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CYTOCHEMICAL STUDIES OF PLANETARY MICROORGANISMS EXPLORATIONS IN EXOBIOLOGY

Summary Report Covering Period July 1, 1968 to July 1, 1969
For
National Aeronautics and Space Administration
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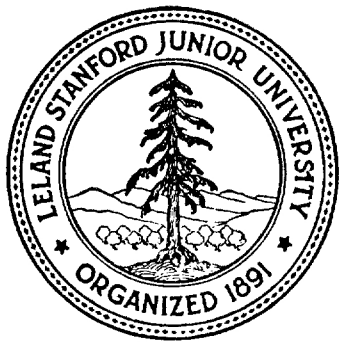

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Report to the National Aeronautics and Space Administration

"Cytochemical Studies of Planetary Microorganisms - Explorations in Exobiology"

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A. INTRODUCTION

This Status Report serves as a programmatic, semi-technical review of the activities of the Instrumentation Research Laboratory for the year July 1, 1968 to July 1, 1969. While the main support of the laboratories' activities during this year has been the NASA grant NGR-05-020-004, some of the research funds have come from other grants, other agencies, and in some cases private institutions.

All the activities have in common the application of advanced techniques of the engineering and physical sciences to problems of biology and medicine. In order to foster as rich an interchange as possible between our NASA directed interests in exobiology (the search for and the elucidation of extraterrestrial life) with other current scientific interests in biological and medical research, we intentionally avoid a clear separation of these lines of work. This has aided the rapid utilization of the ideas and skills developed because of our interests in space missions for medicine and biology in general. Our NASA program also benefits from this exchange. This benefit stems not only by developing sources of new ideas but also by giving us the opportunity of testing instrumentation methods, applicable to future NASA programs, in the circumstances of solving current scientific problems.

For these reasons this report covers all the activities of the laboratory which relate to or which have benefited from NASA support, regardless of their direct support by NASA project funds.

The sense of urgency and commitment to technological innovation associated with the space program are important ingredients of the efforts reported here. Although these pressures are sometimes faulted with the hazard of endangering the most prudent and thoughtful approaches to the solution of concrete problems, they have had the paradoxical effect of protecting and promoting the most innovative experiments.

For example, the large initial investments that are needed to develop computer interfaces to laboratory experiments have discouraged many programs that could, in the long run, create enormous economies and amplifications in scientific analytical techniques. The commitment to solve very difficult problems, even if such investments are called for, has been instrumental in opening up this territory.

We present here a variety of specific programs of instrumentation development. Being at the boundaries of the possible, they are not all strikingly successful. They do help to show us what reasonably can be done by the consistent application of the most advanced physical technology to biological problems.

The general areas of the program resume, Part B of the report, are:

I. Gas Chromatography and Optical Resolution
II. Sequence Analysis of Peptides by Mass Spectrometry
III. Mass Spectral Analysis of Organic Solids
IV. Analysis of Natural Products by Mass Spectrometry
V. DENDRAL - Computer Elucidation of Mass Spectra
VI. Lunar Sample Analysis
VII. Computer Aided Research Instrumentation
VIII. Cell Separation
IX. Optical Data Processing
X. Video Radiograms

B. PROGRAM RESUME

I. Gas Chromatography and Optical Resolution

The aim of our analytical work under grant NGR-05-020-004 is to furnish specific analytical techniques of high sensitivity and optical specificity for the detection of amino acids, peptides and related biologically interesting compounds.

Over the last year some of our new and fast gas chromatographic methods for amino acid analysis, have been used by us for the biochemical screening of blood samples: for high phenylalanine in the detection of phenylketonuria and for the quantitative determination of radiobiological agents in biological materials.

The importance of optical specificity has been well known to biologists since Pasteur's time, but really sensitive methods for optical analysis have only recently been developed. Some of our applications of these techniques have been to the low-level detection of microbial activity and to the optical specificity of several enzyme preparations such as pepsin and chymotrypsin. Since the discrimination of the D and L optical isomers by gas liquid chromatographic separation of diastereoisomers can be explained in terms of conformational influences on intra- and intermolecular hydrogen bond formation, the order of emergence from a g.l.c. column can be used to deduce the stereochemistry at the asymmetric carbon atom. We have now used this procedure to determine the absolute configuration of asymmetric organics. The method correlates the configuration of one compound with that of a homologue, on the basis of the chromatographic behaviour of diastereoisomers derived from the two compounds. This technique is of special value for all those compounds which lack a chromophoric moiety, since here the use of other methods for configurational assignment, such as ORD and CD, is not possible.

Finally the work on amino acid analysis has led to a simple preparative method for the cleavage of peptide from the Merrifield solid phase support. The reaction which involves a simple transesterification reaction offers a convenient route to fully protected peptide intermediates and greatly extends the usefulness of the "solid-phase" method of peptide synthesis.

II. Sequence Analysis of Peptides by Mass Spectrometry

The determination of the amino acid sequence of a particular protein is often necessary for an increased understanding of a biological process. Present sequencing techniques are time consuming because they rely on a stepwise chemical degradation procedure. Recently several mass spectrometric methods for sequencing have been proposed which are based on the fact, that the structure of a linear molecule A-B-C-D-E-F can be deduced from the masses of the intact molecule and of the five fragments of the molecule containing part A (A, A-B, A-B-C, etc.). Several methods for marking the end of the peptide chain are possible, but so far only high resolution mass spectrometry, combined with a sophisticated computer program provides a general method for sequence analysis. In view of the large capital investment in such an installation, we have concentrated on the use of low resolution mass spectrometry for this purpose. Our approach involves the incorporation of an enamine-ketone moiety into the N-terminal amino acid of the peptide chain. The chemical operation involved takes only a few minutes; and the correct amino acid sequence of model peptides containing up to 10 amino acids have been deduced from these mass spectra.

III. Mass Spectral Analysis of Organic Solids

The laser/mass spectrometer solids microanalysis system has been developed to routine operational capability. The system is promptly interconvertible between the laser beam mode and the conventional crucible mode. The laser mode is designed to allow small parts of an extended sample to be scanned for molecular composition. At present, its spatial resolution, in working with bulk samples, is 50 microns. The laser system sensitivity is approximately 1×10^{-9} grams.

The working mass range has been extended to just over 1000.

The ACME computer facility is utilized as a data storage, display and retrieval system. Further refinement of both the spatial resolution and the sensitivity should be achievable, through redesign of the optics, introduction of a laser with shorter pulse duration than the present 1 millisecond, and by increasing the on-time for the electron ionizing beam. However, these innovations would require a larger investment than we can afford to make at the present time.

The system has proved particularly useful for the analysis of small quantities of thermally refractory materials. Of particular interest has been the application to a number of porphyrin compounds synthesized by G. Hodgson (see Lunar Sample section) to reflect particular biogenic and chemical-evolutionary features. Porphyrins occur in a variety of molecular configurations and it is necessary to develop analytical methods for determining whether or not such compounds found in given samples are derived from living organisms. This is of direct interest in examination for extraterrestrial life. In less direct fashion, it relates also to the chemical evolution of life processes. Particular attention has been directed to the association between porphyrins and amino acids, corresponding to the association between chlorophylls (and hemes) and proteins in living organisms. The laser has proven capable of vaporizing intact large porphyrin-amino acid molecules

when these are coupled through regular peptide bonds. Identical treatment of similar molecules coupled through secondary-amine bonds gave strikingly different fractionation patterns, which if details are confirmed by subsequent studies would constitute one means of differentiating between the two classes of coupled molecules.

The suitability of the laser-mass spectrometer system for sample analysis in fiber chromatography was investigated in collaboration with E. Jellum. System sensitivity and lack of suitable porosity in non-vaporizable substrates constituted limiting factors in the applications considered.

B. Halpern has commenced the chemical extraction of samples separated by gas-liquid chromatography on silica gel for analysis by the laser system. This procedure enables several spectra to be readily obtained from a small amount of material with less danger of losing the sample than would be the case in a more conventional mode of operation.

An effort has been made in collaboration with the pathology department to distinguish between normal and lesion human tissues by use of the laser system. Particular attention was directed to the histamine content of the tissues. A similar attempt was made to distinguish between normal and iron deficient blood. The spectra obtained from normal and abnormal samples derived from the same patient positively correlated with one another. Unfortunately, characteristics distinctive of abnormality could not be established.

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. Mass spectrometry is ideally suited to this task because of the extremely small samples size necessary for the recording of a mass spectrum and the quantity of significant information extractable from the spectrum. This is of obvious value in those instances where only a minute amount of natural product can be isolated from a particular source.

