GENETICS

Technical Report No. IRL 1123

CYTOCHEMICAL STUDIES OF PLANETARY MICROORGANISMS EXPLORATIONS IN EXOBIOLOGY

Status Report Covering Period July 1, 1970 to January 1, 1971
For
National Aeronautics and Space Administration
Grant NGR-05-020-004

Instrumentation Research Laboratory, Department of Genetics
Stanford University School of Medicine
Stanford, California 94305

Report to the National Aeronautics and Space Administration

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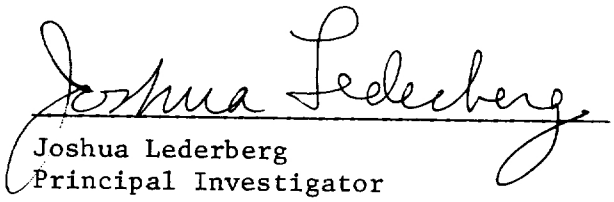
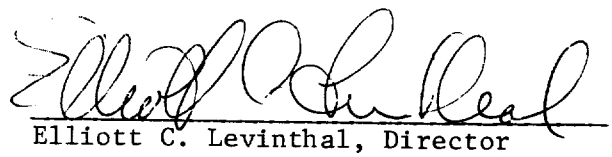


Joshua Lederberg
Principal Investigator



Elliott C. Levinthal, Director
Instrumentation Research Laboratory

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A. INTRODUCTION

This status report covers the activities of the Instrumentation Research Laboratory during the two quarter period from July 1, 1970 to January 1, 1971. It includes activities of the laboratory which relate to or have benefited from NASA support regardless of whether or not they have received direct support by this NASA grant.

During this reporting period Dr. Halpern has left the laboratory to return to Australia. His responsibilities have been assumed by Dr. Alan Duffield. Dr. Duffield retains some responsibilities in connection with Professor Djerassi's mass spectrometry laboratory in the Chemistry Department. This permits close cooperation and collaboration of two groups which have common interests in instrumentation and analysis through the NASA program, DENDRAL and various NIH grants.

The general areas of the Program Resume, Part B of the report, are:

I. Constituents of Urine

II. Chlorination of DNA Bases

III. The Hydrolysis of the Cyclamate in Acid and Chlorox Solutions

IV. Analysis of Natural Products by Mass Spectrometry

V. DENDRAL

VI. Computer Aided Research Instrumentation

VII. Cell Separation

VIII. Mariner Mars 1971 Orbiter Photography

IX. Other NASA Activities

B. PROGRAM RESUME

I. Constituents of Urine

Chemical methods are being developed for the analysis of the basic and acidic constituents of urine. The bases are present in far smaller amounts than are the acids and the isolation of the former requires maximum sensitivity from the gas chromatography-mass spectrometry system. To accomplish this the interface between the gas chromatograph and mass spectrometer has been redesigned. Included in this development has been the construction of an all glass, remotely controlled, high vacuum valve. Chemical derivatization of the acidic and basic components of urine has been developed to enable gas chromatographic separation of the constituents.

II. Chlorination of DNA Bases

Chlorination studies of the bases present in DNA under biological conditions using aqueous chlorox solutions have continued. An assay for nitrogen bound chlorine shows that the products from this reaction are N-chloroamines. The isolation of these compounds from aqueous solution is being investigated. Because of the inherently unstable nature of the produced N-chloroamines chemical reactions are being developed which would transform the unstable product into chemically stable compounds, the isolation of which from aqueous solution would present no problems.

III. The Hydrolysis of the Cyclamate in Acid and Chlorox Solutions

Chlorination in aqueous solution with chlorox or acid hydrolysis of cyclamate results in the formation of cyclohexyl amine. Cyclohexyl amine is known to have a deleterious effect in man. A new quantitative spectrophotometric method for the detection of cyclohexyl amine has been developed. This technique is an improvement over existing methods and allows the detection of cyclohexyl amine in aqueous solution in the 10^{-11} gram range. Using this technique, we have measured the rate of hydrolysis of cyclamate to cyclohexyl amine in acid solution.

