Report to the National Aeronautics and Space Administration

"Cytochemical Studies of Planetary Microorganisms - Explorations in Exobiology"

NsG 81-60

Status Report Covering Period April 1, 1967 to October 1, 1967

Instrumentation Research Laboratory, Department of Genetics

Stanford University School of Medicine

Palo Alto, California

Joshua Lederberg
Principal Investigator

Elliott C. Levinthal, Director
Instrumentation Research Laboratory

A. INTRODUCTION

This Status Report covers the activities of the Instrumentation Research Laboratory from April 1, 1967 to October 1, 1967. Major technical efforts are described in separate technical reports and papers. The status report refers to these and summarizes continuing projects.

Work under grant NsG 81 includes areas of research that are closely related to efforts being carried out in the Department of Genetics under other grants or contracts. This includes Air Force Contract AF 49(638)1599 for "Molecular Biology Applications of Mass Spectroscopy", National Institute of Neurological Diseases and Blindness Grant NB-04270 entitled "Molecular Neurobiology" and work carried out by the Advanced Computer for Medical Research (ACME) program supported by the National Institutes of Health, Division of Research Facilities and Resources under Grant FR00311-01. There is collaboration with the work in the Computer Science Department on artificial intelligence carried out under support of the Advanced Research Projects Agency SD 183. In addition, work is being done on "Genetic Studies of Mammalian Cells", National Institutes of Health, under Grant CA04681-08. The relationship of the work carried out under this NASA grant to these other activities continues to prove of great mutual benefit in all cases. The Air Force Contract AF49(638)1599 terminates in December 1967.

The general project areas of the program resume, part 3 of the status report are:

I. Gas Chromatography and Optical Resolution
II. Mass Spectrometry
III. Computer Managed Instrumentation
IV. Prebiological Evolution
V. Pasteur Probe

These projects contribute to technical mastery of problems in exobiology by furnishing specific analytical techniques of high sensitivity and discrimination for the detection of exotic life. In addition, the management of instrumentation in many laboratories via a time-shared computer (the ACME project) is a system prototype for the automated biological laboratory.

During the six month period described above, ten papers were submitted to journals for publication, in addition to those of Professor Djerassi's laboratory, and two technical reports prepared. A listing of these papers and reports is included in this status report.

B. PROGRAM RESUME

I. Gas Chromatography and Optical Resolution

a. Determination of the Configuration of Asymmetric Compounds by Gas Chromatography of Diastereoisomers

Since the resolution of camphor by Casanova and Corey (1) in 1961, there have been many reports of the GLC resolution of enantiomers either as diastereoisomeric derivatives or on an optically active stationary phase. The advantages of the method over polarimetric measurements in the determination of optical purity are that chemical and optical impurities are separated on the column and analyses can be carried out on the microgram scale.

In resolving N-TFA-L-prolyl-DL-amino acid methyl esters on short, packed columns, we found that the DL diastereoisomer always had a shorter retention time than the LL compound (2). Wieland and Bende have suggested that DL dipeptides exist in a stabilized ring form, while the LL dipeptides exist in open chain conformation (3). If this is also true in the TFA-peptide esters, then the greater volatility of the DL diastereoisomer could be due to a smaller molecular volume. The consistency in the order of GLC retention times suggested the application of this method to the assignment of configuration to other asymmetric compounds.

A number of amino acids were converted to their α-chloro acid analogues with retention of configuration and coupled with amino acid methyl esters. GLC analysis again showed the DL derivatives to have the shorter retention time (4). In addition, different N-chloralkanoyl valine methyl esters were examined by GLC to determine the effect of steric bulk of the alkanoyl group on the resolution of diastereoisomers. A striking correlation was found between Newman's "six number" and the ratio of retention times, with increased crowding of the amide

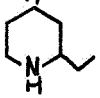
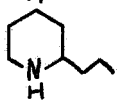
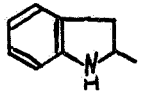
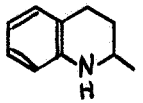
carbonyl resulting in greater differences in the relative volatility of diastereoisomers (5).

In the 1-methylalkylamine series, the TFA-L-prolyl-(+)-amides consistently had the longer retention times. As (+)-2-amino-3-methylbutane and (+)-2-amino-4-methylpentane have been related to the absolute L-configuration of the corresponding α-amino acids, this behavior is again consistent with the dipeptides and it appears that all (+)-aliphatic-2-amino alkanes have the absolute L-configuration (6).

Gas chromatography has been used to correct the assignment of configuration of α-alkylphenylacetic acids by resolution with S-(+)-2-methylamino-1-phenylpropane. Our results show that all (+)-acid-S-(+)-amides of this series consistently have the lower retention volume and since the absolute configuration of hydrotropic acid is known to be S-(+), we conclude that all (+)-α-alkylphenylacetic acids have the S-configuration (7). This confirms the assignment indicated by O.R.D. and Freudenberg's "rule of shift".