A survey of the mass spectrum of an unknown compound often results in the identification of the specific class of organic compound to which it belongs. In favorable circumstances one may be able to postulate a structure for an unknown from an examination of its mass spectrum. Mass spectrometry then is an indispensable tool enabling the natural product chemist to quickly evaluate the results of chemical modification of an unknown compound and to plan future experiments so they will proceed in the most productive direction.

We have also been concerned with the behavior of relatively small organic molecules in the mass spectrometer. This investigation has involved study of the mass spectra of isotopically labeled (deuterium, carbon 13 and oxygen 18) compounds in order to construct basic rules for their fragmentation. These principles are then applied to the interpretation of the mass spectra of unknown compounds of more complex structure.

Interfacing the MS-9 high resolution mass spectrometer with the ACME 360-50 computer system has now been completed. This system will allow very rapid accumulation of complete high resolution mass spectra which will be particularly important to continuing studies of the analysis of natural products. It will be possible to unambiguously assign an empirical composition to an unknown natural product in under one minute using far less than one milligram of material.

Publications

Diekman, J.; Thomson, J. B.; and Djerassi, C.: Mass Spectrometry in Structural and Stereochemical Problems. CLV. The Electron Impact Induced Fragmentations and Rearrangements of Some Trimethylsilyl Ethers of Aliphatic Glycols and Related Compounds. J. Org. Chem., 33, 2271 (1968).

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Duffield, A. M.; Shapiro, R. H.: Mass Spectra of Quinoline and Isoquinoline N-Oxides. Tetrahedron, 24, 3139 (1968).

Buchardt, O.; Duffield, A. M.; and Djerassi, C.: Mass Spectrometry in Structural and Stereochemical Problems CLVII. A Study of the Fragmentation Processes of Some N-Acyl-2-indolinols Upon Electron Impact. Acta Chem. Scand., in press.

Duffield, A. M.; Djerassi, C.: Mass Spectrometry in Structural and Stereochemical Problems CLXVI. The Electron Impact Remoted Fragmentation of Some Aliphatic 1,2-Glycols. Org. Mass Spectry., in press.

Kossanyi, J.; Morizur, J. P.; Furth, B.; and Wiemann, J; Duffield, A. M.; and Djerassi, C. Mass Spectrometry in Structural and Stereochemical Problems. The Electron Impact Promoted Fragmentation of the Aliphatic 1,2-Glycols. Org. Mass Spec., 1, 777 (1968).

Carpenter, W.; Duffield, A. M.; Djerassi, C.; Mass Spectrometry in Structural and Stereochemical Problems Effect of Fluorine Substitution on the McLafferty Rearrangement of Aliphatic Ketones. Org. Mass Spec., 2, 317 (1969).

Buchardt, O.; Duffield, A. M.; and Djerassi, C. Mass Spectrometry in Structural and Stereochemical Problems. A Study of the Fragmentation Processes of Some Benzoazepines Upon Electron Impact. Acta Chem. Scand. (in press).

V. DENDRAL - Computer Elucidation of Mass Spectra

The "scientific method" involves two very different kinds of intelligent operation, sometimes called induction and deduction respectively. A theory is somehow "induced", sometimes out of sheer spectulation, sometimes in order to account for some hitherto baffling or provocative observations of nature. Then, the theory is applied deductively, i.e., logically or mathematically rigorous conclusions are made: if the theory is true, then certain results must be found. Philosophers of science are now generally agreed that a theory can never be proven or logically derived from factual data. We accept a theory as true when it has made some new predictions, different from the predictions of other theories, which survive the test of experimental measurement.

The process of logical deduction follows rules which, at least at an elementary level, are well understood. Correspondingly, computers have been extensively used for deductive calculations: for example, to predict the path of a ballistic projectile in the gravitational fields of the solar system. When discrepancies are found, the theory must be questioned at some level; for example, the mascons are postulated as a "simpler" explanation of certain perturbations, rather than a revision of the laws of gravitational attraction.

Scientific induction remains a mysterious process, connected to the most "creative" aspects of human thinking, and the most difficult to implement on the computer.

The DENDRAL project aims at emulating in a computer program of inductive behavior of the organic chemist in an important but sharply limited area of science, organic chemistry. Most of our work is addressed to this problem:

Given: the data of the mass spectrum of an unknown compound

To Induce: a workable number of plausible solutions, that is a small list of candidate molecular structures.

In order to complete the task, the DENDRAL program also

> Deduces the mass spectrum predicted by the theory of mass spectrometry for each of the candidates, and
>
> Selects the most productive hypothesis, i.e., the structure whose predicted spectrum most closely matches the data. This part of the program entails no conceptual difficulties, and would be more typical of well-established uses of the computer.

We do not pretend to have achieved any important new insights into human creativity. We have, however, succeeded in designing, engineering, and demonstrating a computer program that manifests many aspects of human problem-solving techniques. It also works faster than human intelligence in solving problems chosen from an appropriately limited domain of types of compounds, as illustrated in the cited publications.

Some of the essential features of the DENDRAL program include:

1) A conceptualization of organic chemistry in terms of topological graph theory, i.e., a general theory of ways of combining atoms.
2) Embodying this approach in an exhaustive hypothesis-generator: this is a program which is capable, in principle, of "imagining" every conceivable molecular structure.
3) Organizing the generator so that it avoids duplication and irrelevancy, and moves from structure to structure in an orderly and predictable way.

The key concept is that induction becomes a process of efficient selection from the domain of all possible structures. "Heuristic" is an adjective in computer-jargon for "efficient selection".

4) Most of the ingenuity in the program is devoted to heuristic modifications of the generator:
5) early pruning of unproductive or implausible branches of the search tree,

6) consulting the data for cues (pattern analysis) that can be used to re-schedule the generator for a more effective order of priorities,

7) incorporating a memory of solved sub-problems in a dictionary that can be consulted so as to look up a result rather than compute it over and over again,

8) providing easy facilities for entry of new ideas by the chemist when discrepancies are perceived between the actually functioning of the program and his expectation of it.

Two further design principles have emerged:

a) It is absolutely essential that the program's copy of the "theory of the real world" be centralized and unified. Otherwise, during the evolution of the program it will inevitably accumulate psychotic inconsistencies - like expecting organic compounds possibly to contain sulfur in one module of the theory, and denying it in another.

b) It is advantageous to derive cues for plausible attack by introspection from the program's own theory, rather than from external data which may not yet have been assimilated into the theory. The success of the program depends in every case on the validity of the theory, so there is no use going beyond it. Then, it is more efficient for the computer to play internal games to look for patterns (like mass number 45 corresponds to -COOH) than to wait for experimental data. The theory should be responsive to the data; then the list of preliminary cues should be generated from the theory.

The attached references report the practical application of DENDRAL as an aid in solving problems of chemical structure. While our main interest in DENDRAL is as a prototype of scientific induction, it may have specific application in guiding the closed-loop automation of an analytical mass spectrometer/GLC or general chemical fractionation system. This would be landed on a planet with the instruction:

"describe the overall pattern of natural product chemistry", and waste no undue time in repeating patterns of analysis already completed. At a simpler level we are working on the closed loop operation of an MS which is guided to concentrate its attention on resolving those mass-peaks which are critical to solving the structure.

This work has been supported mainly by a grant from the Advanced Research Projects Agency of the Office of the Secretary of Defense (Grant SD-183) with further contributions from the present National Aeronautics and Space Administration program.

VI. Lunar Sample Analysis

Organic substances will be present in returned lunar samples, and it will be necessary to determine whether these are fossil compounds from extraterrestrial life or ordinary organic compounds synthesized under extraterrestrial conditions. In the present study, attention is directed to a specific class of moderately complex organic substances - the porphyrins. These highly colored substances are synthesized from simple compounds containing nitrogen, carbon and hydrogen under high-energy conditions. Specific examples of porphyrins are used by all living organisms for vital life processes - photosynthesis in the case of plants using chlorophyll, and respiration in the case of animals using hemoglobin. Thus, because of chemical evolution from available complex organic molecules, living organisms apparently developed an ability to synthesize porphyrins, and these pigments persist in the environment of living plants and animals as fossil biochemicals in terrestrial rocks.