IV. Analysis of Natural Products by Mass Spectrometry

During the past year research has continued on the structural analysis by mass spectrometry of natural products isolated from plant, animal and marine sources. A list of publications resulting from this experimentation follows:

"Application of Ion Cyclotron Resonance to the Structure Elucidation of the C_3H_6O. Ion Formed in the Double McLafferty Rearrangement" by G. Eadon, J. Diekman and C. Djerassi. J. Am. Chem. Soc. 92, 6205 (1970).

"Mass Spectrometry in Structural and Stereochemical Problems - CLXXXIX: Electron-Impact Induced Fragmentations and Rearrangements of Alkoxycyclohexanol Trimethylsilyl Ethers and Alkoxycyclohexyl Trimethylsilanes" by R. T. Gray, J. Diekman, G. L. Larson, W. K. Musker and C. Djerassi. Org. Mass Spectrometry 3, 973 (1970).

"Mass Spectrometry in Structural and Stereochemical Problems - CLXXXIV: Charge Localization in Fragment Ions of Amino Ketones and Esters" by J. Cable, G. W. Adelstein, J. Gore and C. Djerassi. Org. Mass Spectrometry 3, 439 (1970).

"Mass Spectrometry in Structural and Stereochemical Problems. CLXXXI. Further Studies of Remote Group Interactions after Electron Impact in 4-Substituted Cyclohexanones" by R. T. Gray, R. J. Spangler and C. Djerassi. J. Org. Chem. 35, 1525 (1970).

V. DENDRAL

An evaluation of new high performance mass spectrometers was carried out to plan new instrumentation for this program.

This system plans to incorporate a high degree of computer control. The goal of the instrumentation project will be to combine the analysis of the DENDRAL computer program with the data acquisition and control capability of the computer. It is planned to do a fast preliminary data acquisition, let the DENDRAL program determine what additional data and data mode is desirable, have the computer control the instrument mode and data scan, and return the pertinent data to the DENDRAL program. Further iterations of this cycle can be repeated as long as the sample persists.

It is planned to connect a GLC to the inlet of the mass spectrometer. The persistence of a given sample is determined by the duration of a GLC peak, a few seconds to a very few minutes. The mass spectrometer of the kind we are considering could usefully take data in many modes; low, high, ultra-high resolution and meta-stables, high or low ionization potential, etc. It cannot acquire all this data in the time span allowed by a single GLC peak. Hence it is required that the computer determine, during the limited sampling time the most useful mode of operation, and then implement this optimum mode.

The DENDRAL research effort has continued to develop along several dimensions during this period. The mass spectra of some previously uninvestigated compounds were recorded. The computer program has been extended to analyze the mass spectra of a more complex class of compounds, using new kinds of data. And the artificial intelligence work on theory formation and program generality has progressed.

Many mass spectra were taken to gather more data for the DENDRAL Program. The analysis of the mass spectra of carbamates and methoxyoximes provided general mass spectrometry rules for the computer program. The spectra of many steroids were taken to elucidate the mass spectrometry of steroids and to provide data for a problem area new to DENDRAL.

The steroid problem is new in several respects: First, in working with steroids, the program deals with much more complex molecules than ever before; second, the computer program uses element maps from high resolution data to resolve ambiguities; and third, the program uses metastable peaks to determine parent-daughter relationships between ions and thus to distinguish molecular ions and their primary fragments. Programming for the preliminary analysis of steroid spectra is nearing completion, and will be useful in the laboratory even though the complete computer program for analyses of this complexity has not been finished.