Asymmetric alcohols have been used extensively in the optical resolution of amino acids and α-hydroxy acids by GLC (8). In this series, the LL diastereoisomer has the shorter retention time but generally the ratio of retention times (α-value) of diastereoisomeric esters is less than for amides. We attribute this difference to the greater rigidity of the amide bond. The importance of conformational immobility in the resolution of diastereoisomers is further confirmed by the high α-values obtained with the cyclic amines (9). We have now applied this method in an attempt to correlate the configuration of some alkaloids with amines of known configuration and the results are presented in Tables I and II.

Table I. GLC separation of cyclic asymmetric amines as their N-TFA-L-prolyl derivatives*

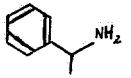
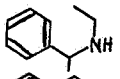
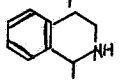
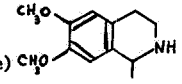
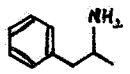
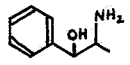
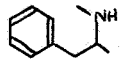
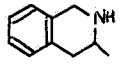
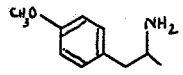
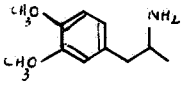
Amine		Retention data of diastereoisomers						α-values
		First GLC Peak			Second GLC Peak			
Name	Structure	Sign of Rotation of Amine	Configuration of Amine	Retention Time, T_1 (min)	Sign of Rotation of Amine	Configuration of Amine	Retention Time, T_2 (min)	$\frac{T_2}{T_1}$
2-methyl-pyrrolidine		+	D	3.2	-	L	3.55	1.11
2-methyl-piperidine (pipecoline)		+	D	3.6	-	L	4.0	1.11
2-ethyl-piperidine		+	D	4.0	-	L	4.7	1.18
2-propyl-piperidine (coniine)		+	D	4.4	-	L	5.0	1.14
3-methyl-piperidine		-		3.6	+		3.9	1.09
2-methyl-indoline		+		7.7	-		9.8	1.27
2-methyl-1,2,3,4-tetrahydro-quinoline (quinaldine)		+		5.85	-		7.30	1.25

* GLC analyses were carried out on a Varian Aerograph 1200 equipped with a flame-ionization detector. The 5' x 1/8" stainless steel column was packed with 5% DC LSX-3-0295 on 60/80 mesh AW/DMCS chromosorb W. During the analyses the nitrogen flow was 30 ml/min and the temperature 210°.

The first series of compounds to be tested were the α-substituted pyrrolidines and piperidines and it was found in all cases that when the racemic amines were coupled with TFA-L-prolyl chloride and analyzed by GLC, the (+)-amine derivative always preceded the (-)amine derivative. Since (-)-2-methylpyrrolidine has been related to L-proline (10) and (+)-coniine and (+)-α-pipecoline have been related to D-(+)-pipecolic acid (11). In this series of compounds the LD-diastereoisomer again has a shorter retention time than the LL. From our results we would predict that (-)-3-methylpiperidine has the D-configuration. It is impossible to assign the configuration of the indoline and tetrahydroquinoline derivatives until we have analyzed compounds in this series of known configuration.

We have also examined a number of asymmetric α- and β-phenylethylamines and their cyclic analogues, the tetrahydroisoquinolines (Table II). The first compound in this series, (-)-α-phenylethylamine has been shown (12) to be configurationally related to L-(+)-alanine and can therefore be written in the L-configuration. However, if the requirements of the Fischer convention for C-1 (methyl group) of the main carbon chain to be at the top are obeyed, the symbolism is reversed and the compound falls into the D-series (13). To overcome this difficulty, the unambiguous R-S system of Cahn, Ingold and Prelog (14) is used throughout this series. N-ethyl-α-phenylethylamine was prepared (15) from α-phenylethylamine of known configuration and the two compounds compared by GLC analysis. In both cases, the TFA-L-prolyl-R-(+)-amide had a shorter retention time than the L-S diastereoisomer. In the β-phenylethylamine series, the (+)-forms of α-methyl-β-phenylethylamine and desoxyephedrine have been shown by Leithe (16) to have the S-(or L) configuration. (+)-3,4-dimethoxy-α-methyl-β-phenylethylamine has been related to L-(+)-alanine and shown (17) to also have the S-configuration. When these compounds were analyzed by GLC, the results were in agreement with the α-phenylethylamines

Table II. GLC separation of α- and β-phenylethylamines as their N-TFA-L-prolyl derivatives*

Amine		Retention data of diastereoisomers						α-value
		First GLC Peak			Second GLC Peak			
Name	Structure	Sign of Rotation of Amine	Configuration of Amine	Retention time (min), T	Sign of Rotation of Amine	Configuration of Amine	Retention time, T_2	$\frac{T_2}{T_1}$
α-phenylethylamine		+	R	4.8	-	S	5.8	1.21
N-ethyl-α-phenylethylamine		+	R	7.1	-	S	7.4	1.02
1-methyl-1,2,3,4-tetrahydroisoquinoline		-	S	10.7	+	R	12.0	1.12
Salsolidine Salsoline (O-CH_3 derivative)		-	S	16.7	+	R	19.1	1.14
α-methyl-β-phenylethylamine (Amphetamine)		-	R	5.45	+	S	5.85	1.07
Ephedrine (O-TMS derivative)		-	R	8.45	+	S	10.65	1.26
Desoxyephedrine		-	R	9.25	+	S	10.25	1.11
3-methyl-1,2,3,4-tetrahydroisoquinoline		+	S	12.0	-	R	14.0	1.17
4-methoxy-α-methyl-β-phenylethylamine		-	R	10.7	+	S	12.1	1.13
3,4-dimethoxy-α-methyl-β-phenylethylamine		-	R	3.6	+	S	4.0	1.11