Extraterrestrial rocks (meteorites) contain porphyrins also, and there is evidence to suggest that similar compounds exist in interstellar dust. It is not yet clear whether these compounds should be regarded as evidence of extraterrestrial life, and the major object of the present study is to develop criteria for differentiating between biogenic porphyrins and non-biogenic porphyrins. To this end, methods are being developed to examine the structure of the extraterrestrial porphyrins on the basis of molecular size, structural configuration, and association with other biochemical compounds - specifically, amino acids, the building blocks of proteins. Magnetic circular dichroism, fluorometry and spectrophotometery are being used to define structural configuration of the organic molecules, and gel permeation chromatography and mass spectrometry are used in determining molecular size. Current data indicate small but significant differences between fossil porphyrins of terrestrial rocks and porphyrins recovered from extraterrestrial meteorites, from which the tentative conclusion is

drawn that extraterrestrial porphyrins are probably not biogenic, but represent an early stage in the chemical evolution of life.

The great abundance of life on earth for the bulk of its history - at least 70% of its history - determines that essentially all terrestrial porphyrins are biogenic. Current analyses of terrestrial lava flows comprising basaltic material similar to that of the lunar surface as revealed by Surveyor analyses, show that porphyrins are present. These are probably also biogenic, but it is possible that they may represent early formation of biochemical compounds. Related studies show that porphyrins combine fairly readily with amino acids, and as a result, a method of formation of porphyrin-protein complexes is illustrated in the context of the chemical evolution of life.

VII. Computer Aided Research Instrumentation

The work on computer aided research instrumentation is largely focused on mass spectrometry and is closely coupled to the Medical School's ACME (Advanced Computer for Medical Research) program with a time-shared IBM 360/50 computer. The long range interest relates to the need for a computer controlled, automated, reprogrammable laboratory for the biological exploration of the planets. Our reports of the achieved systems and methods have generated a great deal of interest and have offered concepts and direct aid to many other brances of scientific effort. Some of these proposed applications have been diverse as mass analysis of newborn babies' urine for detection of birth defects, detecting components of flavors in the food industry, and the computer drawing of illustrations for medical illustrations.

The concept of ACME, had its beginnings in the convictions that the automated laboratory must have an integrated computer. The human researcher would communicate through the computer. The computer would be able to accept gross, concise commands from the human, do the detailed and routine instrument operation, log and process the raw readings from the instrument, and then analyze the data, all as directed by the scientific researcher. An example of this concept is diagrammed in figure 1.

ACME combines the power of a large computer with the ability to serve individual laboratory instrumentation tasks in a time shared mode. In this manner the benefits of an expensive large computer can be economically spread out over a number of users. ACME can routinely service up to twelve instruments, and in addition, about twice as many other programmer applications in a manner that makes it appear to each user that ACME gives him its undivided attention. ACME is an IBM 360/50 computer with just over 2 million words of core memory. The time shared system of ACME has been devised here at Stanford.

It is now about four years since the inception of ACME; the hardware was actually installed about three years ago. In this last year it has started to displace small computers of limited capability which had been dedicated to individual applications. One paper this laboratory presented in 1969 reports the experience we have had in this field with mass spectrometers and is titled "Mass Spectrometers in a Time Shared Computer Environment". It is reproduced at the end of this report as Appendix A.

The MS-9 discussed in that paper is a high resolution mass spectrometer used to obtain the physical data related to the work on DENDRAL and on the analysis of natural products use with the computer. This mass spectrometer-computer system interfaces with the ACME time shared system with its high level computer language ability. It is expected that this has great potential for expanding its capability. We are encouraging the chemical researchers who use the system to exploit the capability of ACME to program more and more sophisticated data analysis. That would be the last two steps illustrated in figure 1. This type of advance in its use will be of interest to the scientific community at large.

The general nature of the instrumentation on the MS-9 mass spectrometer made it quite convenient and economical to relate these developments to Magnetic Circular Dichroism (MCD). It provides a significant increase in the sensitivity of MCD instrumentation. This made it feasible to aid in a porphyrin study of lunar samples. This topic is elsewhere described in this report under Lunar Sample, and a more complete description of the instrumentation is given in Appendix B.

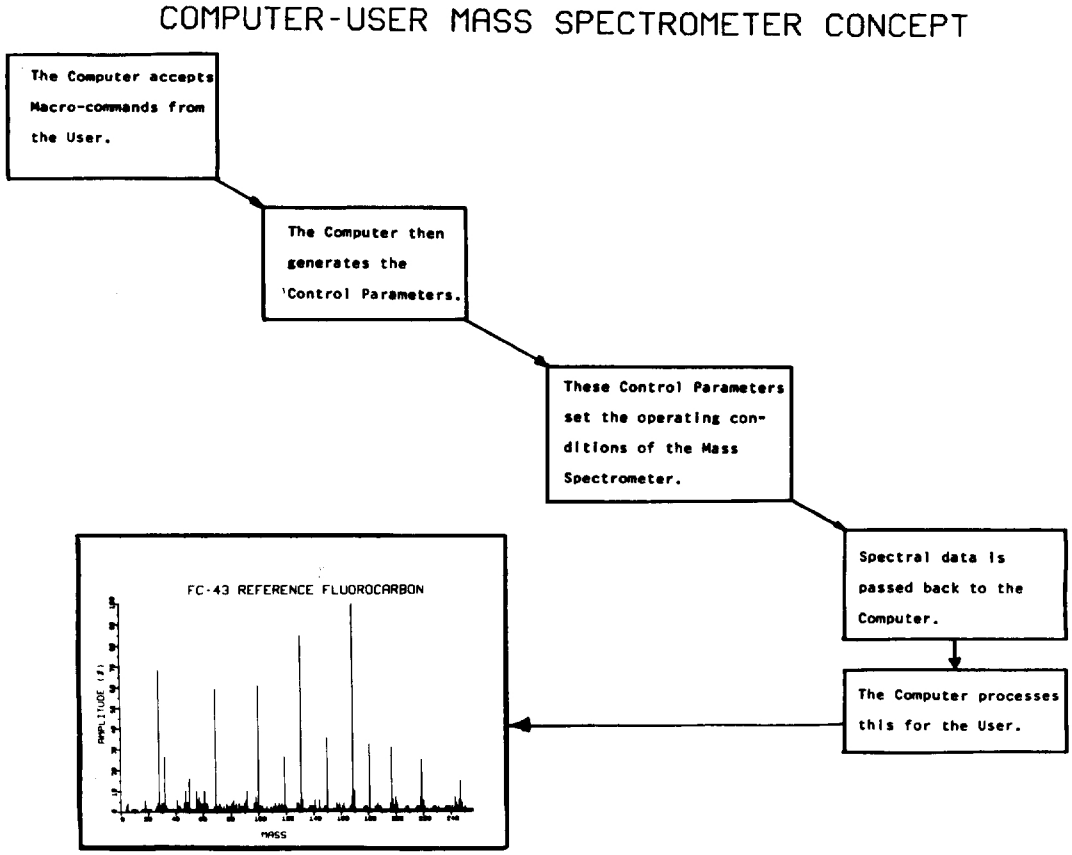
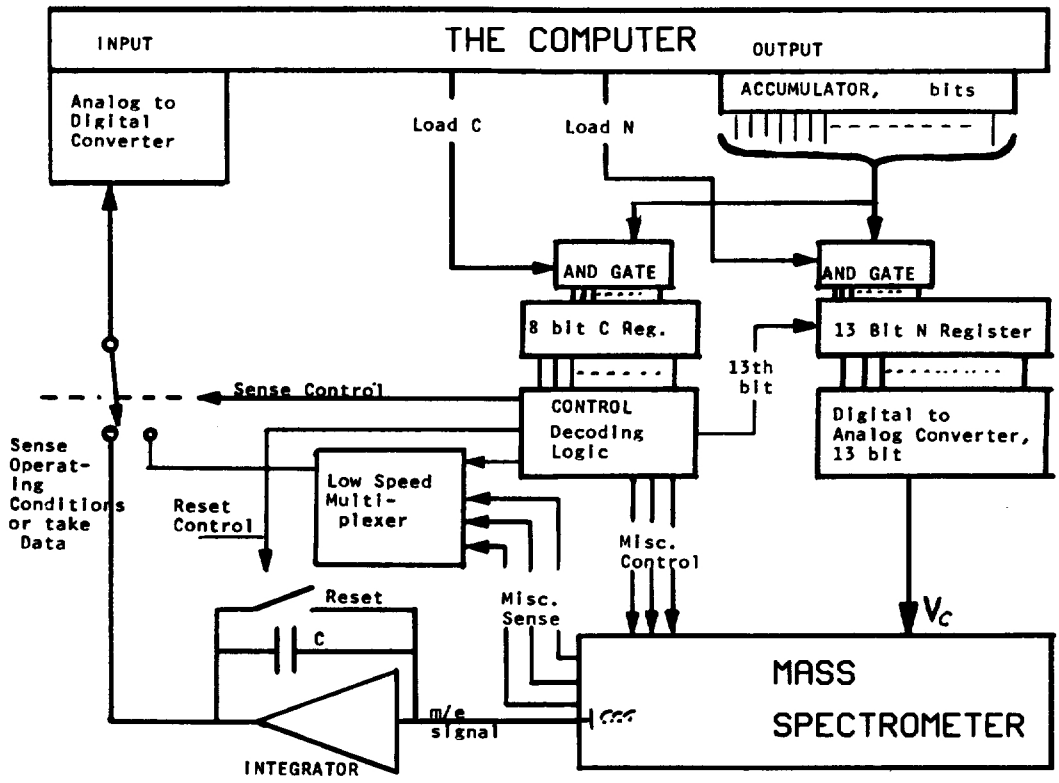


Figure 1. Basic human-computer interaction philosophy.