The artificial intelligence interests of the DENDRAL group are reflected in recent work in program generality, partly described in reference (A), and in the program we call meta-DENDRAL described in reference (C), which will infer mass spectrometry rules from collections of data. Parts of the meta-DENDRAL program have been written which codify observations about mass spectrometry, and work has started on the succeeding phase of the program which will generalize these observations into tentative rules.

A. Feigenbaum, E. A., Buchanan, B. G., and Lederberg, J. "On Generality and Problem Solving: A Case Study Using the DENDRAL Program", in Machine Intelligence 6, B. Meltzer and D. Michie (eds). Edinburgh University Press, 1971.

B. Buchanan, G. and Lederberg, J. "The Heuristic DENDRAL Program for Explaining Empirical Data". To be presented at the 1971 Congress of the International Federation of Information Processing Society (August, 1971) and published by North Holland Publishing Co. (in press)

C. Buchanan, G., Feigenbaum, E. A., and Lederberg, J. "Beyond Heuristic DENDRAL". To be presented at the International Joint Conference on Artificial Intelligence (September, 1971) and published in the Proceedings.

VI. Computer Aided Research Instrumentation

This period has been marked by the growing cooperative efforts with other governmental and academic groups in both the developing and utilization of computer techniques in scientific instrumentation. The principal efforts in the development of new techniques have been in

a) The development of dispersed computer elements in time-shared computer systems for instrumentation, and

b) Continuing work in programming for data acquisition and reduction from the low resolution magnetic mass spectrometer class of instruments.

Dispersed Computer System for Instrumentation

The project for development of dispersed computers is the "smart"* terminal project introduced in our last report being carried out in collaboration with Professor Melvin Schwartz of the Physics Department. Professor Schwartz's interest concerned a Direct Memory Access (DMA) for a PDP-11. This forms an important portion of our "HIQ" terminal concept. This phase has been essentially completed during this reporting period.

*Also referred to as the HIQ terminal.

The HIQ terminal is the result of our experience in connecting scientific instruments to various computers and the current supply of powerful but economical mini-computers. These computers have desirable attributes for dedicated real-time instrument interaction. However, when used alone they can require costly software developments. Current time-shared computers such as ACME have very efficient programming generating capabilities. In the present configuration the HIQ terminal utilizes a PDP-11 mini-computer. The mini-computer is tied to the ACME time-shared computer. The system facilities for the mini-computer will lie wholly in the larger time-shared computer: program manuscript editing, compiling or assembly, and the handling of files. All these functions benefit by the programming efficiency of the larger computer. In addition the time-shared computer replaces local tape and disc units.

By the end of 1970 the prototype HIQ using the PDP-11 as a Local Processing Unit was connected to ACME via our existing data lines. Program writing code assembly, and a degree of control by ACME, the time-shared computer, has been achieved.

Next in development will be the use of the HIQ to preprocess data from the existing mass spectrometer data channels in our Chemistry Department. After that we will integrate the Direct Memory Access (DMA) unit for use with the next generation of mass spectrometers, develop telephone line communication, and other "front end" modules.

VII. Cell Separation

This work, initiated on the subject grant, is now almost completely supported by NIH grant GM 17367 and NIH contract 69-2064.

A. High Speed Fluorescent Cell Sorting

This unit is designed to measure the fluorescence of cells in a jet of liquid, break up the jet into uniform drops and collect the drops in a series of containers, with those drops containing cells of the same fluorescent characteristics collected together.

The sheath flow system using the blue laser, mentioned in the last report is now operational and has increased the sensitivity as hoped. Several experiments are underway in which cells with specific immunological properties are rendered fluorescent by reacting with fluorescein conjugated antigen, or with fluorescein conjugated antibody to an antigen with which the cells have reacted. Isolation of such cells can be of great assistance in solving a wide variety of immunological problems, and the instrument has aroused widespread interest. A second unit with two optical channels, is being built.

