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CYTOCHEMICAL STUDIES OF PLANETARY MICROORGANISMS

PROPOSED CONTINUATION

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

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For The Period

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PROPOSAL

This proposal is a request for the continuation of Grant NGR-05-020-004 to the extent of $110,000 for the period December 1, 1977 through November 30, 1978.

In addition to outlining the proposed activities for the twelve month period beginning December 1, 1977, we have also briefly reviewed some of the accomplishments of the previous twelve month period.

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GENERAL INTRODUCTION

As has been the case for some time, our research effort is divided into a) microbiological, and b) analytical-chemical studies. These are discussed in two main partitions of this renewal application and progress report. On this occasion, all of the methodologically oriented efforts of past years have been 'spun off' into programs supported from other sources; our current proposals --truncated by progressive inflation at level-dollar support -- converge on the theme of the origin of life and fundamental issues in the evolution of primitive microorganisms.

The overall program of work summarized here far exceeds what could be accomplished within the actual budget requested from NASA. We have the advantage, however, of considerable momentum from previous experience and equipment. We also anticipate an advantageous overlap with support that we are planning to request from ERDA and from the NIEHS with respect to health- and energy-related aspects of the biology and chemistry of polymeric carbon. (Only the most preliminary contacts have been made with those agencies at this time.) The funds requested will enable us to sustain the key professional staff during this transition, and to continue to focus on those issues that are of specific interest to the NASA program office. If other support cannot be obtained, we will be constrained to an opportunistic pursuit of only the most promising research gambles that present themselves during our explorations.

RESEARCH PLANS 1977-78.

A. Microbiological and Genetic Studies

For several years, the main focus of microbiological research in this laboratory has been on the construction of simple model systems for the genetic diversification of simple genetic codes. This work entailed the use of 'recombinant DNA' technology to construct bacteria that had simple monotonous synthetic sequences, whose further evolution could then be studied in detail. The technical task has proven to be far more difficult than was originally envisaged; but on the way we have made a number of important advances in a) methods of DNA segmentation and implantation, b) understanding of the conditions for expression of genes exchanged among widely diverse bacterial species, and c) the prevalence of mechanisms for promiscuous mixing of genes among bacteria believed to be quite separate in an evolutionary sense -- e.g. Bacillus and Staphyloccus. In a sense this last finding may unexpectedly prove to be the most important of our contributions to the theme of the origin and evolution of life (Green, 1964).

In the light of the unexpected ferocity and hostility of contention about the public health hazards of work with recombinant DNA, -- although we have endeavored to stay as far as possible from situations with credible pathogenic potential -- we have decided to phase out our work under NASA support that directly involves recombinant DNA; and this will no longer be part of our continuation proposal. However, we will continue efforts along the lines of c) above, namely to seek still broader understanding of the extent to which genetic information is shared with a common gene pool among microbes. Julian Davies, for example, has long speculated that antibiotic-producing streptomycetes are the primary reservoir of plasmids that recur in a wide range of pathogenic bacteria, and by conferring resistance to the antibiotics result in serious clinical problems. However, direct support for this degree of panmixis has yet to be achieved experimentally. In the course of our observations of the Staphylococcus x Bacillus exchanges mentioned above, we believe we may have some hints how to overcome these experimental barriers, and will apply these ideas to building general "plasmid-traps" to capture the movement of plasmids among a variety of species in their natural soil habitats. The insight sought here would of course be of the greatest importance for a) the theory of evolution of microbes (if not eukaryotes as well); b) practical problems of the sources of antibiotic-resistance; and c) common-sense tempering of regulatory controls that have become a serious hindrance to research in this field.