The computer aided researcher concept as presented in Figure 1, has been described by others and further developed in this laboratory. We first applied it to a low resolution mass spectrometer. This was reported in Technical Report IRL 1062, and at several scientific meetings. The hardware computer and connections are diagrammed in Figure 2. This method of control and instrument computer interconnection was conceived for the specific use of a Mars lander automated laboratory and relates quite closely to the present interests of the Viking mission in gas chromatography-mass spectrometry instrumentation.



THE ELECTRONIC INTERFACE

Figure 2. Block diagram of the hardware connections successful in integer resolution mass spectrometer automation.

This instrumentation has proven to be exceedingly useful in our laboratories and has greatly aided the researchers in the Gas Chromatography and Optical Resolution work given elsewhere in this report. This initially was based on the coupling of a small LINC computer developed by NIH, with a small quadrupole mass spectrometer.

In this last year the system has been transferred from the small computer to the ACME system. This additional computer power enabled a two-fold increase in mass range, and increased amplitude accuracy. The method, especially when the mass spectrometer is connected to a gas chromatograph, has stimulated a great deal of research and industrial interest.

VIII. Cell Separation

A. High Speed Fluorescent Cell Sorter

This unit is designed to measure the fluorescence of cells in a jet of liquid, break up the jet into uniform drops, and divert those drops containing cells with particular fluorescent characteristics into a different container than the remainder. It has been tested on suspensions of cells from mouse spleens, a small fraction of which were producing a specific antibody to sheep red blood cells. It proved able to increase the relative concentration of these cells by factors of ten or more over that in the initial suspensions. So far as we know this is the only device capable of rapidly sorting individual cells of about the same size but with different biological characteristics. Such a sorter would be useful in detecting specific properties of microorganisms and other particles on extraterrestrial surfaces. It also seems applicable to many biological and medical problems on earth and we are attempting to obtain funds from appropriate sources to explore such applications further.

B. High Speed Volumetric Cell Sorter

This unit measures the volume of cells by determining the change in electrical resistance in a small orifice containing a flowing conducting liquid as the essentially nonconductive cells pass through. A jet is formed downstream of the orifice and the liquid broken into droplets. Those droplets containing cells of specified characteristics are deflected as in the previous instrument. The unit works quite well but we have had trouble with the cells dying after passing through the system. This appears to be connected with the low concentrations of protective protein we have been forced to use to prevent clogging of the jet nozzle. Changes in the shape of the channel between the orifice and the jet have alleviated the problem and it is hoped that it will be resolved by use of a sheath flow system which encloses the stream of fluid containing the cells in a larger stream of inert liquid. This

permits use of larger jet nozzles less subject to clogging, and higher protein concentration in the central stream.

The sheath flow system is also being applied to the fluorescent separator, to minimize optical problems in looking through the cylindrical stream. In addition we are designing a sheath flow system using both volumetric and fluorescent detection in the same instrument. This will permit use of two criteria for sorting rather than only one.

C. Use of Fluorescent Techniques to Test Immunological Compatibility

The fluorescent assay we use in the fluorescent cell sorter lends itself to a test for cell wall integrity (fluorochromasia*) useful in immunological assays. The fluorescence is built up inside cells with intact walls by enzymatic action on a nonfluorescent substance. The fluorescent product leaks out rapidly if the cell wall is damaged. Such damage is characteristic of immunological rejection (of the type found in heart transplant cases, for example) but does not happen if immunologically compatible suspensions are mixed. A manual test based on this characteristic is used to determine such compatibility but it is quite laborious. Preliminary experiments indicated that a modified fluorescent cell sorter could be used to make such tests, and we have received a contract from the National Institutes of Health to explore the automation of the test further.

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* Originally designed by Dr. B. Rotman, formerly on the Genetics Department staff. (B. Rotman and B. W. Papermaster, Proc. Natl. Acad. Sci., 55, 134, 1966.)

IX. Optical Data Processing

The Mariner Mars 1971 Orbiter program calls for the placement of two spacecraft into orbit around Mars. Members of this department are involved with the interpretation of the photographic data that will be radioed back to earth from these spacecraft.

The photographic data will be received in digital form and thence fed into a high speed digital computer for data manipulation and image generation. The time required for the computer to fully process an individual image can range from several minutes to upwards of one hour, depending upon the complexity of the corrective and manipulative operations performed.

It will not be possible during the planned 90 days of mission orbital lifetime to perform extensive computer processing of more than a small fraction of the pictures. We are therefore investigating the feasibility of supplementary data processing by optical methods.

It is possible to construct optical systems capable of performing a variety of transformations of images derived from film transparencies. The input to such a system in the present instance would be minimally processed images generated by the computer. Among the operations that can be performed are corrections for a variety of aberrations of the optical transfer function, including those resulting from irregular spatial frequency response and camera motion. It is possible to suppress the periodic structure characteristic of pictures generated from discretely sampled scenes. It is furthermore possible to perform cross correlation and convolution operations.

The optical systems can involve the use of either monochromatic or incoherent light sources. We are currently directing our attention to the more powerful monochromatic techniques involving the use of laser light sources. The effort at the moment is to determine the extent to

which the various available optical processing procedures can be utilized to advantage in operating on the particular data in question and under the real-time constraints of the mission.

We have constructed a nineteen foot optical bench and are now conducting experiments using as input data the photographs derived from the 1964 Mars flyby.

X. Video Radiograms

The Scintillation Camera is an instrument which is capable of recording dynamic events within the body after administration of radioactive tracer material. Rapidly occuring events, such as the movement of a radioactive bolus through the heart and lungs may be studied by making exposures manually every few seconds or by recording the final oscilloscopic display on a time lapse or movie camera. Both of these methods of recording leave much to be desired. The former is cumbersome and nonreproducible. If the times selected do not happen to be exactly right, the value of the study is lost and must be repeated, thus exposing the patient to unnecessary additional radiation. In the case of the latter method, the individual frames do not have adequate information for diagnostic purposes. It seemed essential that a new method of recording be devised which enabled one to record all the information continuously on video tape for the full duration of the study and then be able to replay this information while at the same time taking exposures at new desired times and for any desired interval.

A sub-system was devised and developed to augment data obtained from a scintillation camera. In order to clarify the purpose and merit of this sub-system a simplified description of a typical scintillation camera is initially presented. Radioactive media of short half life in liquid suspension is injected into a patient under study. Differential uptake and flow of the media in vessels or organs of the body is demonstrated by differing radioactive event rates from small areas of the organ under study. Conventional optical or electro-optical techniques are not feasible because of the energy spectra involved; the system presently discussed uses a lead collimator to "image" the radioactive phenomena on a light emitting scintillation crystal. A convenient, though not exact analogy is that of pinhole camera operation. The lead collimator represents the lens, and the scintillator crystal the screen. Whenever a radioactive event occurs within the body of the patient a spatially corresponding flash of light is generated by the

scintillator crystal. An array of photomultiplier tubes detects the emission of light by the crystal, each separate tube furnishing an electrical signal which is fed to a matrixing network. The output of this network consists of a pair of rectangular pulses occurring coincidentally. One pulse defines the location of the event in the X axis, the other pulse in the Y axis.

The two pulses are finally applied to the horizontal and vertical plates of a display oscilloscope tube operating in blanked mode. When the two pulses have reached their maximum value the oscilloscope tube is unblanked for a few microseconds. The observer then sees a bright focused point of light whose position on the tube face represents a point in the body of the patient. The random nature and scintillating appearance of this display, especially at low dosage levels makes analysis of flow patterns very difficult without some integration of the information. In practice some form of photographic storage is used, where appropriate portions of the experiment are integrated for a fraction of a second to a few seconds. Since count rates may vary over a very large range the photographic exposure times are difficult to predict and loss of valuable information may occur during film loading.