B. High Speed Volumetric Cell Analyzer

This instrument and its applications have been described in a paper recently accepted by The Review of Scientific Instruments.[1]

1. "An Improved Cell-Volume Analyzer" by J. T. Merrill, N. Veizades, H. R. Hulett, P.L.Wolf and L.A.Herzenberg. Rev.Sci.Instr.,in press (1970).

C. Use of Fluorescent Techniques to Test Immunological Compatibility

This unit has been built and is now being tested. We have had trouble in distinguishing between fluorescent cells and oil globules picked up from a film of oil spread over the samples to prevent evaporation. This problem seems now to have been resolved. Tests are continuing.

VIII. Mariner Mars 1971 Orbiter Photography

1. General Description of the System

The work reported in the last status report is being continued pursuant to a "production" phase beginning in November 1971 and lasting 90 days. As previously reported, interest lies in the area of the automated analysis of images to be returned from Mars by the MM'71 orbiting spacecraft. Major topics of concern are: geometric and photometric alignment of images, the analysis of image differences, image contouring and feature recognition, and associated problems of data compression and file management.

An important element of the system will be the ease with which the scientist is able to interact with the data and the flexibility with which information can be extracted. Figure 1 is a graphical representation of this interaction and other communication paths in the system.

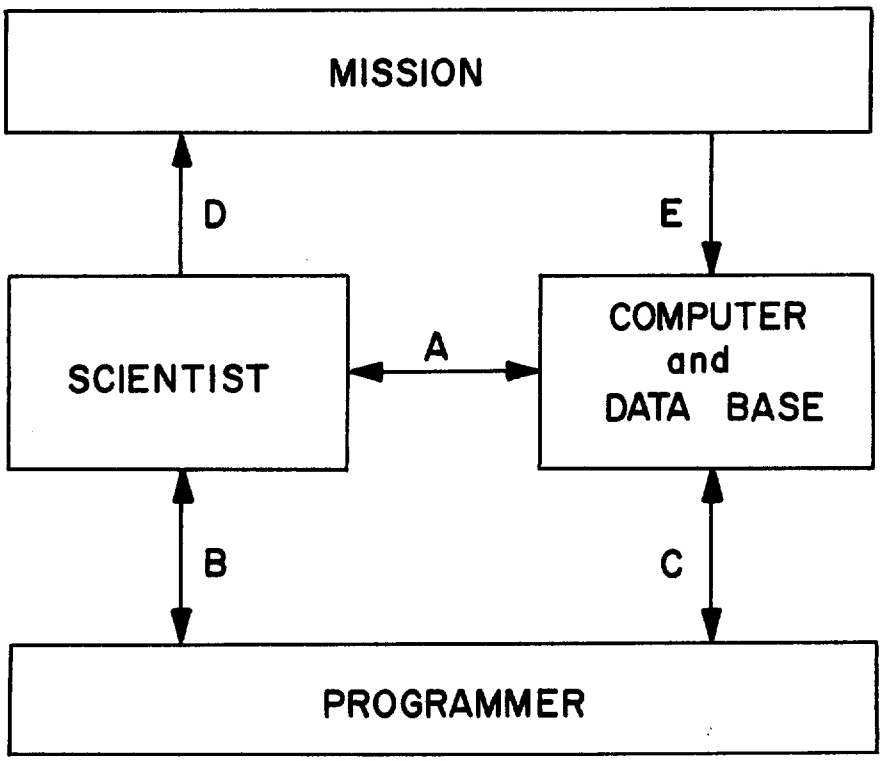
MISSION
D
E
SCIENTIST
A
COMPUTER
and
DATA BASE
B
C
PROGRAMMER

Figure 1

The following is a description of the illustrated paths.

A. The path between the scientist and the data will utilize an interactive computer terminal and a variety of graphic output devices.

B. The scientist-programmer path will provide for determining what new hypothesis can be practically tested using automated techniques.

C. The path between the programmer and the computer is again the interactive terminal where new programming can be developed during the mission in response to new requirements.