This shift of emphasis to natural habitats is coupled with an effort to get a better understanding of the role of solid surfaces in microbial physiology, ecology and genetics. Some years ago, in connection with studies that helped to prove that penicillin acted as an inhibitor of cell-wall synthesis, I was impressed by the role that solid-agar had in controlling the morphogenesis of spheroplasts or L-forms in E. coli. Knowing that many, perhaps most, of the microbes resident in soils have never been isolated in pure culture, I wondered whether much of laboratory microbiology -- using homogeneous liquid media -- is not an artefact, far removed from conditions of the natural habitat. In the interval, many soil microbiologists have made similar observations (summarized in Marshall's book), at least of the influence of interfaces on the growth and physiology of particular species. The most extreme speculation that I propose to investigate is whether there are not many species that have an absolute requirement for a solid substrate -- from which it would follow that conventional methods have been actively selecting against the recognition of an important part of our biosphere. An even more interesting proposition is that these organisms may also include representatives of the simplest, most primitive categories that could give further clues about the baffling missing links in the 'Scala Naturae . Some (feeble) support for such a speculation can be gathered from the fact that Mycoplasmas, which have the smallest genome-sizes of any presently known freeliving organisms, lack a rigid cell wall. Some of these have, to be sure, been cultivated in suspension, but often only with difficulty; and analogous L-phases of larger bacteria generally require an agar substrate. The implication is that a rigid wall, and the ability to proliferate readily in suspension, are later evolved attributes of the 'typical' bacteria compared to more primitive forms. So far, the most primitive mycoplasmas still have the complete repertoire of the genetic code, and therefore can give us little insight into its primary evolution (unless one takes this as evidence of special creation or of panspermia!).

We must concede that we have made little progress since Oparin in bridging the hiatus between the chemosynthesis of nucleotides and their assembly into meaningful, autocatalytic sequences -- in part for lack of observable representatives of the intermediate stages.

While this would be the most rewarding outcome, one does not have to stake one's faith in that speculation to justify the research plan. The strategy will be to construct media and devices that offer large surface areas to organisms in soil habitats, but which can be extracted for closer study-- e.g. glass films either bare or with various mineral coatings. These will be manipulated so as to encourage organisms that have a preference for sessile growth on the surface, and then sampled further for organisms that may have a near- or total- dependence on such surfaces. (One may not have to look far: witness algal and cyanophytic films in aquaria; but I do not know of critical comparisons of their growth potential in adherent vs. suspended mode. It is known that the sheathed habit, associated with attachment, of Sphaerotilus natans, is suppressed by high nutrient concentrations -- there may well be many organisms that correspond to the attached phase of Sphaerotilus, being unable to grow except as epibionts. (The association of bacteria with surfaces has also attracted renewed interest in recent years in connection with dental microbiology, and with other aspects of infectious disease.)

The associations of blue-green algae with fissures and voids in the immediate sub-surface of dry Antarctic soils has been pointed out, by E.I. Friedmann, to refute the asserted sterility of this terrestrial habitat, and to point to some caveats in the design of life-detection strategies for planetary exploration. Saprophytic bacteria that would exploit the primary production of the algae would be very difficult to ascertain by conventional methods if they were interface-dependent. This gap in our appreciation of the soil habitat is thus equally important for laying the groundwork for future planetary biological science and engineering. Furthermore, there are many anecdotal observations of the enhanced resistance to thermal sterilization of microbes in soil matrices; but the phenomenon has not been systematically investigated with regard to mechanism. Thus, our projected studies of interface biology bear on problems of survival in hostile environments (terrestrial and extra-terrestrial) as well as upon practical problems of disinfection, e.g. for planetary quarantine. The methods of microscopic examination of soils that will be developed for these studies should also have some bearing on planning for instruments appropriate to future planetary and return-sample missions from an exobiological perspective.

Finally, few students of the origin of life have FAILED to comment on the probable role of clay and other heterogeneous surfaces for the local concentration of chemosynthetic precursors of life. We thus note that, while the central ideas of this research plan are widely entertained, they have only rarely been the subject of concerted investigation: doubtless because of the overwhelming convenience of working with transparent, homogenous media.

Besides the minerals commonly associated with soil, we will also give particular attention to polymeric carbon as an interphase -- the prevalence, and adsorptive and catalytic properties of this material which justify this choice are elaborated in part B of this proposal.