* GLC conditions were the same as Table I for all compounds except 3,4-dimethoxy-α-methyl-β- phenylethylamine and Salsolidine which were analyzed at 250°.

with the (R)-amine derivative having a shorter retention time than the (S)-amine derivative. The hydroxyl group of ephedrine was converted to the trimethylsilyl (O-TMS) ether prior to coupling with TFA-L-prolyl chloride in order to make a more volatile derivative. (We know that the β-asymmetric center is not resolved under these conditions (2b).)

Finally, we investigated a number of cyclic analogues of the α- and β-phenylethylamines. The absolute configuration of (-)-salsolidine and (+)-salsoline have been elucidated by degradation of the bases to N-2-carboxyethyl-L-alanine which was synthesized from L-alanine for comparison (18). (+)-salsoline was O-methylated with diazomethane and compared with naturally occurring S(-)-salsolidine by GLC analysis after coupling with TFA-L-prolyl chloride. The S(-)-salsolidine derivative was the first peak in the analysis whereas methylated (+)-salsoline (or R(+)-salsolidine) was the second peak. This result is the reverse of the sequence found for the open chain α- and β-phenylethylamines and in order to test its validity, we analyzed two other cyclic analogues, 1-methyl-1,2,3,4-tetrahydroisoquinoline (15) and 3-methyl-1,2,3,4-tetrahydroisoquinoline. The configuration of these compounds have been assigned by O.R.D. (19) and GLC analysis gave the same result as was found for salsolidine with the S-(+) derivative preceding the R-(-) derivative.

In contrast to the open-chain amines, it appears that in the cyclic amines, the TFA-L-prolyl-S-amides have a smaller molecular volume than the corresponding L-R diastereoisomers. This again emphasizes that in correlating configuration by GLC, only compounds of a homologous series should be used.

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G. E. Pollock, V. I. Oyama and R. D. Johnson, J. Gas Chromatog., 3, 174 (1965).
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b. Gas Chromatography of Amino Acids

A recent review on the g.l.c. separation of amino acids (B. Weinstein, Methods of Biochemical Analysis, John Wiley, New York, 1966), cites over 100 references, but so far no entirely satisfactory procedure for a quantitative amino acid analysis is available. The preferred procedure is via derivatisation to the N-trifluoracetyl butyl esters (Gehrke, Biochem. Biophys. Res. Comm., 19, 328, 1965) but our work showed stability problems with the derivatives of the hydroxy and less volatile amino acids. In addition, we found a very short column life, which is due to the lightly loaded polar columns required for the g.l.c. separation.

We are investigating a new approach to this analytical problem, which involves conversion of the amino acids to the non-polar isothiocyanate derivatives. Under the experimental conditions used, all poly-functional amino acids give fully protected stable derivatives and with the exception of arginine all compounds are sufficiently volatile for g.l.c. analysis. The derivatives have been fully characterized by mass spectrometry using the EAI Quad 300 Mass Spectrometer coupled to a Varian 600C gas chromatograph and all the amino acid derivatives have been synthesized separately in essentially quantitative yield for analytical purposes. This instrumentation system was under computer control. It is described in section III of this report and more fully in IRL Technical Report 1062.

In our present work, the amino acid mixture is derivatised on a 1 μM scale and the analysis is carried out on a temperature programmed 5' x 1/8" glass column (5% QF1 on DCMS Areated Chromosorb W). All amino acids can be separated with the exception of leucine-isoleucine, but the latter separation can be done on columns containing SE 52. A study with several of the analytically pure amino acid derivatives showed that 10^{-10}M of an amino acid can be detected with the flame ionization detector. A detailed quantitative study will be undertaken to evaluate the usefulness of this new g.l.c. amino acid analysis.

c. Determination of Steric Purity of Peptides by N.M.R. Spectroscopy

We have recently shown that a series of diastereoisomeric N-acyl-alanyl-phenylalanine methyl esters and N-acyl-phenylalanyl-alanine methyl esters possess different N.M.R. spectra (1). The methyl doublet signal in an L-L compound was at a lower field than the equivalent signal for the D-L analog due to deshielding and offered a potentially convenient means for the quantitative analysis of such mixtures. We have now used this technique to examine the influence of several coupling agents and N-acyl protecting groups in the extent of racemization during peptide synthesis.

Our results (2) show that carbonyldiimidazole and Woodward reagent K are preferable to dicyclohexylcarbodiimide and the water soluble diimides. The optical purity is lower in the peptides derived from N-benzoyl and N-acetyl-amino acids, but the N-formyl group is noted as a potentially useful amino protecting group. The strongly electron-withdrawing N-acyl functions obviously cause a decrease of steric homogeneity in the products, while phenylalanyl derivatives seem more prone to racemize than the alanyl derivatives.

II. Mass Spectrometry

a. Analysis of Natural Products

The Atlas CH 4 Mass Spectrometer in Professor Djerassi's laboratory in

the Department of Chemistry has yielded the results reported in the following papers.