D. The existence of this path provides for the adaptive nature of the mission. As the result of extracting information from previously gathered data, the scientist is able to maximize the quality of the data gathered later.

E. The path between the mission and the data base will make use of either a 50K baud transmission line or air shipment of magnetic tapes.

Fundamental to the success of such a system is the efficient handling of the image data. A data compression scheme has been implemented to increase this efficiency. The method, a modified Huffman coding scheme, provides for reducing the amount of data storage required by about a factor of three without the loss of any information. It is also intended that a computer based "atlas" of Mars information will be developed.

Specific Techniques and Problems

The application of this interactive computer image processing system to the analysis of the TV images of a planet (Mars) has as its purpose the detection of changes on the surface with respect to time (time variable surface features), or other differences between images (such as color, polarization, etc.).

To accomplish this task, the system compares pairs of images which were taken at different times, from different spacecraft positions, and perhaps even different spacecraft.

Since the images are different perspective views of a sphere (ellipsoid), (no surface elevations assumed), a geometric normalization is required to relate common surface points. In particular, each image is transformed to the same orthographic projection of the sphere. If there were no errors in our knowledge of the spacecraft position and orientation, no geometric distortions in the optical and electronic systems of the TV camera, and our object is a perfect sphere, then the two transformed images should be in exact geometric correspondence.

Since the images are taken under different illumination and view angles, a photometric correction is required to relate light intensity levels received at the camera to albedo on the surface. If there were no errors in our knowledge of the photometric function at each location on the planet, and no errors in the photometric response of the vidicon, then in theory we should be able to precisely determine the albedo at each point in the images and perform albedo comparisons to detect variable features.

Unfortunately, all of these sources of error exist, with spacecraft orientation contributing the largest errors to geometric registration, and vidicon response and lack of knowledge of the photometric function contributing the largest errors to photometric registration.

Geometric Registration

In order to remove geometric errors, it is necessary to align geometrically corresponding features (areas) in the two images. A technique was developed which displaces one image relative to the other, searching for a (dx, dy) translation vector which maximizes the cross correlation of the images over a specified area. Maximum cross correlation occurs when the images are properly registered in that area.

If we know the (dx, dy) translation necessary to properly register every point in the geometrically normalized images, then we can analyze the images point by point for differences.

The assumption is made that the misregistration vector as a function of position in the image is a smooth continuous function such that it can be modelled by low order polynomials in two variables. This assumption is realistic if the model of the object is accurate (i.e. surface elevations are small or view angles are similar) and if remaining (existing) optical and electronic distortions are smooth and continuous.

When we in fact perform a least squares fit of low order polynomials in two variables to a set of optimal translation vectors on normalized MM 6 and 7 images we get very small residual errors, in particular, when

fitting 1st order polynomials (which have 3 degrees of freedom) to from 10 to 100 data points, we consistantly get residual alignment errors of less than 1/2 pixel standard deviation. This at least empirically indicates that the predominant sources of error cause a smooth and continuous misalignment between the two images. For Mariner 6 and 7, the known geometrical errors are primarily in spacecraft (camera) orientation which would cause primarily a translational and to a lesser degree rotational misalignment between two images, and therefore 1st order polynomials in 2 variables are a good approximation.

Having an accurate model for the misalignment between the two images, we can recalculate the orthographic transformation for one of the images, taking into account the model. The images are now registered to the accuracy of the (mis)registration model.

Photometric Registration

The variable features detection problem imposes rather difficult photometric accuracy requirements. Some of the variability anticipated is relatively small reflectance (albedo) changes over rather large areas of the planet. These albedo changes may increase the contrast between two areas, or may only change the average absolute light level in both areas. If the errors in the calibration of the camera system are larger than the albedo changes to be detected then we must improve the calibration using information in the images.

