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A CO-SURVIVAL SYSTEM THEORY OF EXOBIOLOGICAL PROPAGATION OF TERRESTRIAL MICROORGANISMS

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INTRODUCTION

Though 'Exobiology' of the definition of Joshua Lederberg refers to the study of extra-terrestrial life, it would be more general to define it as the study of life in extra terrestrial environments; the former may denote only life originating and evolving in extra-terrestrial environments whereas the latter may in addition include terrestrial life imbedded in extra terrestrial environments. In the frame-work of this broader definition, it is interesting to study the exobiological survivability of genetically engineered terrestrial microorganisms for exhibiting maximal compatibility in the new environ.

Exobiological investigations carried out so far invariably confine themselves to the origin, evolution and survivability of individual species of organisms. In the theory advocated in the present paper, it is argued that for exobiological survivability of terrestrial organisms, the concept of 'complimentary co-survivability' of a set of heterogeneously associated organisms is more promising than attempting to assure higher survivability for individual species of organisms. Co-survivability of two or more terrestrial organisms in an alien environment is facilitated by mutually dependent symbiosis or forging heterogeneous association of chimeral pairs.

A straight-forward example is the possibility of co-survival of Blue-green algae and Lichen on Mars. An ideal Martian organism would not require oxygen and grow fast like the Blue-green algae while at the same time have extreme resistance to ultraviolet radiation like Lichen. However, the Blue-green algae is not resistant to ultraviolet radiation whereas Lichen needs oxygen. What is advocated here is not the study of exobiological survivability of the Lichen alone or Blue-green algae alone, but the study of co-survival of the pair together. In the process of assuring such a co-survival, the two strains are sought to be genetically engineered for a symbiotic relationship that endures as a system, on the extraterrestrial environment. Such genetically engineered co-survival can be explored not only between plant microorganisms but also between plant organisms and microbes.

In what follows a comparison is made between the terrestrial and Martian environments with reference to certain life supporting indexes. Subsequently, reasons are advanced for the effectiveness of the 'systemic' genetic engineering of a set of microorganisms for exobiological co-survival. Successful parallel illustrations of experiments for heterogeneous associations of microorganisms are cited. Lastly, a systemic generalization of the theory is attempted followed in an appendix by an advocacy of a system theoretic perspective of Exobiology and Planetary Environment morphosis.

THE MARTIAN ENVIRONMENT

The more important factors that should be considered for studying the survival and growth of organisms are temperature, ultra-violet radiation, water availability, oxygen availability and soil characteristics. On Mars, the lack of atmospheric oxygen would allow growth of only those organisms which do not depend on oxygen like the anaerobes. Mean flux of ultra-violet radiation in the range of 2000 Å - 3000 Å impinging on Mars would decrease their mean survival time to a few minutes at most. The Martian surface temperature range would allow seasonal growth for only 7 hours a day at the equator and mid latitudes. Water in liquid state can exist only in restricted areas. The extent of availability of nitrogen and phosphorous is yet to be accurately assessed. The kinds of terrestrial organisms able to survive in the present Martian environment are quite limited and the growth of even these forms would be quite restricted in vigour and extent, e.g. certain anaerobic cold-adapted terrestrial bacteria.

For higher life forms, an oxygen-containing environment is essential. There are strong indications that the present mass of terrestrial atmospheric oxygen was biologically produced by algal photosynthesis among others. It fixes carbon dioxide and water into a carbohydrate by chlorophyl containing organisms in the presence of visible light. As a byproduct of photosynthesis, oxygen, derived from water, is produced. In principle all the ingredients of photosynthesis - light, CO_2, H_2O - are available on Mars. The question is which of the available terrestrial photosynthetic organism might be best fitted to survive, grow and generate oxygen on Mars. The closest terrestrial environment to Mars are the dry valleys of Antarctica in which are found four major photosynthetic groups with characteristics as shown in the Table below:

