SIGNS OF LIFE CRITERION-SYSTEM OF EXOBIOLOGY

By Prof. JOSHUA LEDERBERG

Department of Genetics, Stanford University School of Medicine, Palo Alto, California

THE imminence of interplanetary traffic calls for systematic criticism of the theoretical basis and operational methods of 'exobiology', the initial search for and continual investigation of the life it might encounter. Very little science is totally irrelevant to it, and the policy-maker must face a riot of potential approaches to space flight experiments. By every standard, this is an epochal enterprise: a unique event in the history of the solar system and of the human species, and the focus of an enormous dedication of cost and effort. It requires a new perspective in experimental policy. The broader interfaces of eso-(Earth's own) biology, by contrast, permit its fruitful growth within the context of methodologies and instruments that can lag behind broadly established needs and imaginative possibilities. A system for orderly appraisal of the problem would rationalize the partition of labour, our only means of managing a complex problem.

Mars is our prior target. Our premissed information is only: (1) terrestrial observation: esobiochemistry; (2) the implications of Mars being a 'terrestrial planet'; (3) a very small body of definite observational results. The choice of our first experiments must take account of a wide range of theoretical possibilities not yet narrowed by the experimental process. Over this broad reach, logical necessity rarely coincides with logical sufficiency. The most compelling inferences might stem from the least likely event. Our speculation will be narrowed and policy simplified by tangible information about any aspect of Mars, especially if it encompasses the variability of the planet's features in space and time.

Evolutionary Stages and the Definition of 'Life'

Fundamental to all biological theory, eso- or exo-, is the evolutionary principle. As is now commonplace, we recognize the following stages in the Earth's history:

(A) Chemogeny (organic chemistry). The production of complex organic compounds by a variety of non-replicative mechanisms—the primitive cosmic aggregation, photochemistry of isolated atmospheres, thermal and spontaneous reactions of inorganically catalysed, previously formed reagents. (B) Biogeny (biology). The replication of a specifically ordered polymer, DNA being the terrestrial example, which specifies the sequence of its own replicas, and of the working materials, like RNA and proteins, from which cells and organisms are fashioned. Random experiments of error in replication, and natural selection of their developmental consequences, result in the panoply of terrestrial life.

(C) Cognogeny (history). The evolution of the mechanisms of perception, computation, and symbolic expression and interpersonal communication, whereby tradition can accumulate, culture unfold.

Mars must be supposed to have had an initial history similar to Earth. To ask whether Mars has life is to ask how far has its chemogeny gone; how like and how unlike the Earth's; has its evolution passed through the biogenic (ordered macromolecular) stage? Then through the cognogenic?

In evaluating a complex set of possibilities it is helpful to find classifying parameters which can be scanned systematically, if sometimes only implicitly, to generate a probability space. In this case, the evolutionary principle furnishes the parameter: chemical complexity.

The cosmic abundance of these elements is relatively high, and there is every reason to believe that Mars is at least as richly endowed as Earth in them. If the initial budget of carbon has not, like that of the Earth's crust, been completely requisitioned by life, then what form will we find it in ?

Chemogeny generates a vast mixture of products through the level of random macromolecules. Mars must have nurtured such chemistry, whether or not it had progressed to biogeny. A negative assay for organic materials would preclude biology, but could we believe such a result? It would properly be blamed on deficiencies in the particular sample. The positive assay, if it told something of the concentration and composition of

organic molecules, would add to our understanding of Mars's development, and would contribute to our judgment of the life-detection problem. But it would not answer it. On the other hand, once life has appeared on a planet, it would dominate its organic chemistry—most carbon compounds would be witnesses of biogenic (or cognogenic) specificity. The cataloguing of organic molecules is a description of the consequences of evolution and must make up a large part of our effort.

The Chemical Scan

To promise an actual complete scan of hypotheses of molecular complexity would be pretentious and witless, notwithstanding that a computer can now be programmed to visualize all the possibilities. However, the fantasy of such a scan is a constructive exercise in evaluation of evidence for life. For each chemical species the imagination of the specialist might be challenged to ask: (a) is there any information concerning the existence of this item relevant to scientific inference in exobiology; (b) what are my prior expectations on the distribution of this species, with and without life; (c) what other data could contribute; (d) how would the observation be interpreted from a terrestrial foray; (e) what special methods are available or could be devised to detect the species ?