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E. Chemistry of Polymeric Carbon

1. Introduction

The methodological emphasis of our previous work is outlined in the progress report (see Appendix 1). We believe that GC/MS techniques have now progressed to the point where scientifically useful applications should be the main stress of our effort, namely on the carbon chemistry of the solar system. We propose specific areas of study which bear directly on the origins of carbon compounds, and their interactions and reactions in both ancient and modern environments. These studies complement directly Part A of this proposal in that we will investigate carbon substrates and adsorbed material as an environment for the growth of microorganisms.

2. Summary of Proposed Studies

We propose to investigate certain aspects of the chemistry of polymeric carbon. Polymeric carbon, defined in more detail in the subsequent section, includes a variety of polymers, such as graphite and carbon black, characterized in part by high carbon content, high structural order at the molecular level and high surface area. The role played by polymeric carbon as a participant in the evolution of carbon compounds over geologic time is poorly understood. Yet it is the thermodynamically favored form of carbon under many conditions of temperature and pressure in reducing atmospheres, and is stable to decomposition under many other conditions because of high activation energies toward decomposition.

There are several reasons for our interest in polymeric carbon:

a) Extraterrestrial Carbon. The carbon chemistry of the moon is quite complex and not fully understood. A similar situation pertains to Mars, where the carbon content is unknown but reactions of carbon compounds in the Viking biology experiments produce unexpected, and unexplained results. An understanding of behavior of low molecular weight carbon compounds and their interactions with polymeric carbon under various conditions might facilitate an understanding of some of the observations.

b) Prebiotic Carbon. There is considerable controversy over the presence of polymeric carbon in prebiological times. No experiment of which we are aware dealing with condensation of gases to yield precursors of biopolymers has considered the effects of polymeric carbon, although the effects of clay on condensation reactions have been studied. Similarly, complex models are proposed for subsequent concentration and condensation of e.g., amino acids without attention being paid to the extreme adsorptive power of polymeric carbon and its catalytic activity. Again, however, other mineral surfaces have been proposed to act in similar roles.

c) Geo- and Biochemical Significance of Carbon. Significant quantities of the world's carbon are cycled through polymeric carbon, for example in natural or man-made combustion products, and in compaction and diagenetic modification of organic compounds into coal or related polymeric carbon deposits. Yet the origins, distribution and fate of this material are not well known. For example, we have no reliable information on the pathways by which charcoal generated by forest fires reenters the global carbon cycle, although this must represent a substantial percentage of the total annual photosynthate. The role of polymeric carbon in mediating chemical reactions in the biosphere and the ability of soil organisms to utilize carbon for support and/or nutrition are not well understood. Previous work in this laboratory, some years ago (Schneour, 1966) did not elicit convincing evidence of microbial conversion of charcoal on a significant scale.

Our present speculation is that soil protozoa and other invertebrates must be involved in ways yet to be experimentally examined.

We do not claim that polymeric carbon was necessarily an essential ingredient in evolution of carbon compounds or in the subsequent origin of life. But the combination of its properties, its intimate relationship to other forms of organic carbon and the lack of attention which has been given to its role in the above problems demands some investigation. We propose to begin some preliminary studies, described in the following section.

Because our proposed studies deal with mixtures of organic compounds, GC/MS will be the primary analytical method for characterization of mixtures and identification of compounds. Thus, maintenance of our current capabilities in GC/MS and computer techniques is necessary. In addition, we will have to carry out some development work in both hardware and programs to support the new studies.

3. Proposed Research Studies in the Chemistry of Polymeric Carbon

Polymeric carbon is defined as those natural and synthetic materials which consist structurally of fused polycyclic ring systems composed predominantly of carbon. These materials range in homogeneity from graphite to coal and soot. (For present purposes we ignore the crystalline form of diamond.)