MacLeod, J. K., and Djerassi, C. Mass Spectrometry in Structural and Stereochemical Problems. CXXXVI. Primary Hydrogen Isotope Effects in the McLafferty Rearrangement. J. Am. Chem. Soc., 89, 5182 (1967).

Green, M. M., and Djerassi, C. Mass Spectrometry in Structural and Stereochemical Problems. CXXXVII. Examples of Interaction of Remote Functional Groups After Electron Impact. J. Am. Chem. Soc., 89, 5190 (1967).

Duffield, A. M., Djerassi, C., and Sandström, J. Mass Spectrometry in Structural and Stereochemical Problems. CXXXVIII. Fragmentation Processes of Some Thiocarbonyl Compounds on Electron Impact. Acta Chem. Scand., in press.

Schroll, G., Lawesson, S.-O., Duffield, A. M., and Djerassi, C. Mass Spectrometry in Structural and Stereochemical Problems. CXXXIX. The Fragmentation of Some Cyanohydrins and O-Acylcyanohydrins upon Electron Impact. Arkiv Kemi, in press.

MacLeod, J. K. and Djerassi, C. Mass Spectrometry in Structural and Stereochemical Problems. CXL. Competitive McLafferty Rearrangements in Bifunctional Compounds. J. Org. Chem., in press.

Briggs, W. S. and Djerassi, C. Mass Spectrometry in Structural and Stereochemical Problems. CXLII. Electron Impact Induced Analogies to Thermal Elimination Processes in S-Methyl Xanthates and Esters. J. Org. Chem., in press

Carpenter, W., Duffield, A. M., and Djerassi, C. Mass Spectrometry in Structural and Stereochemical Problems. CXLIII. Unusual Fragmentations in the Mass Spectra of Some Aliphatic Ethers. J. Am. Chem. Soc., in press.

Carpenter, W., Duffield, A. M., and Djerassi, C. Mass Spectrometry in Structural and Stereochemical Problems. CXLIV. Unusual Fragmentations of the Low Voltage Spectra of Aliphatic Ketones. J. Am. Chem. Soc., in press.

Carpenter, W., Duffield, A. M., and Djerassi, C. Mass Spectrometry in Structural and Stereochemical Problems. CXLV. Factors Governing the Preferential Loss of Small versus Large Radicals in Ketones, Schiff Bases and Ethers. J. Am. Chem. Soc., in press.

Brown, P., and Djerassi, C. Mass Spectrometry in Structural and Stereochemical Problems. CXLVIII. Electron Impact Induced Fragmentations of Cyclic Sulfides and Carbonates. Tetrahedron, in press.

Duffield, A. M., Djerassi, C., Mazerolles, P., Dubac, J. and Manuel, G. Mass Spectrometry in Structural and Stereochemical Problems. CLII. Electron Impact Promoted Fragmentation of Some Substituted Germacyclopentanes and Germacyclopentenes. In preparation.

Sengupta, P., Chakraborty, A. K., Duffield, A. M., Durham, L. J., and Djerassi, C. Terpenoid Studies. LXI. Chemical Investigations on Putranjiva Roxburghii. The Structure of a New Triterpene, Putranjivadione. Tetrahedron, in press.

Buchardt, O., Duffield, A. M., and Shapiro, R. H. Mass Spectra of Quinoline and Isoquinoline N-oxides. Tetrahedron, in press.

Duffield, A. M., Cymerman-Craig, J., and Kray, L. R. Electron Impact Promoted Fragmentation of Some Phenothiazine Derivatives. Tetrahedron, submitted for publication.

b. Mass Spectral Microanalysis of Organic Solids

We described in the last report (Technical Report No. IRL-1056) an application of optical ray brightness invariance that lead us to an improved laser-optical design concept suited to vaporizing organic materials in our time-of-flight mass spectrometer. The system, illustrated in Figure 1 (reproduced from the last report), has been constructed and is now in use. The design optimizes delivered energy density, with full energy delivery, by maximizing the solid angle of condensed radiation. Practical constraints necessitate a sufficiently long distance from the condensing lens to the working point that a lens aperture larger than the laser beam is required.