The known repeatable sources of photometric errors are such things as vidicon shading (non-uniform response) and residual images, both of which can be handled in a systematic manner. The primary sources of error which cannot be corrected are an absolute system gain error, and an absolute offset error resembling scattered light. These errors give a system output Y as a function of light input X as: $Y = aX + b$.

Using this model for the photometric misregistration function, we can choose areas in the two images which are assumed to be photometrically equivalent (absolute or relative) and solve for a combined gain and offset error which will minimize the difference between the areas using conventional least squares techniques.

Variable features detection

Once two images are both geometrically and photometrically aligned, the analysis of differences between the images can begin. Certain classes of known differences are expected. These include albedo differences due to errors in the photometric model of the planet, variations of the photometric function from place to place on the planet, and errors due to the effects of slopes (such as crater rims) on the photometric function.

Given images which were taken with approximately the same illumination and view angles (and hence phase angle) the above sources of error should be minimized. The MM 71 Mission B High Sun TV images are intended to satisfy the above requirements, and hence these sources of error (except for regional variation of the photometric function) can

be largely ignored.

The remaining albedo differences can be attributed to temporal variations of the photometric function due to such things as clouds, dust storms, etc.

Science analysis of these albedo variations requires that the pixel by pixel albedo differences be reduced to area differences and other graphical representations of the differences. One useful form of area difference display is a drawing of albedo difference contour lines.

Another useful display of differences is a graph of the size of an albedo difference area versus time (for a fixed difference level) or a graph of the magnitude of the albedo difference versus time (for a fixed area).

The most appropriate representation of differences depends on the nature of the differences and the insight of the scientists who are trying to understand them.

It is the ability of an interactive time-shared computer system with appropriate graphics devices and image processing software to easily carry out new experiments with the data and to provide new representations to the experimenter which makes such a system invaluable to interactive planetary exploration.

This work has been large supported by JPL Contract 952489. Professor Lederberg is a principal investigator on the MM'71 Television Team and Dr. Levinthal is a co-investigator on that same team.

IX. Other NASA Activities

Professor Lederberg is an investigator on the Biology Science Team for the Viking '75 orbiter and Dr. Levinthal is an investigator on the Lander Imagery Science Team for that same mission. Walter Reynolds of our laboratory is a consultant to Martin Marietta Corporation under a contract with Stanford. Applying the work we have done on computer controlled science instruments he has assisted in defining requirements for the Science Evaluation and Integration Laboratory (SEIL) for the Viking'75 mission.

Three reports were delivered on site in Denver in December 1971, "General Impressions of SEIL Planning," "Software Requirements," and "Description of suggested SEIL-LPU Hardware Configuration." Figure 2 is a diagram from that last report. This is a computer facility designed for the checkout and evaluation of the engineering test models of the scientific instruments to be furnished for the Viking '75 mission. This closely parallels our work here at Stanford.

Dr. Levinthal served on an advisory committee to review a study of Applications of Bio-Space Technology to Patient Monitoring Systems.

