well below that detectable by existing biological techniques, such as plating; Lederberg[14] believes that existing techniques can be immediately extended to detect one microorganism $m^{-2}$, but considerable further refinement would be required to detect $10^{-2}$ $m^{-2}$ where subsurface sample-gathering is also required. Cosmobiota and remnants of indigenous lunar organisms, if such exist, would be sequestered almost exclusively at much greater depths below the surface than would deposited terrestrial microorganisms. We conclude that the probability of biomictic contamination of the Moon is very low.

Since a typical bacterium has a mass of roughly $10^{-12}$ gm, the amount of organic matter deposited as microorganisms by impacting vehicles is completely negligible compared with the amount of indigenous organic matter which has probably survived from the early history of the Moon.[15] A similar conclusion follows for organic matter arising from vehicle structural elements. We conclude that the probability of sapromictic contamination is negligible.

An investigation of the production of organic molecules in the primitive lunar atmosphere[15] has indicated that as much as 10 gm $cm^{-2}$ of organic matter may be buried beneath the present surface of the Moon at a depth crudely estimated at a few tens of meters. Subsurface temperatures are known to avoid the extremes of lunar surface temperatures,[5] and a mechanism has been proposed whereby biologically optimum temperatures may be provided by radioactive heating, and localized at some depth by a hydrostatic pressure-induced increase in the thermal conductivity of the dust.[16] At such temperatures and depths, moisture should be expected, arising from meteoritic and organic bound water. A viable terrestrial microorganism introduced into such an environment might reproduce very rapidly. The extent of phagomictic contamination would depend on the degree to which concentrations of organic matter are in mutual contact under the lunar surface, on possible self-limitation of the reproduction rate by accumulation of catabolites, and, of course, on the presence of the specific growth requirements for individual varieties of microorganisms. It is very improbable that a given organism deposited near the surface would find its way to a depth of tens of meters, but when $10^{14}$ microorganisms are deposited, the situation is very different. Although the presence of appropriate temperatures, moisture and organic matter for terrestrial microbiological multiplication remains to be demonstrated rigorously, at the present writing the likelihood of phagomictic contamination of the Moon is not negligible.

Because of its great potential importance, the admittedly very speculative possibility must be raised that life arose on the Moon before the secondary lunar atmosphere was lost to space. Conditions on the Moon $5 \times 10^9$ years ago were probably not very different from conditions on the Earth $5 \times 10^9$ years ago. Recent thinking on the origin of life on this planet is increasingly inclined toward a very rapid origin of the first self-reproducing system. If a similar event also occurred on the Moon, natural selection may be expected to have kept pace with the increasingly more severe lunar environment, at least for some period of time. If subsurface conditions exist similar to those described in the preceding paragraph, then the possibility of an extant lunar parabiology must not be dismissed in as cavalier a manner as it has been in the past. Even if indigenous lunar organisms exist, the occurrence of ecomictic contamination will depend on such matters as the stereo-

chemistry and detailed ecology of the autochthons; but in our present ignorance the possibility of ecomixy cannot be excluded. Of all the kinds of biological contamination, this would represent the greatest loss.

To date, two man-made objects have impacted the Moon, the instrument package and the 7,700 kg carrier rocket of Lunik II. According to reports from the Soviet Union, both were sterilized.[17] It is recommended that all future lunar probes be scrupulously decontaminated.

*Summary.*—The probability of survival of a terrestrial microorganism, accidentally deposited on the Moon by an impacting lunar probe, has been computed. A population of the least radiosensitive dormant anaerobic microorganisms, if exposed to solar ultraviolet radiation, would be completely killed in hours. The resulting organic dissociation products would remain intact for much longer periods of time—0.1 to 10 years if the lunar surface magnetic field strength is much less than $10^{-2}$ gauss (so incident solar protons are magnetically deflected), and $10^4$ to $10^5$ years if the field strength exceeds $10^{-2}$ gauss. Organisms shielded from solar illumination, perhaps in congealed dust matrix interstices, might survive cosmic radiation for $10^9$ years or more; lunar subsurface temperatures are too low to impede survival. The possible circumstances of lunar biological contamination are then discussed. It is concluded that the probability is very low that deposited terrestrial microorganisms and organic matter will be confused with indigenous lunar organisms or organic matter; but that the explosive reproduction of terrestrial microorganisms in indigenous lunar organic matter, and the disruption of the ecologies of hypothetical lunar organisms are remote but nonnegligible possibilities.

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## *INTRAMOLECULAR MODELS DEPICTING THE KINETIC IMPORTANCE OF "FIT" IN ENZYMATIC CATALYSIS**

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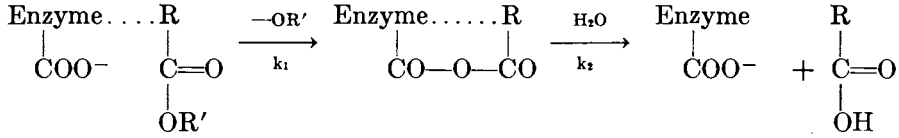
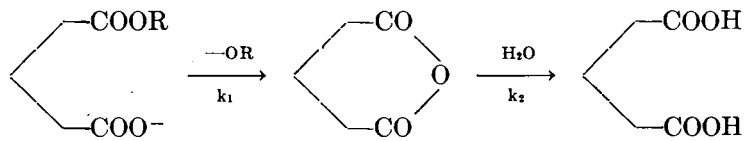
This communication describes a few of the intramolecular models which have been studied in this laboratory in order to ascertain the kinetic effect of steric compression on an intramolecular catalysis of the hydrolysis of an ester bond. In enzyme catalysis the bond making and breaking processes occur within a complex of enzyme and substrate (ES) presumably *via* the participation of particular functional groups at the "active site." For the case of certain esteratic enzymes an intracomplex nucleophilic displacement of —OR′ from R − CO − OR′ to give an acyl enzyme as an intermediate is involved. Thus, for the enzyme ficin, a carboxyl anion has been suggested to be the intracomplex participant.[1] The reaction may be pictured schematically as (I):




ES Acyl Enzyme

(I)

The similarity between (I) and an intramolecular nucleophilic catalysis of ester hydrolysis (as II) has been pointed out:[2–6]




(II)

When the intramolecular model (II) is compared with its bimolecular counterpart (i.e., the catalysis of ester hydrolysis by acetate anion), it is found that II is much more efficient, due to a decrease in translational entropy in the formation of the transition state.[2,6] By analogy, the high efficiency of enzymic reactions must be due, at least in part, to a similar phenomenon, in which the ES complex brings the reacting groups together. It is commonly accepted that the enzyme must also align the reacting groups in a particularly favorable steric conformation to allow the