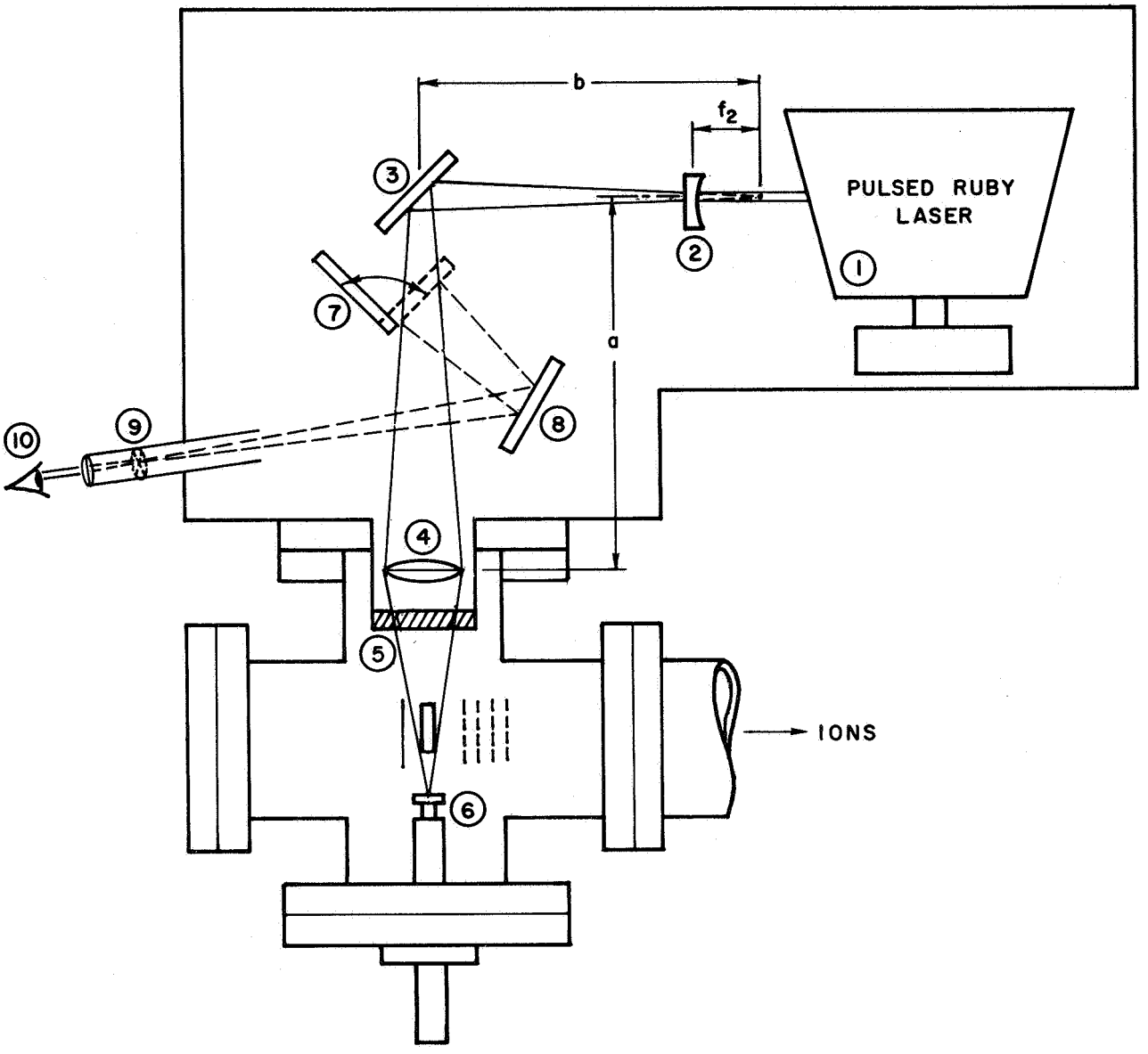


FIGURE 1

Schematic of Laser Optical System

The laser beam is expanded to fill the condensing lens by placing a diverging lens immediately in front of the pulsed ruby laser.

The system delivers approximately a 1 millisec - 10 millijoule pulse of ruby radiation to an approximately 70 micron diameter spot. The present optical configuration involves the placement of a planar glass vacuum port within the cone of rays converging to the target. A significant contribution to the observed spot size derives from the spherical aberration contributed by the window.

We have found that the optical pulse is of insufficient magnitude to vaporize bulk reflective samples. Such samples are handled by depositing them in such a manner on a black oxidized copper support that thermal vaporization is accomplished by thermal transfer from the copper to the sample.

Placement of the sample at the point of concentration of the laser radiation is achieved with mirror 7, in Figure 1, rotated to its phantom position. The sample target point is placed so that it is imaged at the cross hair 9 of the eyepiece. The optical configuration is such that the portion of the sample imaged on the crosshair will be at the focal point of the condensed laser radiation when the system is fired.

Figure 2 illustrates the probe that has been constructed to provide the three degrees of freedom required to properly position the sample. The probe consists of a hollow tube containing a centrally located stem penetrating through a 0.010 inch thick stainless steel diaphragm brazed to the stem and the tube. The sample is placed on the upper end of the removable stem tip. The shoulder on the probe base structure seats on the vacuum lock of the mass spectrometer source. The sample is raised or lowered relative to the shoulder by rotating a concentric wheel engaged to the threaded base of the tube through an