One way to obviate these difficulties would be to record the entire set of experimental data in a suitable medium, then select desired areas of the record for later detailed analysis. Although no currently available acquisition and storage system met fully the ideal parameters it was thought that closed circuit television camera and recording techniques offered the optimal solution to the problem, and promised the greatest scope for future improvements. A fully transistorized compact television camera complete with precision synchronising generator was mounted at 90° with respect to the scintillation camera display tube to conserve space. The optical path from tube screen to camera lens was completed by a swing out 45° mirror, and occluded from room light by a detachable hood (Figure 3). The vidicon camera tube has a persistence of about 1/30 of a second so that the very short duration

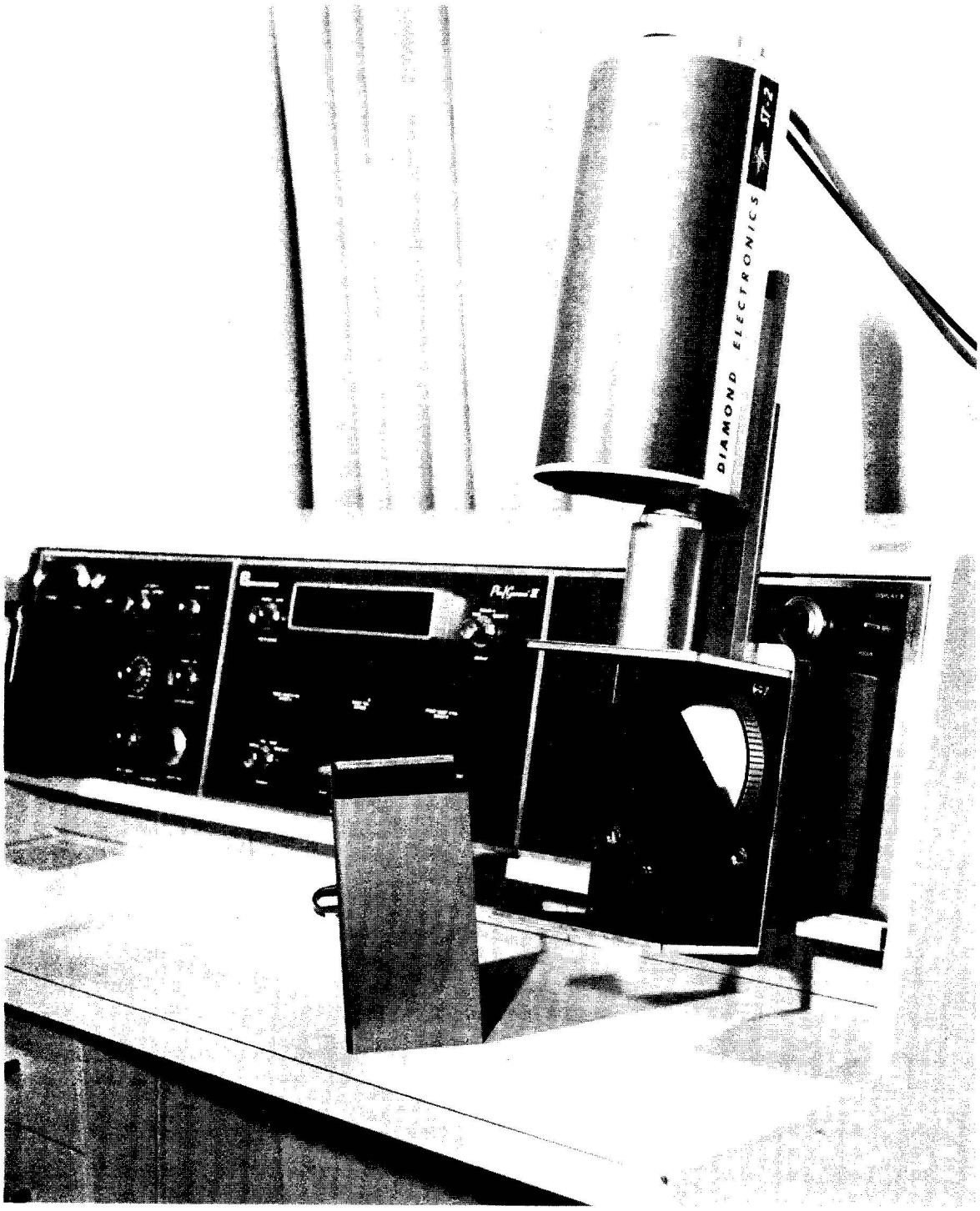

Figure 3. Television camera mounted above scintillation camera display tube.

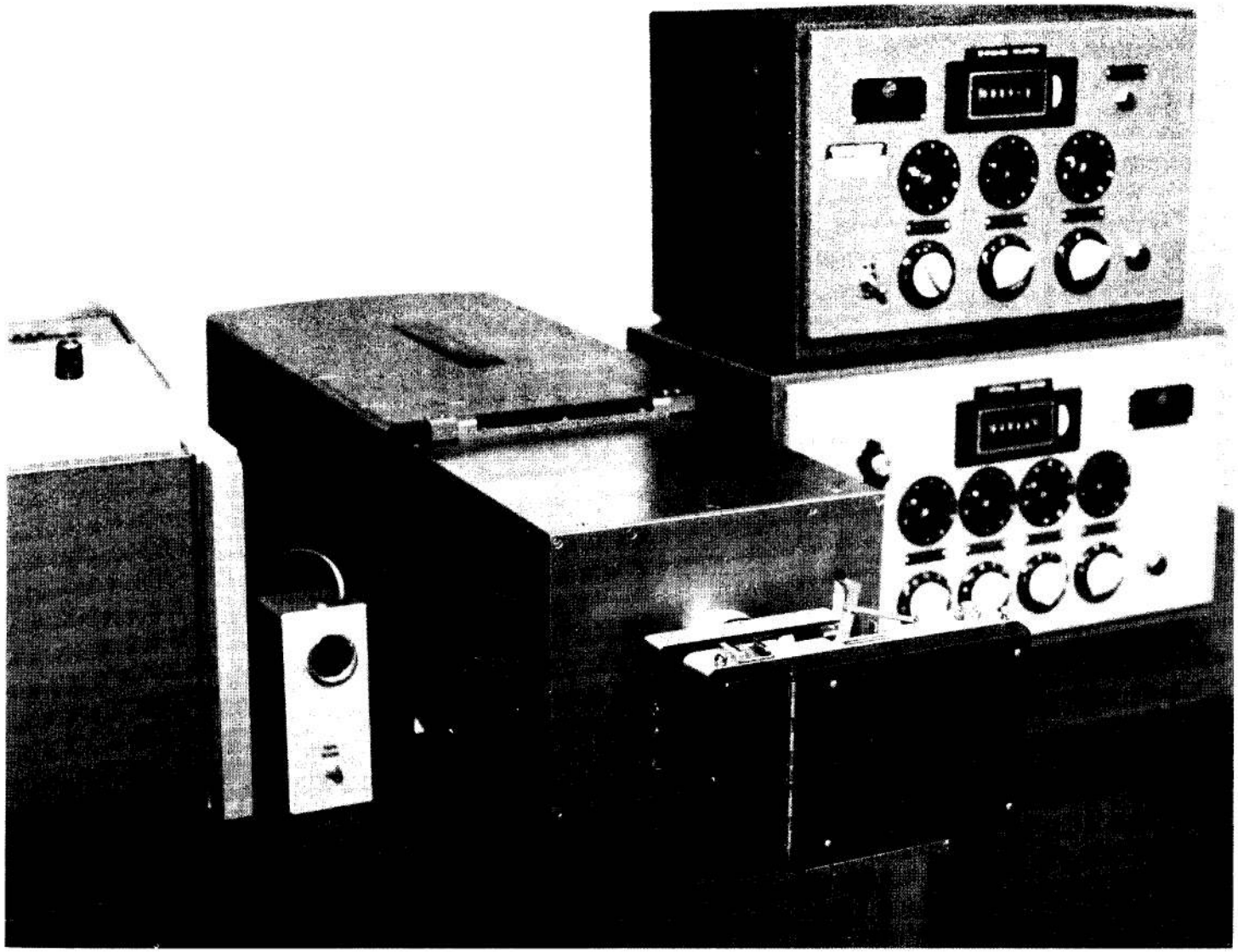

Figure 4. Audio cue generator unit, television monitor, interval and exposure counters.