TABLE 1

Organism	Requires Oxygen	Extreme Resistence to Ultra-violet radiation	Extreme Resistence to drying	Growth rate	Growth habitat
Green Algae	Yes	No	No	Fast (hr)	Soil (surface & subsurface)
Blue-green algae	No	No	Yes	Fast (hr)	Water (surface & subsurfaces)
Lichen	Yes	Yes	Yes	Very slow (yr)	Surface
Moss	Yes	No	No	Slow (wk)	Moist surfaces
The Ideal Martian organism	No	Yes	Yes	Very fast(min)	Soil (surface & subsurface) water

It can be seen that no terrestrial photosynthetic organism so far examined can match the charecteristic of an ideal Martian organism. However, the Blu-green Algae and the Lichen come the closest, the former being susceptible only to ultra-violet radiation and the latter only in its requirement of oxygen.

The cyanophytes (blue-green algae) have been extensively studied by Brock(1,2), James(3), Cameron(4), Lund(5), Godward(6) and Fogg(7) some of whose results are summarised in Table 2.

TABLE 2

	Lower limit	Upper limit
Temperature (survival)	4 K (Cameron)	373K (dry soil),(Lund(5)) 363K (wet soil),(Lund(5))
Phototrophic metabolism	243K (Lichens) (James)	346L (Brock(2))
Growth : pH	pH 4.0	pH 11.0
: Oxidation potential	-190 mV at pH 4.0	+ 700 mV at pH 4.0
: Salinity	0.001%	30.0% (Brock(1))
Resistence to Ultra-violet radiation	--	$> 10^6$ rads of γ-irradiation (Godward(6))

Cyanophytes are phototrophs which utilize carbon dioxide almost exclusively as their source of cellular carbon.

The Lichens has been extensively studied by Johns(8), Kappen(9), Rao and LeBlank(10), Lange(11), Buttner(12), Siegel and Daly(13), Blum(14), Bliss and Handley(15) among others. These investigations have given results that determine the extreme limits of survival and growth of lichens with reference to growth rate, lack of water, temperature, ultra-violet radiation and photosynthetic production of oxygen.

Lichens have shown favourable response to very low temperatures - the tolerence increasing with drying. Some species after cooling to 77K showed normal CO_2 uptake immediately after rewetting at 283K according to Kappen. Lange recorded photosynthesis at 268K proceeding at 50 percent of the max. rate.

The growth rate is extremely slow. However, tolerance to ultra-violet radiation is extremely high somewhat mitigating the effect of slower growth rate. Siegel and Daly report that the continuous exposure of at least one species to radiation of over 2500 Å at 2.5×10^6 ergs/cm^2/min for 24 hours resulted in no observable effect upon respiration. As of now the exact mechanism of this resistance is not known, though some bacteria exhibit molecular repair mechanisms for damage by ultra-violet radiation.

Lichens can function by absorbing water vapour; although Blum(14) has shown that a major portion of water is probably only gained in this fashion from atmospheres in which relative humidity is very high. It is known that blue-green algae phycobionts can hold large amounts of water in their thick gelatinous sheeths.

Studies on photosynthetic production in lichens by Reid gave a range for the maximum net photosynthetic rate as 0.34 - 3.2 mg CO_2/50 cm^2 surface area covered/hour. Lange found that net photosynthesis is appreciably affected by moisture content. Kershaw and Rouse(16) showed that lichens assume a state of suspended animation below a critical moisture content. Lichens can reproduce by means of spores which are resistant to environmental extremes and are disseminated easily.

An important lacuna in the study of lichens are the experiments on the adaptation of lichens to environmental extremes concerning the ability of these organisms to survive and grow under anaerobic conditions. Another lacuna is that the mechanisms which regulate and control growth in lichens are unknown.

THE CONCEPT OF EXOBIOLOGICAL CO-SURVIVAL

Investigations concerning the habitability of Mars, to the best of the knowledge of the author, have been confined to the induction into the Martian environment of species of individual microorganisms. Some of the recent research reported like that from NASA(17) regarding the habitability of Mars is also of this nature.