The major forms and methods of preparation of carbon are as follows:

a) Graphite. Graphite consists of large two dimensional sheets of fused aromatic rings. The layers are held together by weak van der waal's forces which give the material its characteristic properties - lubricating ability and softness. It is found naturally in a variety of places including deep Earth deposits and meteorites. The majority of the graphite in commercial use is prepared synthetically by high temperature conversion from less pure carbon black or coke (Holliday et al., 1973).

b) Carbon Black. These materials, which are prepared by partial combustion of various petroleum feedstocks, consist of fairly pure carbon (90-100 per cent C) and structurally are composed of microcrystalline graphite-like particles. Carbon blacks have high adsorptive properties and have impurities which depend on the feedstock, pyrolysis method and post-treatment with oxygen, sulfur, nitrogen and occasional inorganic elements (Schubert, et al., 1969).

c) Activated Carbon. These materials are prepared by controlled pyrolysis of various organic substrates including sugar, bone, and coconut. They are activated by partial oxidation to yield materials of optimum adsorptive properties. This preparation leaves this carbon form very porous. Depending on the feedstock activated carbon is more or less pure carbon (Hassler, 1974).

d) Coal and Petroleum. These biogenic materials vary greatly in their carbon content and their degree of polymerization. They are formed over geological time by deposition and modification of biological materials followed by alteration by heat and pressure (Eglinton and Murphy, 1969).

e) Other Carbons. Any incomplete combustion process will leave a residue of carbon of variable form. Major processes of this type include soots from petrochemical energy production and uncontrolled fires.

There are several methods of natural production of polymeric carbon which may have been important to the early carbon chemistry of the Earth. Among the most important are:

a) Pyrogenic. Due to interest in the preparation of carbon black this area has received considerable attention (Cullis, 1976; Abrahamson, 1977). The generally accepted view is that acetylene is formed as an intermediate by cracking of larger molecules or dehydrogenation of methane. Soot formation (polymeric carbon) then occurs as a stepwise process of polymerization of acetylene and partial dehydrogenation.

b) Cosmological. Below 930 degrees C carbon monoxide is unstable with respect to disproportionation to carbon and CO2. However, this reaction is very slow and needs catalysis to achieve reasonable rates. Catalysts for carbon monoxide decomposition include palladium, iron oxides and graphite itself. The mechanistic aspects of this formation of carbon are no doubt quite complex and have not been studied.

c) Thermal. Once complex molecules have been formed, whether abiogenically or biogenically, thermal processes serve to modify them further. Such reactions have been important in more recent geological periods in the formation of petroleum and coal and in present-day production of activated carbon. Carbonaceous materials formed by pyrogenic or cosmological reactions are also subject to thermal alteration, however, and such processes were presumably active in early Earth history.

4. Specific Projects

a. Chemical Characterization of Polymeric Carbon.

We propose to develop chemical methods for detection and characterization of polymeric carbon. Detection is important for those problems such as analysis of lunar or chondritic material (and presumably martian soil also) where the levels of carbon are relatively low and no direct methods for measurement of polymeric carbon exist. Characterization of the structure of polymeric carbon is important because of the widely differing physical and chemical properties of the various structural forms.

i. Detection of Polymeric Carbon. The analysis of low levels of graphitic carbon has traditionally been accomplished using difference methods. For example carbon black in rubber is analyzed by dissolving away the organic matrix with hot nitric acid and weighing the residue (Schubert et al., 1969). Graphitic carbon in lunar soil was estimated after removal of volatiles, materials rendered volatile upon acid treatment and materials rendered volatile below 750 degrees C. The residual carbon was assumed to be graphitic and could be oxidized to CO2 at 1050 C (Chang et al., 1970).

Our method for the analysis of carbon is based on unpublished work from our laboratory. We have found that activated carbon upon oxidation with chromic acid or potassium permanganate gives rise to oxalic acid and a series of benzene polycarboxylic acids (BPCA's). Similar results had previously been described upon oxidation of graphite with sulfuric acid (Mantell, 1968) and of coal gasification residues with oxygen in a basic solution at elevated temperatures (Eisenberg and Solomon, 1977).