We might nurture a hope of turning up a special treasure, a rare example of a molecule which would reveal something about the evolution of the planet and help narrow our choices among the confusing array of possible targets. In practice this advantage does not materialize so easily, for the hope is false. Not that no chemical

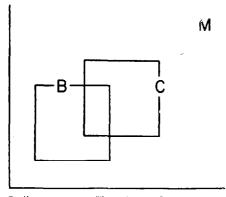


Fig. 1. Contingency space. The universe of possible observations I includes the overlapping domains B and C, the predictions of biogeny and chemogeny, respectively. The remainder is M, contradictions of these models, and possible consequences of cognogeny. Should this also be bounded?

species is potentially informative; paradoxically, every one is.

Consider hydrogen. In terms of the simple Venn diagram (Fig. 1), most expected observations would fall in the region $(B \cdot C)$, that is, would be consistent with biogeny but not imply it. However, the sensible absence of hydrogen from Mars' surface would fall in region $(-B \cdot C)$, that is, virtually preclude life. But if we could produce no plausible physical model for the disappearance of hydrogen, we would have to reconsider the region $(I \cdot -C)$, that is, to ask whether the anomaly implies a biogenic or cognogenic sequestration of the element. On the other hand, certain microscopic distributions of H are hard to reconcile with any chemogenic model, and point to the region $(B \cdot -C)$, that is, an inference in favour of biogeny. This verges on morphology, but can still be formulated as molecular statistics.

On another tack, suppose a specimen consisted of pure protium, ¹H, to the exclusion of deuterium, ²H. The price of pure protium on the terrestrial market hints at the obstacles to a chemogenic model. Apart from cognogenic activity, if a biogenic system were exquisitely sensitive to deuterium-toxicity it might evolve a discrimination against it.

The arguments have been laboured, but are quite typical of those that discovery of any other species would arouse.

Entropy or Unlikelihood ?

Given the evolutionary continuity of life and our understanding of the organism as a chemical machine, there can be no absolutely distinctive signature of life. Some conjunctions-like a planetary depot of protiumwould be so unaccountable to our present model of chemical behaviour that we would feel obligated to postulate the operation of a goal-directed system (biogeny or cognogeny) rather than accept the improbability of such a conjunction by chance. This choice plainly depends on our freedom of choice of models. For example, our present knowledge of chemogeny permits a wide latitude of hypotheses as to the range of molecular species that atmospheric photochemistry might generate. Further developments in our knowledge of chemogeny or of the available chemical and physical resources of Mars might confer useful constraints on the data that might now be 'explained away' as chemogeny, and thus cannot yet make a crucial contribution to our search.

From terrestrial experience we judge that the occurrence of any of a number of compounds in high purity is a sign of life. Such deposits at a macroscopic level tend to signify cognogeny—a smelter, a chemical laboratory, a communications cable, rather than biogeny—organic

structure usually being built of microscopically defined components. Negentropy is a necessary, but not sufficient, sign of life. However, it can help filter out the most promising situations. Then only the details of experience or confident use of available theory can decide whether the eddy has a chemical-kinetic explanation or a bio- or cogno-genic one. Lacking our experience, a Martian visitor might credit diamantane carbon to some mysterious biogenic function, inhibited by Y chromosomes; if he were cleverer, to the General Electric Co. He would need very special knowledge of the Earth to predict that diamonds would be found in the ground (and even more to understand why men dig them up, only so that women will wear them).

Kinetic instability in the context of local chemical and physical conditions is another clue: For example, cover of photosensitive pigments (witness terrestrial chlorophyll) requires special attention to the magnitude of plausible synthetic processes, atmospheric-chemical versus biogenic, by which their steady-state concentration could be maintained. Analogous reasoning would apply to compounds which are thermolabile in relation to the ambient temperature, or chemically unstable species which should reach equilibrium with coexistent oxidant. Do we see a forest fire? Then we must think of the efficient system of photosynthesis which will restore the steady-state vegetation. Top-heavy structures, which high-altitude reconnaissance could perceive even without resolving single trees, houses, or bipeds, likewise tell of kinematic instability, and in turn, some process to reraise what must some time fall. But geophysics competes with biophysics, and we have to discriminate life from vulcanism and orogeny.