No studies appear to have been made concerning the distinct possibility of the increased co-survival of heterogeneous associations including symbiotic sets of microorganisms.

If the Martian Environment is not subjected to a climatic metamorphosis, the author conjectures that the best terrestrial candidate for photosynthetic biota of Mars is a heterogenous association of Cyanophyte and lichen through genetic modifications for optimal complementarity in facing the rigours of the Martian environment. It can be seen from the previous section that cyanophyte suffers from ultra-violet radiation damage which Lichen does not whereas lichen requires oxygen which cyanophyte does not.

One idealized physical model we can suggest for such a symbiotic co-survival on Mars is the growth of lichens on the surface with the cyanophyte phycobionts underneath the surface with the spores or cortical hairs of the former and the gelatinous sheaths of the latter in a cooperative orientation. The lichens would protect the cyanophyte from ultraviolet radiation while the cyanophytes can provide oxygen to the lichens. Symbiotic relationships between the two are not unknown however. It has been demonstrated that air-dried thalli in a latent state can become reactivated and reach photosynthetic rates close to optimal. It appears that the algal member of the symbiosis derives protection from its fungal partner, which provides a buffer to rapid changes in humidity. In the proposed structure, cooperation should be such as to assure a net photosynthetic release of oxygen into the atmosphere. This symbiosis would require extensive genetic engineering on both the cyanophyte and the lichen.

To understand the methods for forging such a heterogeneous association, three recent experimental techniques developed in contexts different from the present one are outlined below. It is cautioned here that their description is only meant to give the broad concepts and general directions for approaching the problem of heterogeneous association between cyanophyte and lichen and is not meant as a direct suggestion for an experiment.

Associations between cells of different genotypes to be constructed in vitro, is made possible by tissue culture methodology. In this system, the spectrum of such associations is limited only by the growth requirements of the cultured cells and not by the complex developmental and morphogenetic processes which are naturally restrictive.

Many plants are chimeral associations of genetically dissimilar cells. An experimental system may be designed by the use of calluses grown in culture in which genetically distinct cells are able to proliferate contiguously and form a chimeral association. There is a distinct possibility that the basic concept behind chimeral culture, so far used in relation to higher plants, can be applied to the association of lower organisms like the lichens and the cyanophyte, using genetic engineering.

In addition to constructing associations between plant cells of different genotypes, in vitro culture methods may be used to bring together cells of more distint origins. A case in which this has been accomplished in nature is the symbiotic relationship between many plant species and bacteria capable of reducing atmospheric nitrogen. Artificially forging such associations with lower organisms may again require the application of genetic engineering.

Although one cannot confidently predict all applications of the ability to genetically engineered micro-organisms, some applications are readily apparent and others, are more speculative. Certainly chimeric micro-organisms could be developed in appropriately designed symbiotic associations. The cloning of specific DNA sequences in micro-organisms could also be used to genetically manipulate species to associate in complementary symbiotic relationships to increase survivability and growth in environments beyond the range of endurance by the individual partners. The discoveries leading to the ability to couple genes from organisms that do not normally exchange genetic inofrmation and to introduce them into micro-organisms where the foreign genes can be cloned and ultimately expressed has profound significance to the forging of symbiotic microorganisms for survival on other planets.

THE CONCEPT OF 'SYSTEMIC' GENETIC ENGINEERING

The above arguments in favour of terrestrial symbiotic pairs of photosynthetic micro-organisms genetically engineered for maximal co-survival on other planets leads us to a further generalization. A set of dependences between inorganic substances like carbon, nitrogen and sulfur, organic substances like organic macro-molecules, small organic molecules like RCOOH, RCH_2OH etc., micro and macro nutrients, as well as micro-organisms can be designed for desired regenerative cyclic mode of the biota so as to maximize the co-survival and growth of the entire system.

Based on certain well known dependences suggested for the biological recycling of carbon, nitrogen and sulfur in a primitive environment, a graph representation of inorganic, organic and biotic dependencies is given in Fig.1 around the Lichen-Cyanophyte symbiotic system proposed in the previous section for the Martian environment.