These acids are conveniently identified and quantitated after conversion to their methyl- or trimethylsilylester derivatives. The method will be calibrated using known amounts of carbon. We will study what, if any, interference is caused by the presence of clays or other inorganic materials. This will lead us to a method for measuring the polymeric carbon content of soils.

There is evidence in the literature that different forms of polymeric carbon will react differently to the oxidizing conditions of our experiment. Chromic acid has been reported (Oberlin and Mering, 1964) to oxidize graphite to CO2 while carbon black is relatively resistant to such treatment. Also, Eisenberg and Solomon (1977) have noted variations in the pattern of BCPA's formed upon oxidation of different coal-gasification residues.

The only non-polymeric materials which might give rise to benzene polycarboxylates in this experiment are small to medium size aromatics. The presence of these materials can be checked by extraction and volatilization techniques.

ii. Analysis of Carbon Structure. The many forms of elemental carbon (graphite, carbon black, activated carbon) are normally characterized in terms of their methods of preparation, particle size, surface area and other gross properties (Schubert et al., 1969). The types of molecular sub-structures present and elemental analyses of various carbons are also well-known (Garten and Weiss, 1957). We would like to extend the knowledge of carbon structure to include estimations of the size distributions of the individual polymeric molecules present in carbon structures. Two methods for approaching this problem are:

1) Solubilization with phenanthrene - it is known that hot phenanthrene will solubilize 90% of the material present in coal (Haredy and Fugassi, 1966). Once in solution, the carbon molecules could be sized by gel permeation chromatography.

2) Chemical modification - we have speculated that a reaction which increases the polarity of the individual polymer molecules might increase their repulsive interactions and allow for a more facile solubilization. Possibilities include sulfonation, mild oxidation to phenols or a nitration/reduction scheme to yield a polyammonium species. Indeed, Boehm, et al. (1962) investigated the breakdown of graphite into individual layer molecules upon oxidation. He prepared graphite oxide, a partially oxidized material which retains all of the original carbon atoms. After dispersion as a dilute suspension in base, he reduced the material to carbon with hydrazine to form what were believed to be crystals of only a few layers of carbon each. Further characterization by X-Ray

analysis proved inconclusive in terms of determining the layer diameters within the crystals.

We propose to repeat this experiment with graphite oxide but do the sizing experiments on the oxide itself rather than the rereduced material. Presumably the oxygen functionalities on the surface of the graphite oxide (phenols, ethers, quinones) will disrupt the interlayer attractive forces and facilitate the production of single layers. We will size these by liquid phase methods (gel-permeation chromatography). The data so generated will not be easily correlated to an absolute molecular weight due to the possibility of holes in the graphite polymer (Hutcheon, 1970). However, this method should allow for characterization of different forms of polymeric carbon and such data will be important in tracing the genesis of unknown carbons found in soil and other complex media.

b. Gas/solid Carbon Chemistry

We hypothesize that polymeric carbon played (and continues to play) a significant role in the carbon chemistry of earth in particular and the solar system in general. Thermodynamic calculations (Urey (1953); Dayhoff, et al. (1964); Eck, et al. (1966)) reveal the stability of "asphalt-like" mixtures under conditions simulating solar nebulas and planetary atmospheres. Yet such studies have not performed the more complex calculations needed to deal explicitly with elemental carbon (Dayhoff, et al. (1964); Eck, et al. (1966)). Few laboratory simulations of condensation of model planetary atmospheres into simple organic molecules examine carbon polymers which are formed, although "aromatic polymers" have been noted in at least one case (Anders, et al,. 1973). No one to our knowledge has studied the effects of polymeric carbon on such condensations although other solid supports have been included in some studies (Harada and Fox, 1964). Seuss has discussed the participation of solid forms of carbon in early earth history (Seuss, 1975). Evidence from carbonaceous chondrites and the moon (which might be considered modern representatives of early earth history) (Eglinton et. al., 1972) indicates a very complex carbon chemistry involving in part polymeric carbon. The known adsorptive and catalytic properties of polymeric carbon suggest that, if present, it can participate in a variety of chemical reactions involving carbon compounds.