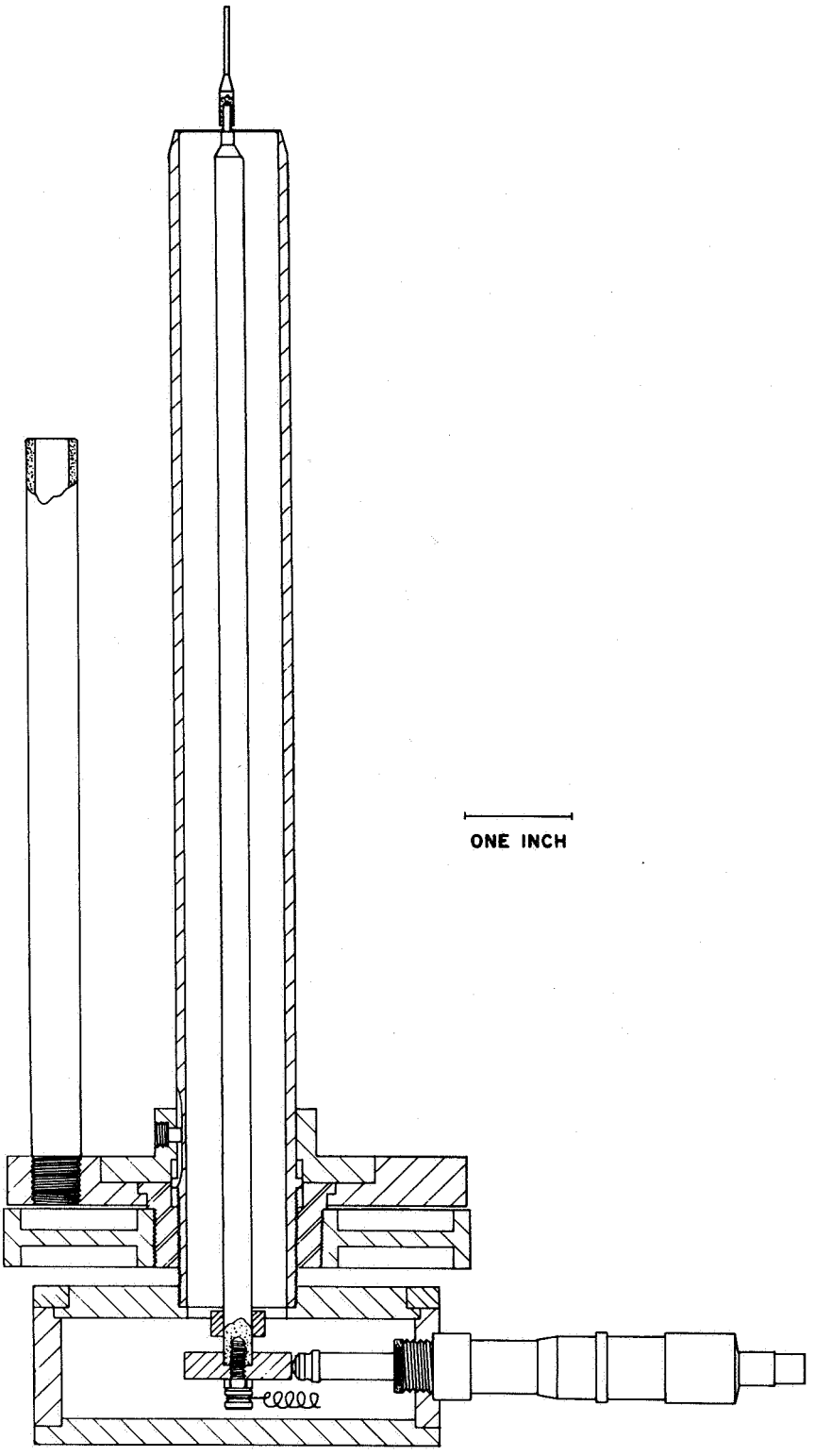


FIGURE 2

Mass spectrometer sample probe
for laser induced vaporization

intermediate annular key. The sample is displaced perpendicular to the tube axis by two mutually orthogonal micrometers that pivot the stem about the flexible diaphragm. The micrometer driven motion is reduced 10:1 at the sample. The diaphragm tolerates 2×10^{-2} radians of angular displacement from equilibrium before acquiring a set.

Figure 3 is a schematic illustration of a control unit now being constructed to expedite the recording of output from the laser-mass spectrometer system. When the start button is pressed with the unit in "normal" function, a "background" number of reference gas traces is recorded across the top and then again across the bottom of the scope face, the laser is then fired, and after the designated number of mass spectrometer "cycles delayed" (100 microsec/cycle), the "sample" number of successive cycles of mass spectral analysis of the laser induced output is recorded in an equally spaced display between the upper and lower background traces.

We have commenced the mass analysis of selected organic samples with the completed laser-optical system. Our first results have demonstrated that we are readily able to detect pronounced parent molecular peaks for the four nitrogenous bases adenine, guanine, thymine, and cytosine. We have also demonstrated that the base peaks are conspicuous for the nucleosides deoxyadenosine, deoxyguanosine, and deoxycytidine. We also find peaks at the base masses in the two nucleotides we have examined, deoxyguanosine monophosphate and thymidine monophosphate.