flashes which make up the original scintillation image are retained until the camera scanning beam reads out the information. The output of the T.V. camera is fed to a videotape recorder and to a T.V. monitor, enabling the progress of an experiment to be visually checked during recording. Simultaneously with the recording of scintillation information the pulses which synchronize the vertical sweep of the T.V. system are impressed on the video tape. Upon playback of the tape these recorded pulses accurately identify the time at which associated information was recorded. In an experiment of 20 minutes duration there would then be 36000 fields of uniquely defined information. Any single field or any sum of fields may be readily identified and selectively displayed by a simple counting and gating procedure. We used two presettable industrial counters (Figure 4) for this purpose, one to count off time intervals from the beginning of the record, the other to count off the selected number of frames of interest. To avoid the necessity of total tape rewind during analysis an audible cue generator was provided. After review of the complete record one or several cues may be recorded to cause the counting system to automatically start the preset sequence. During the counting of frames from the start of a tape record, or from an audible cue the T.V. monitor is blacked out, and a Polaroid camera with opened shutter is focused on the screen. When the preset area of interest is reached, as determined by the interval counter the exposure counts commence a pre-selected count. The T.V. monitor is brightened to a suitable level and exposure of the film commences. At the end of exposure count the T.V. screen is darkened again, and the integrated film may be removed for study. During this cycle of events a hinged hood removes extraneous light from the optical part, multiple exposures are readily achieved by repeating the procedures, but with a suitable change in interval time. As noted, intentional multiple exposures can be made by recording the required number of audible cues.

The initial results in patients have lived up to and exceeded expectations. It now seems probable that many cardiac and pulmonary diseases are amenable to diagnosis by simple and non-traumatic

techniques which requires only two minutes of the patient's time. The modified scintillation camera is called the variable time-lapse scintiscope. A description of the instrument has been published (1). The use of the equipment in the diagnosis of many different forms of heart disease has been repeatedly proved. Several reports have either appeared, are in press, or have been presented before national meetings. (List of publications and abstracts follows).

A grant has been obtained from the Easter Seal Research Foundation to continue some of the work, especially that dealing with heart disease in children.

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APPENDIX A

The text of a paper describing the Instrumentation Research Laboratory's basic work in connection with mass spectrometry and other laboratory instruments with a large computer used in a time-shared mode.

This paper was given at the 17th Annual Conference of Mass Spectrometry and Allied Topics at Dallas, Texas, in May 1969.

Mass Spectrometers in a Time Shared Computer Environment

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Genetics Department, Stanford University School of Medicine
Stanford, California

ABSTRACT

The use of a time shared computer system for mass spectrometer data acquisition and/or instrument control is described. The computers involved are an IBM 360/50 with an interconnected IBM 1800 and a classic LINC. The use described is in a time shared mode, where numerous data processing and instrumentation users are simultaneously served. The mass spectrometers connected are a mix of a high resolution double focusing, a medium resolution single focusing, and a quadrupole instrument in diverse areas, ranging to 1500 feet from the central computer. These and other instruments are served without any prior scheduling or the particular knowledge of other users. Programming is done on remote terminals in a high level language. The experiences in these modes enables comments to be made concerning the relative merits and useful roles, deficiencies, and benefits computer time sharing can offer. This work was sponsored in part by National Aeronautics and Space Administration Grant NGR-05-020-004 (Genetics Department), NIH Grant AM 04275-07-S1 (Chemistry Department), and NIH Grant 5 P07 FR00311-02 (Medical School-Computation Center, Advanced Computer for Medical Research).

In 1965 a group of Stanford researchers and computer science planners formed a committee to establish the basic design of a time-shared computer system that would not only provide the time shared terminal access as had been pioneered by MIT and others, but also would include comparable time shared data channels with connections directly into the laboratory. Early large computers and batch processing had separated the user from the computer. Time shared computers later restored the user to direct contact with the computer. Somewhat in the same manner it is hoped to free the laboratory instrumentation from the constraints of the small computer, or from the restrictions and delays of submitting recorded data to a batch system. The time shared large computer should be able to give acceptable real time service to the instrument in the laboratory.

In Figure 1 the Stanford system, ACME, is shown composed of a 360/50 with 2,065,000 bytes of core with a satellite IBM 1800. The latter functions only as low speed instrument data channel. I have included the Genetics Department's classic LINC computer in the general mass spectrometer systems. It can operate in the system or stand alone. There are three distinct instrumentation data paths possible from a laboratory to the central computer:

1. A high speed unit, up to 25 K bytes/second, called the 270X-270Y. The 270Y is remote in the laboratory. The 270X is local to the computer and has access through the multiplexer.

2. Low speed, up to 1 K samples or words per second, via the 1800 inputs.

3. Last, if used, the a-to-d converter of the LINC.

There are output channels via each path that have analogous attributes. On the left side of Figure 1 are the principal mass spectrometers of this system.

1. Double focus, High Resolution, AEI MS-9.

2. Single focus, Integer Resolution, ATLAS CH 4.

3. Quadrupole, Integer Resolution, Finnigan 1015.

4. Time-Of-Flight, Integer Resolution, Bendix Mod. 12.

A typical laboratory instrumentation center in our concept would contain a data terminal, a keyboard for programming and control, and a graphical output capability. This is functionally arranged in Figure 2.

The computer complex and domain is below the dotted line. Physically the computer connections are in the laboratory, but design, maintenance and specifications of the terminal lie with the computer administration. The special adaptors and additions

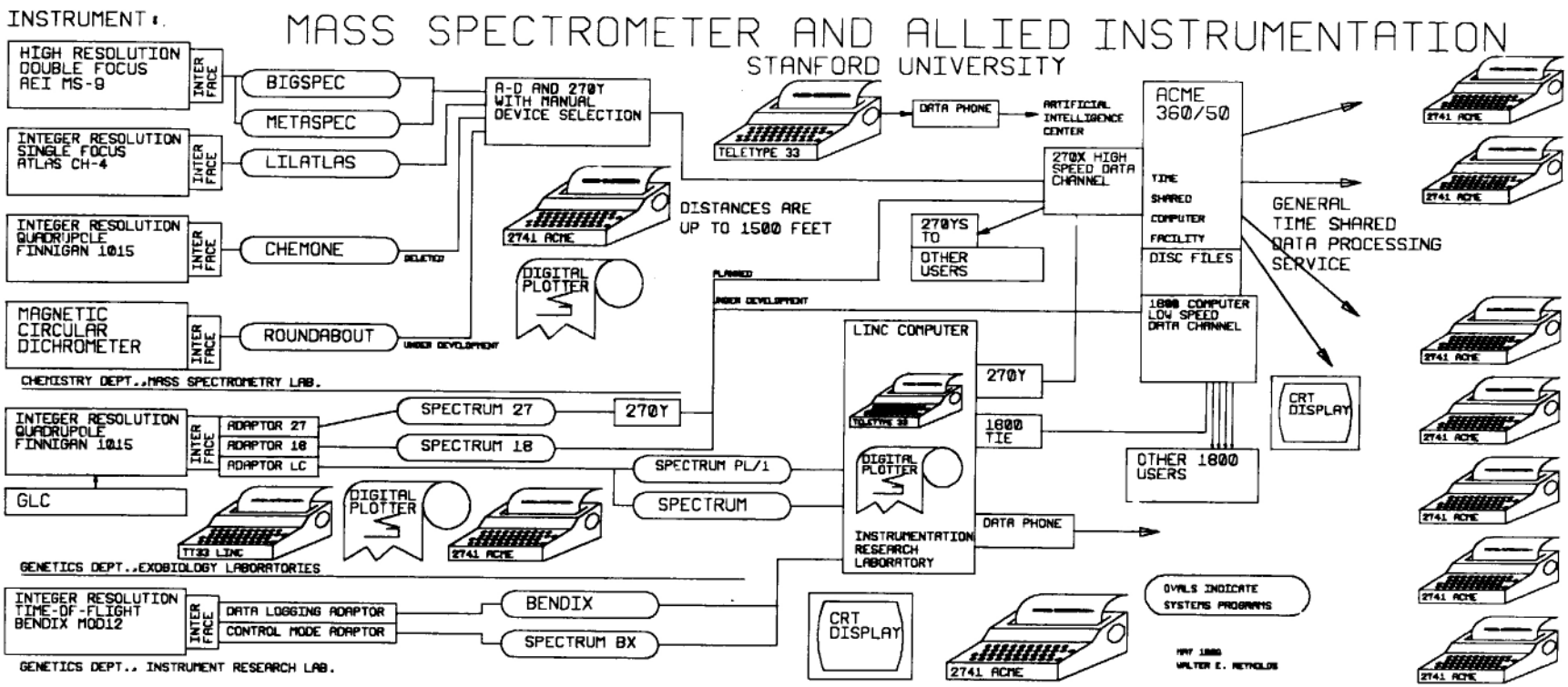


Figure 1

A diagram of the units in the mass spectrometer system employing the time-shared 360/50 computer.

needed to connect the mass spectrometer, are identifiable and indeed built, as a separate special purpose interface. Design, maintenance and even computer interchangeability are enabled or simplified by this arrangement.