In sum, unlikelihood in terms of the chemogenic model gives weight to any finding as a datum for exobiology. It should be possible to quantitate chemogenic likelihood, essential if a datum is to be given a measured value in any decision-making programme. The resolution of the measurement need not be very high to make it still very useful in comparing disparate approaches.

In more general terms, biota have a high density of internal information: the root of our conceptual distinction between matter and life is the rich story that life can tell about itself, a plot the details of which we can scarcely deduce from our simple knowledge of the initial conditions. But there must be a plot, that is, the information must have some interesting pattern, or we would not distinguish a cell from the dislocations in a snowflake.

Optical Activity

Many molecular species can contribute in an important way to our appreciation of life. A priori, we have a very

limited basis to predict which species will be most cogent. We should, of course, give high, but not exclusive, priority to terrestrial prototypes like amino-acids and nucleotides. Fortunately, there is a generic classification of compounds which is relatively independent of detail of structure, yet should pervade a biogenic chemistry. This is optical activity.

The argument for logical necessity of net optical activity has nothing to do with optical rotation. It depends on the crucial role of the informational macromolecule in a definition of life. When tetravalent carbon is incorporated into macromolecular structure, each carbon stands a reasonable risk of being an asymmetric centre, of having a distinctive substituent on each of its four valences. Such an atom is subject to stereo-(optical) isomerism, and its orientation, D- or L-, must be specified if the macromolecule is to be fully ordered, more concretely, if it is to have a well-defined three-dimensional shape. Conversely, biogenic macromolecules, having ordered asymmetric centres, have the necessary information to discriminate among the isomers of monomeric substrates. On Earth, where biogeny has dominated the statistics of organic molecules, we find that the ratio of D- to L-glucose residues is at least 10¹⁵ : 1.

Logical sufficiency can also be argued. Chemical enantiomorphs should be generated in equal proportions except under the influence of a catalytic system which is already asymmetrically organized. The global organization of a planet into one catalytic system of particular orientation is a catastrophe of a magnitude unique to biogeny. Spontaneous resolution might occur locally. Hence this criterion has its greatest weight when applied to a species which flows through the planetary circulation. A paragon would be labile molecules condensed from the atmosphere: optically active smog.

Within the biogenic system, both enantiomorphs of a metabolite might be generated, but this would be in precisely equal amounts with no greater likelihood than any two species designated at random. Chemogeny and sterically ordered biogeny thus give sharply contrasted expectations on these statistics, and biogenic chemistry can hardly avoid becoming sterically ordered.

These lines of inference do not account for such parochialisms as the undeviating series of L-isomers of aminoacids in esobial proteins. For example, D-alanine would be at least as interesting as L-valine in expanding the homologues of glycine. But, for that matter, why is α -amino butyric acid passed over ? We may know better when the rules of polypeptide conformation are better known; more likely, from the details of evolution of amino-acid anabolism and problems of discrimination among analogues. It is precisely at this level that the local biological theory fails, and thereby points to the crucial issues of a cosmic biology.

In view of the theoretical generality and historical tradition of the Pasteurian principle, it is paradoxical that the direct measurement of optical activity is weak by comparison to other instrumental approaches. However, the basic criterion is not optical rotation but molecular statistics. Enantiomorphs can be assayed with optically active reagents to give resolvable diastereo-isomers, and exploit the most sensitive methods known to chemistry.

Macromolecules

Informational macromolecules define the boundary of chemogeny and biogeny, of chemistry and life. Their description on another planet is the fundamental challenge of exobiology. Replication of macromolecules (genes) and the inevitability of random error (mutation) open the door to natural selection and the evolution of more and more complex forms of life. A random polynucleotide is not life; routes to its photochemico-synthesis from simple gases and inorganic phosphate are in sight. Can we deduce the replication of a polynucleotide by any means short of the most recent achievements of direct observation and in vitro enzymology? Historically, we could deduce the informationality of macromolecules just from compositional data. When the same sequence occurs in many molecules-as in a sample of crystalline haemoglobin -we have to invoke an informational process to programme and implement the synthesis of the protein. In fact, only recently and rarely could we gain complete specifications of an actual sequence. This is usually inferred from fragmentary analyses of a fraction found to be monodisperse on a few measures and then assumed to be sequentially homogeneous.