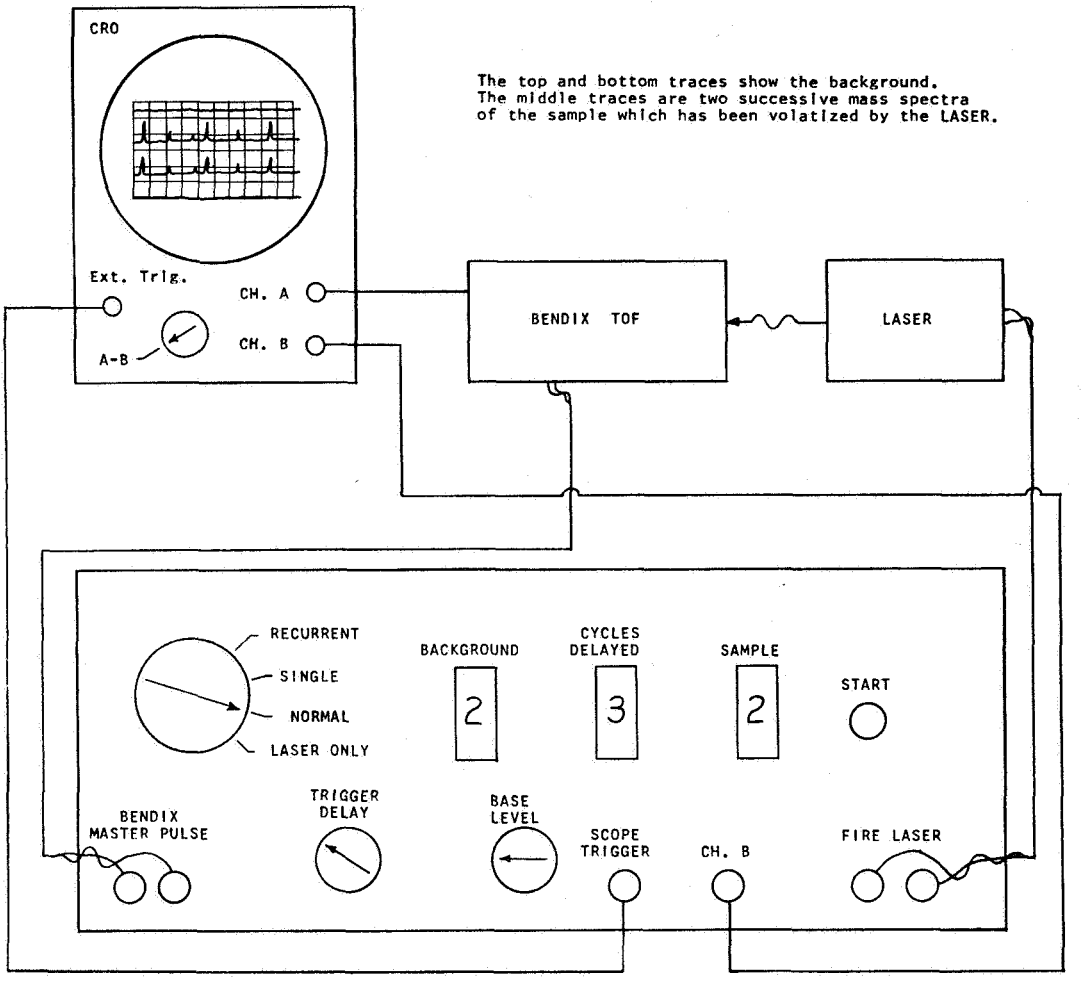


FIGURE 3

Control unit to expedite scope display of laser induced mass spectra

III. Computer Managed Instrumentation

a. ACME Program

The cooperative program between the Instrumentation Research Laboratory and the Advanced Computer for Medical Research Group (ACME) has resulted in the ability to communicate between the IBM 360 computer and laboratory instruments. The IBM 1800 purchased under Grant NsG 81 is used as an input-output computer in this communication.

Currently, analog data are being transmitted from various labs in the Medical School of the 1800. When the 1800 receives a "start" interrupt generated by closing a switch in the lab, the 1800 A/D converter samples up to five channels of data, transfers the data to the time shared 360 where it is processed by the user's program. The A/D converter provides a 14-bit resolution and has a nominal A/D conversion rate of 20 kc; however, when used with the time shared 360, the maximum conversion rate allowed one user is 1 kc. Data are transferred to the 360 in blocks of words. The size of these blocks may vary between 1 and 400 words. Once the data are transferred to the 360 they are referenced by a very simple I/O statement in the user's time shared program. This statement is:

CALL READ (x, array).

Using this statement data associated with an analog, line x, (in some cases a low-speed digital line) is transferred to a user-defined area called "array". The use of the memory of the 1800 allows the user great timing leeway for when the CALL READ is executed.

Prior to data being transferred from the 1800 to the 360, the ACME system waits or services other users until the user has initiated a start interrupt in the lab and data are transmitted to the 1800, converted and transferred to the 360. If the CALL READ is executed

after data transferral to the 1800, the data are retained in a buffer until called for by the user's 360 program. If a sequence of CALL READS progressively lags behind the data transferral, the data are temporarily stored in auxiliary backup buffers in the 1800.

The ACME system has provided the user with a procedure for then processing data from laboratories which is both simple and flexible. The user is, therefore, able to use the analog and digital input capabilities of the 1800 but do all programming in the relatively straightforward language of the 360 (PL/1). A similar system for output is anticipated.

The ability to interface experimental equipment directly with the 360 is also being developed. Applications for which the 1800 system will not provide adequate speed has prompted this development. The IBM 270X Data Adapter Unit described in the previous report will be used with the four recently delivered 270Y Remote Experimental Terminals to serve this function.

The 270Y terminals offer a general purpose digital interface between experimental equipment and the 270X. An eight bit (plus parity) data path is provided between the experimental equipment and the 360. A cable length of up to one mile is provided for between the experiment -- 270Y site and the 270X-360 installation.