It can be seen that Fig.1 represents a complex Feedback System whose analysis would perforce require the methodology of General Systems Theory of the type developed by the author (Seshagiri(21)). For a more realistic characterization of the interactions, Fig.1 will be much more complex than the simplified version given here as an illustration.

Genetic modification of each one of the micro-organism - the Lichen-Cyanophyte association, Clostridium bacillus, Thiobacillus denitrificans, Desulfovibrio,etc. - would necessitate mutually compatible structural changes with the maximization of a common objective function, viz., co-survivability and system growth.

As the entire process of genetic manipulation so conceived is systemic, it may be appropriate to call this field of study as 'Systemic Genetic Engineering'.

A SYSTEM THEORY OF LICHEN-CYANOPHYTE SYMBIOTIC ASSOCIATION

The mathematical model developed by Gates(18,19) and Nobel(20) for studying energy exchanges and photosynthetic production in higher terrestrial organisms is extended here to evolve a system theory of

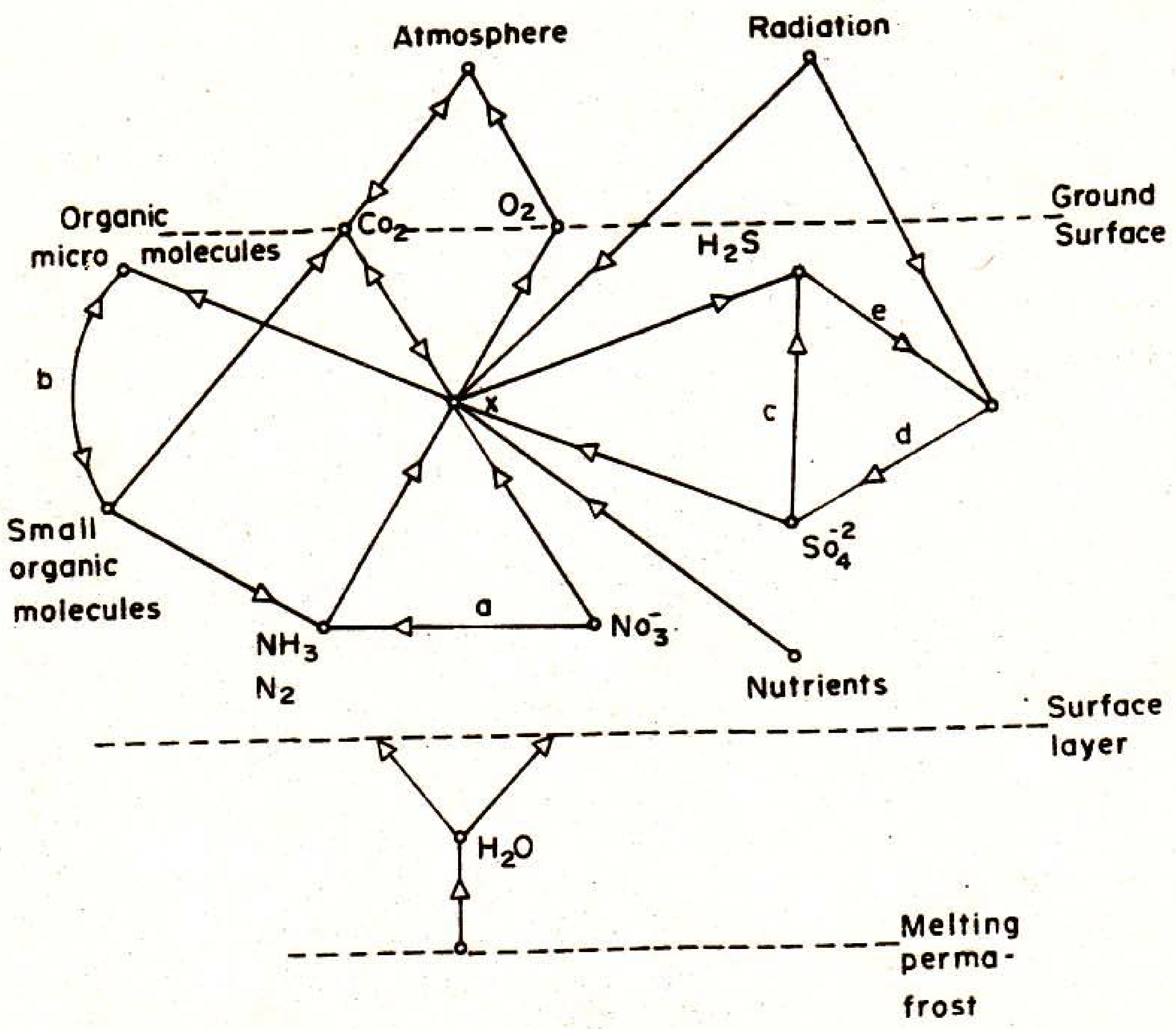

Fig.1. A System of interactive inorganic substances, organic substances and microorganisms with feedback mechanisms.

x : Lichen-cyanophyte Symbiotic Association

a, d & e : Photosynthetic sulfur bacteria, e.g. Thiobacillus Denitrificans

b : Anaerobic and facultative decomposers like clostridium bacillus

c : Desulfovibrio or other similar

of the Lichen-Cyanophyte symbiotic association involving feedback mechanisms.

The symbiotic, characteristics behind the model is as defined in the concept of exobiological co-survival. Temperature of the Lichen-Cyanophyte symbiot in the Martian environment is controlled by solar radiation, convective transport, conductive transport and evaporative cooling. Solar radiation is coupled to the symbiot by its absorptivity α_l for the lichen and α_c for the Cyanophyte. Let β be the symbiotic ultraviolet shielding factor of the cyanophyte by the lichen, then, the radiation absorbed by the symbiot will be