The sequence need not be the gene itself. Macromolecular sequencing is also manifest in gene products, RNA and proteins. It is important that the sequence imply ordering from a template which selects from an abundance of kinetically equivalent choices, not merely a pattern inherent in the chemistry of the monomer, as in crystallization.

Molecular esobiology faces the same methodological problems. This challenge gives us the groundwork for exobiology and assures that any instrumental advances will have redoubled utility. But it is a chastening note that biochemistry has barely reached the point of affirmation that antibody γ -globulin has an informational sequence or is specified by a polynucleotide. That this abundant and medically important molecule can still be so controversial must evoke some humility in our postulations and experimental efforts concerning macromolecules on another planet.

How We Detect Informational Macromolecules

(A) Compositional analysis. (a) Does the sample contain macromolecules ? (b) What is their composition ? (c) Any evidence of informational ordering ?

Esobiology is firmly founded on the isolation of macromolecular species and their purification before attempts at analysis. Some of the most successful methods are empirical recipes of extraction and precipitation.

More rational techniques include diffusional properties of large molecules, free diffusion, sedimentation, dialysis, molecular sieves, and electrophoresis, in principle also vapour phase diffusion (to remove monomers)—molecular distillation, gas chromatography and mass spectrometry. Solution chromatographic methods may also rely on the coincidence of functional groups on one molecule, for example, a polyelectrolyte.

Similar principles underlie non-separative methods of detection which have not been extensively developed to date. Rotational relaxation times can be measured by flow or electric birefringence, or the analogous polarization of fluorescence. Polyfunctionality is tested by intermolecular interactions of adsorbed dyes (for example, optical shifts in acridine orange on DNA) or the monomeric units with one another in special cases (hypochromicity of DNA, diagnosable on heat-denaturation). More direct chemical tests for polyfunctionality also suggest themselves.

The previous methods, in so far as they lack perfect generality, may give only a clue as to the composition of the macromolecule, as well as its molecular size. At the other extreme, we would seek the complete primary structure to emulate the recent *tours de force* of chemical technique. Reasonable inferences might be drawn from less complete evidence of structural individuality, hard to evaluate in advance: homogeneity in molecular weight or end-group analysis, crystallinity, or sharp fractionation by any other procedure. A sharp X-ray diagram of a heteropolymer sample could imply its individuality long before it had yielded to full analysis.

Other partial measures of great utility include the scission of the polymer by specific reagents, especially enzymes, to give a pattern of characteristic fragments (the polypeptide 'fingerprint').

The underlying generalization is 'molecular speciation'. Chemogenic synthesis of macromolecules should generate a continuum of nearly equiprobable forms. Biogeny chooses a few of these and generates a sharply discontinuous polydiscrete spectrum, that is, it speciates. Speciation can be discerned by many measures, for example, the distribution of molecular weight. Thus a sample under analysis by a sophisticated instrument might reveal a sac of haem-polypeptide, containing about

Function	Complex with	Example
Auto-replication	Incipient polymer (same species) and polymer- building monomer	DNA : DNA + deoxynucleo- side triphosphates
Hetero- replication	Incipient polymer (different species) and polymer- building monomer	DNA : RNA + nucleoside triphosphates
Morphogenesis fibres, mem- branes, vesicles	Formed polymer, similar species	Collagen: collagen sub-units
Enzyme	Substrate—catalytic effect Cofactors—to form'holo- enzyme Analogues—complexes inactive, qua enzyme	
Neutralizing	Whatever	Antibody: antigen, that is, any chemical species foreign to the reacting organisms
Transport	Hormones, toxins, nutrients	Serum albumin: hormones, toxins Permeases: nutrients and metabolites for transport in and out of cells

a billion (a thousand million) atoms of iron. Virtually all the iron-polypeptide consists of a single species, that is, almost all the molecules have 2,936 carbon atoms, no more, no less. After removal of iron and porphyrin, equal numbers of sub-units containing just C_{685} and C_{715} are assayed. It would be difficult to escape an allusion to life after a single encounter with the red blood cell that has just been described.

(B) Functional analysis. The adaptive values, the uses that biogeny has discovered for some species of macromolecules, reveal short cuts to their singularity. These functions are all reducible to a structural specification: the stereospecificity of the polymer in reacting with other molecules.

In this list, the enzymatic functions are particularly promising in the light of their specificity and amplifying capability. Many enzymes have turnover numbers of 10^4 substrate molecules/sec'enzyme molecule. If suitable precursors (nutrients) can be defined, integrated enzyme sequences or metabolic systems, like respiration or photosynthesis, extend the versatility of this approach.