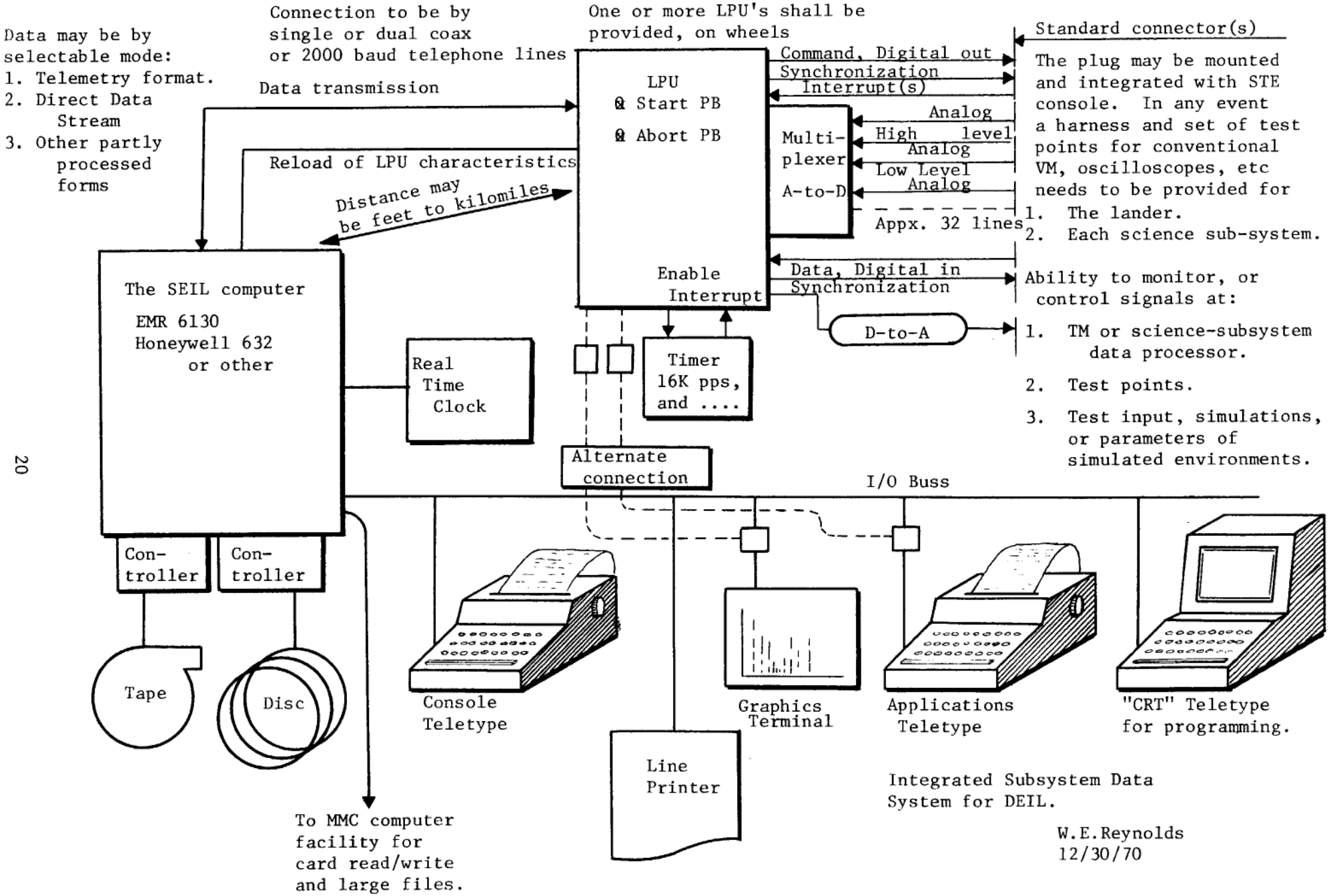
Data may be by selectable mode:
1. Telemetry format.
2. Direct Data Stream
3. Other partly processed forms
Connection to be by single or dual coax or 2000 baud telephone lines
Data transmission
Reload of LPU characteristics
Distance may be feet to kilomiles
One or more LPU's shall be provided, on wheels
LPU
Start PB
Abort PB
Enable Interrupt
Command, Digital out
Synchronization
Interrupt(s)
Multi-plexer
A-to-D
Analog
High level Analog
Low Level Analog
Appx. 32 lines
Data, Digital in
Synchronization
D-to-A
Standard connector(s)
The plug may be mounted and integrated with STE console. In any event a harness and set of test points for conventional VM, oscilloscopes, etc needs to be provided for
1. The lander.
2. Each science sub-system.
Ability to monitor, or control signals at:
1. TM or science-subsystem data processor.
2. Test points.
3. Test input, simulations, or parameters of simulated environments.
The SEIL computer
EMR 6130
Honeywell 632
or other
Real Time Clock
Timer 16K pps, and ....
Alternate connection
I/O Buss
Con-troller
Con-troller
Tape
Disc
Console Teletype
Graphics Terminal
Applications Teletype
"CRT" Teletype for programming.
Line Printer
To MMC computer facility for card read/write and large files.
Integrated Subsystem Data System for DEIL.
W.E.Reynolds
12/30/70