$$R = (\alpha_{\ell} + \alpha_{c}\beta)\, R_s + R_p \qquad (1)$$

where R_s and R_p are respectively the incident solar radiation and absorbed planetary radiation.

For the lichen, the upper surface molecular conduction transports heat down the temperature gradient across a laminar boundary layer of thickness d cm. which is related to a mean wind speed near the surface V cm/sec and the mean lichen dimension L. If the cyanophyte is resident d_o cm, below the bottom surface of the lichen as a colony with effective dimension L_o, we obtain,

$$d = 0.4 \left\{ \frac{L + L_c \cdot \frac{d - d_o}{d}}{V} \right\}^{\frac{1}{2}} \qquad (2)$$

If the near surface air is below that of the symbiot, then the heat is conducted from the latter through the boundary layer and diffused by eddy transport and conversely. The convective - conductive energy transport E_{cc} is given by,

$$E_{cc} = \frac{K_{CO_2}}{d} \left[\frac{T_l + \frac{(d - d_o)(m_c\, T_c)}{d\, m_1}}{\left(1 + \frac{m_c}{m_1}\right)} - T_{CO_2} \right] \qquad (3)$$

where, K_{CO_2} is the thermal conductivity of CO_2 of the Martian atmosphere, $\frac{m_c}{m_1}$ is the mean ratio of mass of the lichen and that of the colony of cyanophyte underneath, T_1 is the temperature (K) of the lichen and T_c is that of the cyanophyte.

Since lichen is in contact with the surface, heat transport by conduction will occur giving similarly

$$E_c = \frac{K}{d_1}\left(T_1 - T_g\right) \qquad (4)$$

where d_1 is the characteristic half thickness of the lichen, K is the corresponding mean thermal conductivity and T_g is the surface temperature.