The simpler the level, the more likely are we to find a metabolic analogue on Mars, for example, for the assimilation of elementary nutrients, C, N, O, S or P, into organic molecules. The next more complex molecules, H_2O , CO_2 , and O_2 and NH_3 , are the most pervasive metabolites of terrestrial life, and the choice among them for searching for evidence of their conversion into other compounds will depend mainly on instrumental considerations. In general, the more complex the metabolite being tested, the less our prior expectation that it was part of an extraterrestrial biogenic system. However, the complete system offers the largest amplification—a single bacterium could grow'and multiply into tonnage masses in a few days, but might make the most exacting demands of the environment.

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Morphology

Biogeny rapidly elaborates higher forms of organization: cells, tissues, organisms, populations, which might be recognizable according to their own forms and to their rectifications of the environment. However, what systematic rules distinguish biological forms in general? Some forms are recognizable, for example, a friend's face, and recognition then contains many bits of useful information. Compound vesicles, apparent cells, are most inescapable in morphogenesis; their absence would at least set an upper limit to the stage of biogeny. Their presence would be extremely provocative, but properly would raise many scepticisms of chemogenic artefact. Nevertheless, esobiology has so many roots in morphology that we could scarcely ignore the insights that our historic practice of it would offer. Any recognizable forms would provoke tangible and hence useful working hypotheses of the Martian system.

Some aspects of morphology can be systematized. As an example which might illustrate speciation, ultrastructural spacings in the range of 20-500 Å could be detected by powerful optical (electron microscope, X-ray diffraction) as well as separative techniques. Approaches so cogent to esobial ultrastructure must play an important part in exobiology. Unfortunately, we have little empirical basis to prejudge the morphological detail that might be exhibited by an infra-biogenic planet, since so much of the chemical diversity of Earth has been pre-empted by life.

As is well known, five-fold symmetries are anathematic in crystallography. Hence, regular pentagonal and dodecahedral forms might occur as elementary units, for example, perhaps a ferrocene, but no simple law of crystal growth could account for their occurrence in diverse sizes. A glen of periwinkles has a deductively simple signature of life.

Signals

So far I have tacitly assumed that whether or not Mars has achieved biogeny, it has not passed to cognogeny. Reaction to the once notorious Schiaparellian *canali* may account for a position which has no rigorous basis. True, we have had no scientifically admissible sign of intelligent activity on or communication from that planet. However, we can only fancy whether an exotic culture would have either the means or the motive to effect recognizable communication. We can generalize that the works of cognogeny would constitute the most startling unlikelihoods, exceptions to biogeny and chemogeny alike.

It is no trivial exercise to speculate how we could most

compactly summarize our scientific culture. For example, a description of DNA and our amino-acids could portray the convergence of physical and chemical ideas in biology, and some of the least predictable aspects of esobiogeny. If we could but do it, a detail of the inter-neuronal synapse and the cytoarchitecture of the cerebral cortex would go even farther. How much of our cognogeny would then be deducible from these facts and our awareness of them?

Purposeful emissions cost enough more than mere listening that we do not undertake them ourselves, but we have made casual efforts to hear them. Further, we might hope to eavesdrop on the internal communications of another planet, perhaps more likely far beyond the solar system. Among other difficulties, efficient information is, by definition, indistinguishable from noise to the unbriefed eavesdropper.

While a rigorous answer to any notions of Martian intelligence is difficult, a realistic policy is not. Cognogeny would reveal itself in divers ways, and, at least for Mars, we have no better recourse than to keep eyes, ears and noses alert for any signs of it as we make progressively closer approaches to the planet.

Instrumentation

The rational classification of existing instruments, or those proposed for analytical purposes, is a task as difficult as it is urgent. The real aim, a classification of possible instruments, requires a total knowledge of physics, and some system for classifying this information that will help us to understand the relationships among existing instruments and suggest new ones. A proposed scan parameter is the energy-level of the transition by which the molecule is recognized. Further parameters include whether photons are introduced or emitted, whether chemical reagents are employed, including auto-reactions, whether the displacement or state of the analysand or of the probe is diagnostic, and, for radiation probes, the role of power, polarization, phase, wave-length, or flux vector of the probe. The first step in a detailed rationalization is to determine whether any more dimensions are needed for our matrix of possible configurations.