We suspect that solid, polymeric forms of carbon also play a role in the carbon chemistry of Mars. Interactions of carbon present on the Martian surface with gases or added nutrients (Viking Pyrolytic Release and Labelled Release experiments, respectively) might contribute to the observed results, which to this date have no widely accepted explanation.

We do not propose to attempt to simulate a variety of planetary atmospheres or soils in order to study the potential role of polymeric carbon. Important laboratory experiments must be performed first to promote our understanding of the physical and chemical nature of polymeric carbon and its interactions with molecules in the gas phase. The experiments discussed below are aimed toward laying the foundation for more complex studies in the future.

We propose to study the interactions of well-characterized polymeric carbons with low molecular weight gases under a variety of conditions. We will obtain representative samples of polymeric carbon of various degrees of purity, including graphite, activated carbon and channel black. We will select samples based in part on how well they have been characterized by the manufacturer in terms of physical and chemical properties. We will characterize the materials further using scanning electron microscopy and by utilizing the proposed chemical procedures for characterizing polymeric carbon (see above, Section II.B.1).

We plan at least the following two specific experiments. The first experiment is to study the interaction of CO and CO2 mixtures with polymeric carbon under moderate temperatures and pressures, with and without heavy metal catalysts. We will utilize 14C labelled gases or carbon substrates to determined adsorbed and chemically bound or reacted species. We will use GC and GC/MS to determine gas mixtures and identities of higher molecular weight materials if any are formed. The results of this experiment may have relevance to observations (by the Viking landers) of Martian soil chemistry.

The second experiment we will perform will be to study the formation of organic materials using mixtures of methane, ammonia, hydrogen, water with polymeric carbon, with and without heavy metals, as a catalyst. An adaption of the apparatus of Harada and Fox (Harada and Fox, 1964) will be used to carry out the reactions. GC/MS will be used subsequent to derivatization of products to determine the amounts and types of materials formed. Comparisons will be made to published results from other methods of simulation of prebiotic synthesis (Calvin, 1969).

c. Liquid/Solid Carbon Chemistry

Solid surfaces have assumed a major role in most accounts of prebiotic chemistry as vehicles for adsorption and concentration of organic molecules (Nissenbaum, 1976) and catalysis of synthetic and polymerization reactions (Poncelet et al., 1975; Paecht-Horowitz, 1975). As the most likely candidates for this activity, the wide-spread clays have received considerable attention (for a review see Anderson and Banin, 1975.)

Although not important surface solids at present, presumably because they are metabolically recycled, the graphitic carbons should have been abundant in prebiotic times and may have functioned for the adsorption (protection and concentration) or transformation of hydrophobic molecules.

There is ample precedent for the catalytic activity of polymeric carbon. For example, activated carbon has been shown to catalyze numerous liquid phase reactions including: 1) racemization of 1,1'-binaphthyl (Pincock et al., 1973), 2) olefin isomerizations and polymerizations (Meier and Hill, 1974), 3) the inversion of sucrose (Puri et al., 1972), 4) the hydrolysis of ethyl acetate (Puri et al., 1972), 5) the reduction of Co (III) by iodide (Murcinik and Spero, 1974).

Activated carbon by itself will also catalyze the oxidation of various substrates by molecular oxygen (Garten and Weiss, 1957). Especially prone to carbon-catalyzed oxidation are difunctional molecules such as amino acids and malonic acids. The use of carbon as a support for metal catalysts (Hassler, 1974 Boersman, 1974) and inorganic reagents (Kagan, 1976) is well known.

Paecht-Horowitz (1976) has shown that montmorillonite clays will catalyze the polymerization of amino acids and derivatives in aqueous solution. The derivatives used were amino acid adenylates in neutral solution. Such derivatives show a natural tendency to polymerize under aqueous conditions, but in the presence of clay both a higher degree of polymerization and a high degree of order in the resulting polymers are obtained.