The symbiot expends energy through thermal emission, at different rates for lichen and cyanophyte and by transmission at different rates. For every gram of water evaporated from lichen and cyanophyte, h_1 and h_c calories of heat are required per unit mass. With the lichen absorbing a part of the heat given out by the colony of cyanophyte below, the total loss of heat energy by the symbiot is given by,

$$E = m_1 h_1 \frac{\delta_\ell}{r_1 + r_a} + m_c h_c \frac{\delta_c}{r_c + r_e} \quad \frac{\tan^{-1} \frac{L}{2d_o}}{1 - \frac{}{2}} \qquad (5)$$

where, δ_ℓ and δ_c are the respective saturation water vapour density in gm/cm^3 at the temperature of the respective organism, r_1 and r_c are the internal resistance offered by the respective organisms to water loss, r_a is the air resistance across the laminar boundary and r_s that of the subsurface environment of the cyanophyte colony. Further, the black body radiation from the organisms is given by,

$$E_b = \epsilon \sigma T^4 \qquad (6)$$

where σ is the Stephen-Boltzmann constant and ϵ is the emissivity. The energy balance equation of the symbiot can now be given as,

$$R = E_{cc}(T) + E_c(T) + E_b(T) + E\ T) \qquad (7)$$

The complete budget of energy exchange between the symbiot and the environment is given by,

$$R = \epsilon \sigma T^4 + k \left[\frac{V}{L + L_c \frac{d-d_o}{d}} \right]^{\frac{1}{2}} \left[\frac{T_1 + \frac{(d-d_o)(m_c T_c)}{dm_1}}{1 + \frac{m_c}{m_1}} - T_{co_2} \right]$$

$$+ \frac{K}{d_1} (T_1 - T_g) + m_1 h_1 \frac{\delta_l - H(\delta_l')}{+}$$

$$+ \left[m_c h_c \left\{ \frac{\delta_c - H(\delta_c')}{r_c + r_a} \right\} \left\{ 1 - \frac{\tan^{-1} \frac{L}{2d_o}}{2\pi} \right\} \right] \qquad (8)$$

where H is the relative humidity of the atmosphere surrounding the lichen, δ_l and δ_l' denote the saturation vapour density at the lichen temperature and air temperature respectively (similarly H_c, δ_c and δ_c').

The quantification of the ability for photosynthesis of the symbiot is a much more involved task. Here we may use a first approximation approach suggested by Gates(19) and extend it to the case of the symbiot. The symbiot's 'effective' photosynthetic rate can be expressed as a function of several major limiting variables, including temperature T, photic intensity I, CO_2 concentration C and water vapour concentration of the organism S. Following Gates(19) we may consider I, C and S as limiting 'substrates' to enzymatically controlled reactions. Though for such symbiotic systems the appropriate kinetics is the more generalized one developed by Seshagiri(21), we may proceed as a first approximation, according to the Michaelis-Menton Kinetics in which the individual influence of each element is represented by a kinetic term of the form, $P = P_m/(1+K/X)$, where P is the photosynthetic rate, P_m is the maximum possible photosynthetic rate, K is the Michaelis constant for the reaction and X is either I, C or S. If the three kinetic terms corresponding to I, C and S are worked out, the Gates hypothesis suggests the product of the three terms as the photosynthetic rate. In the case of the symbiot it would be necessary to add the individual photosynthetic rates with due corrections for the partial shielding effect of the lichen on the receipt of radiation by the cyanophyte as well as the loss of oxygen through the symbiotic transfer of oxygen molecules to the lichen.

CONCLUSION

A co-survival theory of exobiological propagation of symbiotic or other heterogeneous associations of terrestrial micro-organisms is proposed in this paper with specific reference to the Lichen-Cyanophyte genetically engineered symbiosis for survival and growth on Mars. The co-survival theory has been developed by the author to more generality than what is described here, encompassing not only the survival and growth of heterogeneous associations of terrestrial micro-organisms but also the possibility of symbiotic and heterogenous associations as the basis of primitive evolution of life. The latter theory is not described here as the objective of this paper is merely to illustrate the existence of a large number of feedback mechanisms which call for a systematic application of the concepts and methods of systems theory as further elaborated in the Appendix.