Figure 2

C. PAPERS AND REPORTS

REPORTS

1. Lunar Sample Analysis Final Report. Contract NAS 9-9439. Technical Report No. IRL-1111 (1970)

PUBLICATIONS

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2. H. R. Hulett, Anne Coukell and Walter Bodmer. "Tissue Typing Instrumentation Using the Fluorochromatic Cytotoxicity Assay". Transplantation 10 (1), 135 (1970).

3. W. E. Reynolds, V. A. Bacon, J. C. Bridges, T. C. Coburn, B. Halpern, J. Lederberg, E. C. Levinthal, E. Steed and R. B. Tucker. "A Computer Operated Mass Spectrometer System". Analytical Chem. 42, 1122 (1970).

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5. W. Pereira, V. A. Bacon, W. Patton and B. Halpern. "The Use of R-(+)-1-Phenylethylisocyanate in the Optical Analysis of Asymmetric Secondary Alcohols by Gas Chromatography". Analytical Letters 3 (1), 23 (1970).

6. A. Buchs, A. B. Delfino, A. M. Duffield, C. Djerassi, B. G. Buchanan, E. A. Feigenbaum and J. Lederberg. "Applications of "Artificial Intelligence" for Chemical Inference, VI. Approach to a General Method of Interpreting Low Resolution Mass Spectra with a Computer". Helv. Chimica Acta 53, 1394 (1970).

7. B. Halpern, W. Patton, P. Crabbe. "Cotton Effects of Some Isothiocyanate Derivatives of Amino Acids and Amino Alcohols". J. Chem. Soc., 1143 (1969).

8. G. W. Hodgson, M. A. Holmes and B. Halpern. "Biogeochimstry of Molecular Complexes of Amino Acids with Chlorins and Porphyrins". Geochim. Cosmochim. Acta 34, 1107 (1970).

9. F. W. Karasek, W. H. McFadden, W. E. Reynolds. "GC/MS Computer Techniques". Am. Chem. Soc. Short Course (1970).

10. G. W. Hodgson, E. Bunnenberg, B. Halpern, E. Peterson, K. A. Kvenvolden and C. Ponnamperuma. "Carbon Compounds in Lunar Fines from Mare Tranquillitatis-II. Search for Porphyrins". Proc. of the Apollo 11 Lunar Science Conference, Vol. 2, 1829 (1970).

11. H. R. Hulett. "Amino Acid Synthesis in Simulated Primitive Environments". Science, 170, 1000 (1970).

12. P. E. Crabbe, B. Halpern, E. Santos. "Cotton Effect of Dimedone Condensation Compounds with Optically Active Amines. Tetrahedron 24, 4299 (1970).

13. H. Masursky, R. Batson, W. Borgeson, M. Carr, J. McCauley, D. Milton, R. Wildey, D. Wilhelms, B. Murray, N. Horowitz, R. Leighton, R. Sharp, W. Thompson, G. Briggs, P. Chandeysson, E. Shipley, C. Sagan, J. Lederberg, E. Levinthal, W. Hartmann, T. McCord, B. Smith, M. Davies, G. deVaucouleurs, C. Leovy. "Television Experiment for Mariner Mars 1971. Icarus 12, 10-45 (1970).

PAPERS

1. W. E. Reynolds. "Engineering Comments on the Components of Computerized Mass Spectrometer Systems". Pacific Conference on Chemistry and Spectroscopy, Oct. 6-9, 1970, San Francisco, California.