Our proposed experiment is to repeat this polymerization reaction in the presence of suspended polymeric carbon. Catalysis of this reaction by clays was very pH dependent (Paecht-Horowitz, 1976), proceeding only at near neutral pH's due to the competing requirements of protonated amino groups (for adsorption to clay) and free amino groups (for polymerization). On carbon the neutral species will be the preferred adsorbate and this fact may lead to increased rates of polymerization of adsorbed amino acid adenylates.

d. Solid/Solid Carbon Chemistry

i. Experiments on Prebiotic Synthesis. One can speculate that materials such as amino acids generated on carbon surfaces (for example, in gas/solid reactions, Section II.B.2) or adsorbed from aqueous solution might be sufficiently concentrated and oriented so that the probability of thermal polymerization is increased. In a paper on thermal polymerization of amino acids (Fox and Harada, 1958) it was found that an excess of one or two amino acid monomers was necessary to decrease the melting point of an amino acid mixture sufficiently below decomposition temperature. Under these conditions, some polymerization could occur without extreme decomposition. On carbon surfaces we have a very different situation energetically. Crystal forces are unimportant and monomers are free to interact (if the concentration is high enough) at lower temperatures.

We propose to study the thermal polymerization of various amino acid mixtures after adsorption onto polymeric carbon. Analysis of product mixtures for degree of polymerization and for the presence of any induced specificity or preference for certain sequences will be noted.

ii. Carbon as an Environment for Microorganisms. In Part A of this proposal we speculated about the influences of solid surfaces on microorganisms and the potential role of such surfaces as environments for novel organisms. Research planned for the first part of this proposal will address questions of the utility of surfaces such as polymeric carbon for the growth and nourishment of organisms. In this part we propose to examine another aspect of organisms and polymeric carbon, namely, can the carbon itself be utilized by microorganisms as a carbon source.

In experiments conducted in these laboratories several years ago (Schneour, 1966) preliminary evidence was obtained for the microbiological breakdown of elemental carbon. Using radiolabelled carbon, it was shown that a) soil from several sources showed activity toward metabolism of elemental carbon, and b) this activity was enhanced in soils taken from areas expected to have high concentrations of polymeric carbon due to recent forest fires.

We propose to reopen this investigation, using techniques developed in conjunction with gas/solid studies (Section II.B.2) to monitor radiolabelled gases. We will use well-characterized carbons (see Section II.B.1) together with different organisms including those commonly culturable from soils. If activity is found, we will search for intermediate products of carbon oxidation.

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APPENDIX 1

PROGRESS REPORT

Part A. Recent work has been summarized in the introductory and in discussion of the rationale and research plan for Part A. Additional details may be found in the attached reprints.

Part B. We have fulfilled the objectives of our previous research proposal. The emphasis of that proposal was on extending the analytical methodology of GC/MS because we perceived that the proposed extensions would have a strong impact on our chemical and clinical research, partly supported by other grants. We realized at that time that the concepts were sufficiently general that many other studies employing GC/MS would benefit if the concepts were transformed into working systems. We proposed a) to develop methods for quantitative comparison of mixtures analyzed by GC/MS; b) to utilize GC/MS data acquired at both low resolving powers (GC/LRMS) and high resolving powers (GC/HRMS) to determine structures of unknown compounds encountered in complex mixtures; and c) to upgrade and improve our libraries of mass spectral data.

Our efforts have culminated in a series of papers which discuss several aspects of the use of computer techniques to assist in data analysis and interpretation. The first paper (Smith, et al., 1977; Appendix 1) discuss our approach to the quantitative comparison of GC/MS profiles of complex mixtures. This approach embodies the plan described in our previous proposal. GC/MS data are processed to locate points of component elution and to obtain mass spectra free from background and contributions from overlapping spectra (Dromey, et al., 1976) (See Fig. 1 of Appendix 1).