Last, but not least in requirements or expense, is a system to make this work and be useful. The realization is best identified by the computer program written to effect operation. The general use concept is implied in the program. We have tended to identify these systems by their program names. In Figure 1 and Figure 2 these system names appear in the oval boxes that tie together the operational paths.

Our programming is all in PL/1. Programs may and must be written at any of the remote keyboards. Since there is an interactive compiler, programs may be modified, altered and/or controlled right at the using site at any time, even concurrently with operational use. Figure 3 shows an elementary program that would cause 100 a-to-d conversions from a laboratory instrument and then plot these values as a graph on the laboratory plotter.

The eight systems shown in Figure 1 connecting mass spectrometers of the time shared net, are of three classes. The top two, BIGSPEC and METASPEC both are quite special to the MS-9. BIGSPEC is actually a collection of three major programs for the data acquisition and processing of high resolution runs. Element mapping has not yet been included; typed output gives fractional mass position of the found peaks. Provision is being made to send this data directly to other computers at Stanford engaged in the artificial intelligence project and their organic structure elucidation programs. METASPEC is a little more than a slide rule to aid in metastable identification.

LILATLAS and CHEMONE are data logging programs and integer peak identification. This type of program has been well reported in the literature. With only minor changes to accommodate the type of scan, we will use the same program on the magnetic focus, the electrostatic quadrupole and the time-of-flight instruments.

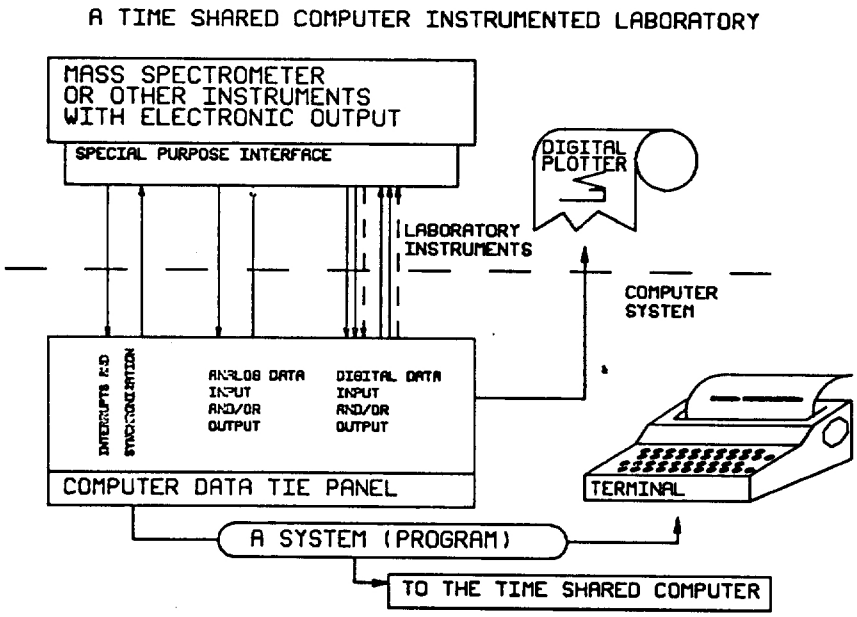


Figure 2

The SPECTRUM series are computer control and data acquisition. SPECTRUM does not use the features of the time shared system, only the LINC. The system was described at last year's E-14 in Philadelphia. The SPECTRUM logic has been rewritten into PL/1 and operates as SPECTRUM PL/1 in the time shared mode. It presently features completely automatic calibration and a mass range from 10 to 500. The other two versions of SPECTRUM will be nearly identical, but differ in the hardware path used. Interchangeability of hardware modes is made possible with the standardization of the CALL READ statement shown in the PL/1 example.

Provision is made to type or plot results in the laboratory. Figure 4 shows two of the standard plot formats we employ for integer resolution data.

An information presentation problem has arisen. Profuse data acquisition and impatience for results have led us to devise interactive data presentation methods. In the SPECTRUM systems the users typically take 10 or 20 spectra of a solid sample, and 200 or more of a GLC run. The results are in the computer and instantly available. But typing or plotting takes time and our users have become impatient with delays of more than 10 or 15 minutes.

We are now working out ways of giving data abstracts to enable the user to "home in" on the spectra or data he wants. This interactive information extraction runs like this:

a. The user asks for a certain small set of data based upon his estimate of what is the pertinent data. If his estimate is vague, presumably he will ask for a condensed set or simply indicates the set or sets.

b. The program responds with a quick presentation.

c. The user may use this computer output to improve his estimate of what he required. Return to a.

This may take the form:

a. Ask for the 8 highest peaks in all 10 spectra taken during a solid sample run.

b. Computer types the 80 items.

c. The user sees that the higher masses did not show up in the sets of 8 high peaks. But he sees that peaks from spectrum 7 onwards are saturated.

d. Reasks for the 8 highest peaks from mass 200 onwards, in spectra 1 to 6.

e. Computer responds with 48 items.

f. User notes that a good fragmentation was indicated in spectrum 4.

g. User asks for a full plot of spectrum 4.

The central time shared computer allows shared files. We are building a file system with standard formats. Each mass spectrometer user's data may be made available to all.

```
 1.000 PLOTSOME:Procedure;
10.000   /*        This is a PL/1 program to take 1000   */;
11.000   /*     sample points and then plot them.        */;
12.000   declare LINE2H (1000);
13.000   CALL READ(18, LINE2H);
14.000   /*  18 addresses the laboratory instrumentation */;
15.000   /*  terminal.                                   */;
16.000   DO I=1 to 1000;
17.000       CALL PLOT(76, I, LINE2H(I),2);
18.000   END;
19.000   /*  76 addresses a specific digital plotter     */;
20.000   /*  in the laboratory.                          */;
21.000 END PLOTSOME;

?
```

Figure 3

A sample program in PL/1 that would accept laboratory data and plot it on the laboratory plotter.

The uses of this are just emerging. Perhaps the first practical use will be that of sending samples to a different mass spectrometer laboratory. The results may be filed by the mass spectrometer operator and the requestor may get his results directly on his own laboratory terminals and process it in his own programs. All the described operations used the computer in a time shared mode and concurrent with other users and other instrumentation. No advance notice is required to operate, other than to request a "line" from the operator. The operator does reserve the right to deny service if the usage is too high. This normally happens only in the teletypewriter ports or when there is some abnormal operating condition of the computer.

Observations based upon our experience with time shared instrumentation:

A time shared large computer does greatly aid in program development. The ability to write instrumentation systems software in a high level language and with on-line compiler does enhance program development. Also since much system improvement will be largely to the software, such improvements may be introduced easier than if machine code or assembly languages had to be used.

It should be noted that much more support and computer capacity are needed for system development than for operational uses. Certainly program changes and documentation is easier with high level languages and hence less costly by an order of magnitude. The large system is peculiarly suitable and capable in this initial phase. After the algorithims are tried and proved usable and experience has been gained in the operational use, the justification of the large computer does decrease. Operations could be carried on largely or solely by a small computer.

Another useful aspect of powerful time shared compilers is the availability of common functions, exponentials, log, trigometric and the like. Also the ease of floating point and double precision does make life more enjoyable. No interaction between user and the system is enhanced. If nothing else, the output formatting ease of high level languages greatly aid programming for user interaction.

It had been our experience that small programs may be written in a tenth of the time in the time-shared mode as compared to small computer assembly languages. However the advantage diminished as the systems get larger and the structure of the high level language program gets complex.