APPENDIX

NEED FOR A SYSTEM THEORETIC PERSPECTIVE IN EXOBIOLOGY AND PLANETARY ECOSYNTHESIS

The main paper develops a new theory of exobiological survivability and growth of heterogeneous associations of terrestrial microorganisms. In the process it is brought out that there are numerous feedback mechanisms underlying the total system. This would be the case for most theories which try to grapple with the complexities of the dependences in exobioloty and planetary ecosynthesis. To give a perspective spectrum of situations where feedback system theory can be of much use to understand the problems better, a set of representative research problems are posed below with specific reference to the three areas: (a) The co-survival system theory, (b) Systemic Genetic Engineering and (c) Planetary climate morphosis.

a) The Co-survival Theory:

1. The oxygen cycle in a primitive environment is a dynamic (evolving with time) feedback system which can be analysed using the methods of Dynamic Programming developed by Richard Bellman.
2. Developing a more detailed combinatorial graph than what is given in Fig.1. by including all the intermediate steps in the interactions and using this as a basis for computer-aided simulation.
3. Characterizing in detail the basic Lichen-Cyanophyte symbiotic relationship of Section-3 by developing combinatorial graphs for Lichen and Cyanophyte separately and identifying the required symbiotic interactions between these two graphs in the background of tha graph representing the Martian environment. Define an objective function for the symbiotic relationship that is required to be maximized. Identify the constraints over this maximization. Reduce the problem

into one of non-linear programming and use known methods like the Box-method.

4. Investigate the structural mechanisms which regulate and control growth in lichens and cyanophytes. Characterize this structure as a control system (substantial work has been reported in the development of control theoretic methods for molecular biological structures like Regulatory enzymes etc.).

b) Systemic Genetic Engineering:

1. Develop a computer-aided simulation approach by modelling the repair mechanism by moving an appropriate set of genes from donor organisms and inserting them into recipient organisms. Try different genetic engineering methods in the simulation like the ones described in the main paper and compare their effectiveness simulationally. Simulate the Lichen or Cyanophyte and study the implications of changing their genetic characteristics for maximal concordance with the characteristics of the ideal Martian organism. Try simulations of genetic engineering techniques whose theoretical feasibility has been established and has been or has not yet been practically realized.

2. Genetic modification of each one of the microorganism - the Lichen - Cyanophyte association, clostridium bacillus, Thiobacillus Denitrificans, Desulfovibrio etc - would necessitate mutually compatible structural changes with the maximization of a common objective function, viz., Co-survivability and System growth. As there are numerous constraints imposed by the environment on the maximization, any quantitative characterization of this problem would call for the methods of Non-linear Programming.

c) Planetary Environmental Morphosis:

1. Develop a global environment model for Mars from the

data realized from Viking missions and study the sensitivity of each environmental parameter on such biologically important environmental factors as the increase of planetary temperature. The environmental model is necessarily a feedback system of high complexity. A general planetary feedback system model has been developed by the author (Seshagiri(22-24)) which can be specialized to the Martian environment.

2. Increasing the amount of carbon dioxide or water vapour in the atmosphere will increase the surface temperature through a 'greenhouse effect'. There are other such feedback couplings which can be studied for the Martian atmosphere with reference to their effect on the biotic survival and growth, e.g.,

Warmer temperature → more water vapour in the atm.
more greenhouse effect

More snow cover → more reflection of insolation
Colder surface temp.

3. Investigate by simulation on a computer the model for a 'run away' greenhouse and study its sensitivity with respect to different biologically important environmental factors.

4. The present climate regime on Mars represents one of the stable regimes. Study the stability of the climatic system as a whole and identify the weak spots in the stability zones. For example, as the atmospheric mass increases, the amount of heat transported from low to high latitudes increases. The mean global temperature does not change but polar temperatures increase while equatorial temperatures decrease. This is because of advective heating. It is conjectured that there exists another stable regime at a polar surface winter temperature of 190 K and at a surface air pressure of about 1 bar. This regime can be brought about by

increasing the effective solar flux over the polar cap by 20 percent which in turn is made possible by reducing the polar cap albedo from the present level of 0.77 to 0.73. Simulate this second stable regime and study its effect on the survivability and growth of different terrestrial organisms.

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