Radiation probes are usually limited, either in selectivity—say, absorptiometry, or sensitivity—say, nuclear magnetic resonance—but they have special value in conjunction with chemical reagents. Absorption (power loss) measurements have dominated instrumental analysis. Conventional optical methods rarely stabilize or measure input power better than 1:1,000, with corresponding limitations to detectivity. For example, optical molar absorptivity rarely exceeds 10^5 so that 10^{-8} molar solutions (6×10^{12} molecules in a $1 \cdot cm^{-3}$ cell) would give the lowest useful signal under the most favourable conditions. By contrast, fluorometric measurement (which can exploit shifts in wave-length, flux vector, polarization and phase) can easily measure 10^8 molecules and can be extended at least to 10^4 . The delicacy of excitation methods (which could also include chemical, nucleonic and thermal excitation) stems from the measurement of the data signal merely against a detector noise background as compared with the much larger power fluctuations of practical probes.

Optical activity is also usually measured via loss of power (attenuation of polarized light by a crossed analyser): the molar rotations are relatively small, present detectivity being about 10^{15} molecules. If some method of transforming optical rotation to an excited signal were developed, it would enormously enhance the power of this technique.

The most sensitive approaches to analysis are two-stage mechanisms: the selective displacement of the analysand, then a sensitive detection. In principle, such methods might detect a single molecule, as in mass spectrometry: selective m/e displacement followed by the sensitive detection of an ion that can be accelerated to arbitrary energy. The potential information content of a mass spectrum is especially high since the theoretically measurable mass of a single molecule is defined to a resolution far better than 10⁻⁶, independent of the variety of energetic states, which broaden other physical features. Existing instruments still lag behind theoretical limits of mass resolution, yet have already demonstrated their power in organic analysis. Further, the mass datum at high resolution for an intact molecular ion is deductively reducible to a molecular composition, unlike the inferential data given by most other spectroscopic techniques, and the statistics of the fragments also give detailed insight into the complete structure of the molecule. From these considerations the combination of a mass spectrometer with a simple, rugged, separative device, like the gas chromatograph, promises to be the most powerful component of analytical systems for biochemistry. However, a science of metrology, the orderly study of methods of measurement, remains to be developed. I can have little confidence that the last word has been said on this issue.

Some Private Thoughts on Exobiological Strategy

The multitude of possible means and detailed ends in exobiology leaves little hope that a brilliant flash will illuminate the whole picture as a happy substitute for the diverse paths of esobiology. Nor should there be any discouragement of the variety of talents and insights that would be needed in any event for the full development of the subject. The overriding problem in planning is, of course, how little we actually know about surface detail

and atmospheric composition of Mars. We are also bedevilled by the uncertain hazards, but immense stakes, to either planet of an intemperate rupture of the interplanetary barrier. Earth-based telescopes can, to be sure, add significantly to our present appreciation of Mars, and hence of the hazards of landing. But the next significant step would be a Mars-orbiting observatory, keeping the planet under a constant synoptic scrutiny from a safe distance, close enough to measure significant surface dotail, and large enough to maintain the most sophisticated instrumentation, telemetry to Earth, and perhaps even some Earth-based regulation of its surveillance schedule and precautions against accidental intact landing.

Such an approach to Mars would also open the way to political agreements to unify terrestrial strategy and can allow constructive co-operations like the International Geophysical Year of recent history, for example, to facilitate the relaying of synoptic data. While it is essential to mount vigorous instrument development efforts to assure that a landing can ever be implemented, the detailed specifications of experiments should take full advantage of the most up-to-date planetological information. That is to say, the final decision to implement a landing on Mars should be suspended until we can have digested the data from a Mars orbiter. This criterion lends further weight to the strategy of designing a generalpurpose laboratory for planetary investigation, in which many investigators can participate, and which has the flexibility to be readily reprogrammed in the light of new data. At present, for a biologist to participate actively in space research requires a commitment to engineering efforts which few are willing to undertake.

The deliberate staging of the exploration of Mars, perhaps with international agreement to proceed first with reconnaissance while preparations are made for comprehensive landed missions, would allow for the widest participation of interested scientists, both in the design of experiments in exobiology and in the prudent determination of global policy for the solar system.

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