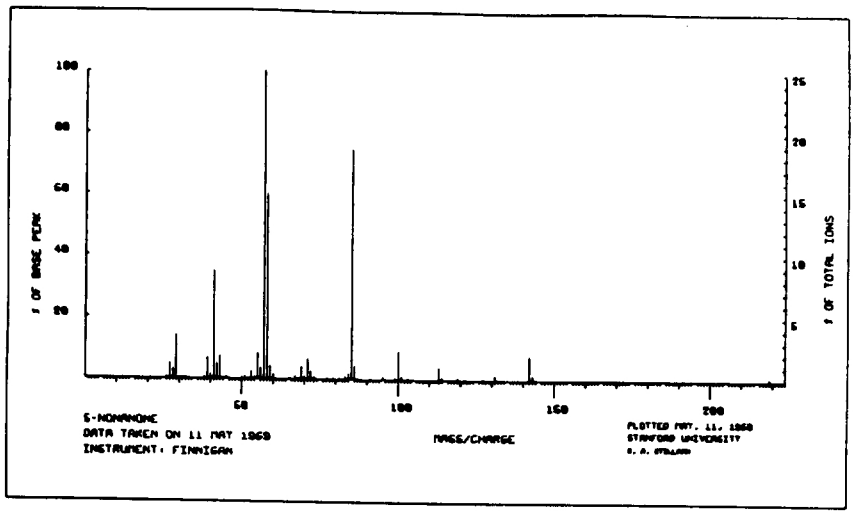
Some weaknesses that have shown up in our system:

1. The lack of overlay capability or object code stored programs or procedures can cause uneconomic or cumbersome operation. Total systems of instrument setup, data acquisition, data processing and/or filing, and data presentation can run to very large proportions. Our systems run 5 to 25 thousand words for just instructions and auxiliary working areas.

There appears to be a fundamental conflict between the incremental compiler used in very responsive time shared programming systems and precompiled program or program segments. In our system, programs are filed on disk as manuscript source code and compiled upon entry into object code. Thus a program or procedure must be recompiled each time it is brought from storage. This forces us to large programs held in core for hours even though the use is intermittent. The penalty to avoid the delays of recompiling is very uneconomical use of core. Our experience clearly indicates the need to swap programs or program segments in the order of seconds.

2. Fast disk or drum data filing should be provided. Most sophisticated time shared systems have very protective file systems that are well calculated never to make an error. But the price paid due to redundancy, flexibility, and cross checks is slow filing. Instrumentation data usually can pay a price of bit errors of the order of one in 10^9 to 10^{12} if speed is gained. And there is the real time need to file data rapidly to avoid large core storage. Again the price paid for slow filing is the large amount of core needed to buffer real time data input.

3. For instrument-computer interaction, fast channel turn around is desirable. This is the ability to do sequential operations of input, then output or vice versa. This is not necessarily allowed with complex computer operating systems. Such systems tend to be oriented to fast data streams in one direction as to or from disk or tape units. If one wished to accept a datum point, and then react with an output, then repeat, it may be found that it takes a big computer many milliseconds to change from input to output and back. Similar difficulties may be found if complex orders of data input/output are attempted.



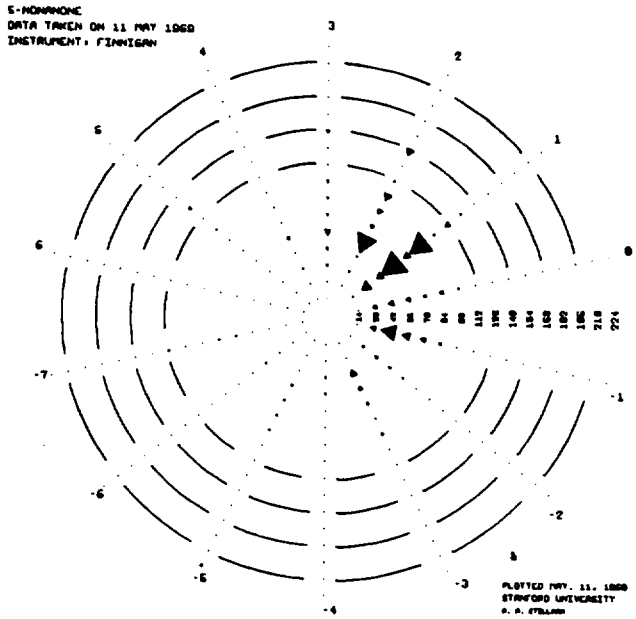


Figure 4

4. At this state of the art large computer systems are prone to "crashes". The complex program that makes a large computer a time shared facility will be faced with conditions or malfunctions it cannot handle. At such times the system becomes inoperative and remains so until operator intervention restores normal operation. Often data or program extras made sometime prior to the "crash" are lost. Also the user's programs normally have to be recompiled. Even a few moments of master computer down time can cost the user hours of his and his laboratory's time.

Despite the limitations described, which largely are the price paid for a developmental system, we feel that the mass spectrometer complex we have is now reasonably current with the state of the art in mass spectrometer instrumentation. But this is not the point of merit. The fact that the system is capable of much development is the most exciting feature. The basic instrumentation approach, the data channels provided, the versatile access to the computer from the laboratory, the general purpose computer service in real time, and high degree of programmability provided, all give us a vehicle especially suitable for continuing the enhancement of mass spectrometer-computer-instrumentation.

INTERACTIVE INFORMATION PRESENTATION, EDITING, AND FILING

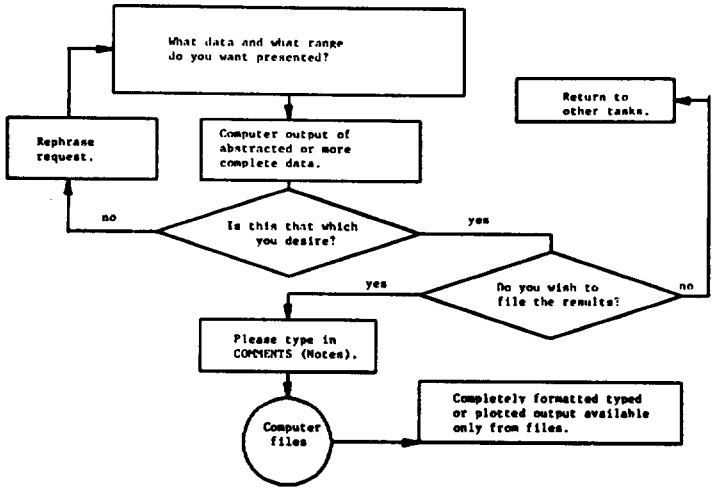


Figure 5

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APPENDIX B

Investigation of Porphyrins by Magnetic Circular Dichroism Spectroscopy

The purpose of the present investigation has been to explore the applications of magnetic circular dichroism as a sensitive spectroscopic technique for the detection of a biologically important class of compounds, the chlorophyll-like porphyrins, which may be present in lunar samples. During this period attention has been directed toward the accumulation of reference magnetic circular dichroism spectra and toward the development of a more sensitive instrument. The results of our work during this period are very briefly summarized in the following paragraphs.

Reference spectra have been collected using synthetic samples as well as extracts from petroleum residues. On the basis of these measurements it is apparent that magnetic circular dichroism is a particularly well suited analytical spectroscopic technique for detecting porphyrins in lunar samples for several reasons. First, since this technique is nondestructive, the sample can be recovered intact for subsequent examination by other workers. Second, magnetic circular dichroism is particularly useful as a companion and confirmatory technique to fluorescence spectroscopy. This situation results from the fact that the magnetic circular dichroism signals observed for metalloporphyrins are much larger than those observed for metal-free porphyrins whereas fluorescence spectroscopy is applicable only to demetallated samples. Thus, magnetic circular dichroism can be applied to extracts obtained one step earlier in the sample handling schedule and the results obtained can be confirmed in the next step by fluorescence measurements on the demetallated sample. Third, the observation of S-shaped magnetic circular dichroism bands (signals) near 400 mμ and near 540 mμ will provide very secure evidence for the presence of metalloporphyrins in lunar samples. The relative magnitudes of these bands will provide

very secure evidence for the presence of metalloporphyrins in lunar samples. The relative magnitudes of these bands will provide further information about the particular type of porphyrins present. Finally, magnetic circular dichroism can be applied to rather crude extracts since experiments on porphyrin-containing petroleum extracts have shown that magnetic circular dichroism is rather insensitive to relatively large amounts of other organic substances.

Although magnetic circular dichroism, as an analytical technique for detecting porphyrins, is second only in sensitivity to fluorescence spectroscopy, the very small quantities of these substances which are likely to be present in lunar samples require that our instrumentation must be as sensitive as possible. In order to achieve this increased sensitivity we have made a number of electronic modifications to an experimental magnetic circular dichrometer as well as interfacing this instrument with the ACME 360-50 computer.