Subsequently, spectra of added hydrocarbons are located and their elution times utilized to determine relative retention indexes (RRI's). Then spectra of internal standards are located and the concentration (based on peak areas) of each component relative to the standard is determined. These data, the collection of "clean" mass spectra, RRI's and relative concentrations, constitute the GC/MS profile for a mixture. We have developed methods (Smith, et al., 1977) for collecting these profiles into libraries to obtain a historical record of past observations. We have also developed the necessary programs for comparing new data to an historical library. The results of the comparison quickly indicate which components are present in abnormal amounts. The collection of programs available in our laboratories constitutes a powerful tool for analyzing and comparing complex mixtures of any organic compounds which can be suitably derivatized for analysis by GC/MS. As Appendix 1 indicates, we have used the historical library approach to validate our analytical procedures for isolation of organic materials in human body fluids. More recently, we have been using the approach to establish baselines on organic constituents of both urine and amniotic fluid.

Two additional papers (Smith and Carhart, 1977; Carhart, et al., 1977; Appendix 1) discuss approaches to interpretation of mass spectral and other chemical data in terms of molecular structure. These studies, carried out together with our collaborators in the SUMEX and DENDRAL projects, have used actual unknowns discovered during the course of our GC/MS analyses of mixtures. GC/LRMS and GC/HRMS experiments provided mass spectral data on unknowns. Subsequently, structural candidates for these unknowns were propoed based on computer-assisted analysis of the mass spectral data together with other chemical information. Additional details may be found in Appendix 1. The techniques discussed in these papers are also quite general, and like the historical library approach, can be used in the study of diverse chemical problems, including those outlined in subsequent sections.

We have made considerable progress in improving and extending our library of mass spectral data. We have added new spectra to the library by running spectra of standard compounds and processing the spectral data with the CLEANUP program (Dromey, et al, 1976). We have improved the quality of existing spectra using the historical library approach outlined above ("HISLIB", Smith, et al., 1977). HISLIB averages spectra of the same compound observed in several GC/MS runs. Thus, statistical variations in ion abundances are reduced as additional spectra are averaged. The resulting spectrum is frequently of much higher quality than spectra in existing libraries. We have implemented a mechanism for adding averaged spectra to or replacing spectra in our primary library. This provides a mechanism for gradual improvement of our libraries with time. In addition, relative retention indexes are included with the spectra now, enabling us to improve the certainty with which subsequent spectra are matched to the primary library.

NASA SRT BUDGET
Period 12/1/77 - 11/30/78

Prepared 8/2/77

PERSONNEL

J. Lederberg	Principal Investigator		$ 579*	
E. Levinthal	Co-Principal Investigator	06	2,509	
D. Smith	Research Associate	25	6,295	
H. Pettigrew	Sr. REsearch Assistant	25	4,416	
W. Fitch	Postdoctoral Scholar	25	3,241	
T. Everhart	Research Assistant	50	7,235	
N. Veizades	Research Engineer	06	1,606	
E. Steed	Research Engineer	07	1,333	
P. Evans	Life Science Technician	25	3,400	
H. Pham	Laboratory Assistant	25	2,788	
W. Harlow	Machinist	03	621	
D. Pearson	Electronics Technician	06	1,047	
H. Bush	Glassware Washer	25	2,403	
M. Wyche	Glassware Washer	50	6,800	
M. Allan	Secretary	20	2,785	$ 47,058

*This portion of Dr. Lederberg's salary will be cost shared by the university. The remaining effort will be without funding from this source.

Staff Benefits 9/1/77 - 8/31/78 at 20% 9/1/78 - 8/31/78 at 20.8%	$ 9,506

EXPENDABLE SUPPLIES

Laboratory Supplies	$ 6,540	
Books	156	
Postage	96	
Communications	1,092	
Miscellaneous office supplies	684	
Equipment Maintenance	5,184	$ 13,752

TOTAL DIRECT COSTS	$ 70,316
INDIRECT COSTS at 58%	$ 40,784
TOTAL BUDGET	$111,100
LESS UNIVERSITY COST SHARING	$ 1,100
TOTAL REQUESTED BUDGET	$110,000