The 270X-270Y hardware has been applied in several ways during the checkout phase. A Sander's 720 keyboard-display unit, a Calcomp 565 digital plotter, and a LINC computer have been interfaced to the 360. The connection of the LINC computer to the 360 will surely be of considerable value. The software for this connection has been written such that the LINC looks to the ACME programmer much like any other 360 input-output device.

The ease with which this interface can be used was demonstrated in the processing of data from a nanosecond fluorimeter. The data was collected by the LINC and stored on LINC tape. It was later referenced by a time-shared PL/1 program for reduction and analysis. The user had no need to learn the rather inconvenient programming language of the LINC but could easily call for the data with simple "READ" statement in his ACME PL/1 program. A further application of the 270Y interface is described in part (4) of this section of the status report.

b. Mass Spectrometers

(1) Computer Manipulation of Bendix TOF Data

The completing of the hardware and software for the ACME 360-LINC communication gives us the capability to process mass spectrometer data gathered by the LINC on the 360/50. Previous status reports have mentioned the handicap posed by the limited computational ability of the LINC. This limitation results from the LINC having a twelve-bit-integer arithmetic system. Most data manipulation steps, therefore, must be done with software routines for double-precision, floating-point arithmetic. The result is that arithmetic operations take place at millisecond rather than microsecond speeds.

Having this ability to communicate, we are experimenting with using the 360 to transform the data collected from the Bendix TOF mass spectrometer into a more usable form. A computational process which required 20 minutes to complete on the LINC was found to require only two minutes when done under the ACME time-sharing system on the 360. In this case the data was sent to the 360 operated on in allocated "time slices" and returned to the LINC for display on the LINC oscilloscope. About half of the two minutes required was the result of the tape reading and writing operations on the LINC.

(2) GCL/MS System

The computer interface and computer operating system of the Electronic Associates Inc. (EAI) QUAD 300 was completed in June 1967. In this

system a computer exercises control, and acquires spectra from a QUAD 300 quadrupole mass spectrometer. It proved useful in aiding gas chromatograph effluent analysis by mass spectral means, as described elsewhere in this report.

The methods and results of this system are separately reported in a technical report No. IRL-1062 dated November 1, 1967. The instrumentation consisted of a quadrupole mass spectrometer, a special small rack of electronics, which we termed the "interface", a LINC computer, and a computer software system written by our group. The following is the abstract of that report.

> "A mass spectrometer-computer system has been devised to utilize the decision-making capabilities of the modern digital computer. The system described assists the researcher user by allowing a computer to query the researcher for operating parameters. The computer translates these into detailed control functions that operate the instrument. The data acquired from the mass spectrometer is made available to the researcher in an on-line system. The system employs a small digital computer and an integer resolution quadrupole mass spectrometer. A reference gas is valved into the mass spectrometer by computer control to permit automatic calibration. Spectra processing of GLC effluent was demonstrated; the means and results are given."

The EAI QUAD 300 mass spectrometer that was used was in our laboratory on loan for evaluation. This evaluation study was completed and the mass spectrometer was returned to the manufacturer late in June. A similar quadrupole mass spectrometer made by Finnigan Instruments Corporation has been ordered and is scheduled for delivery in December.

(Note added in proof)
The Finnigan Instruments Corporation model 1015 quadrupole mass spectrometer was delivered essentially as scheduled and is now (January 1968) being installed and tested. The initial tests indicate that the resolution will allow automated operation to well over m/e 500. Resolution (m/(half width of the peak)) of nearly 1000 has been achieved. Solid sample capability has been demonstrated with one of our typical materials, TFA-L PROLYL-(-) NAPHTHYLETHYLAMINE.

(3) Computer Control and Data Acquisition on the Bendix Time-of-Flight
The original design of the QUAD 300 computer interface made provision for the use of the electronic hardware and the computer programs with the Bendix time-of-flight mass spectrometer as well as the QUAD 300. After the return of the QUAD 300 this interface equipment was installed with the Bendix TOF. The controllable reference gas supply used on the QUAD 300 was also reinstalled to operate in a similar manner on the Bendix.

A voltage boosting circuit was installed in the Bendix analog scan unit to control the Bendix from the computer interface. This and other rework on the Bendix was done in a manner to allow either conventional or computer operation. One of the computer programs, "LOCATE", had to be rewritten to accommodate the square law relationship of mass with time in the TOF mass spectrometer. These items were completed and ready for final checkout by the 1st of October.

This enables the Bendix time-of-flight to be operated from and by the computer in a manner virtually identical to that accomplished on the QUAD 300. The system allows a reasonably fast data acquisition, nominally 4 to 8 seconds per spectra, and an on-line plot or presentation of the spectral measurements. The data acquisition time is compatible with obtaining mass spectra during the period of sample peaks in gas chromatograph effluence. However, at this time no gas chromatograph has been connected to the TOF mass spectrometer.

The completed system of computer control, data acquisition and data presentation is very economical of the mass spectrometer operator's time. It does allow more reliable data acquisition in a fraction, perhaps as little as one tenth, of researcher and technician attendant to the mass spectrometer, chart interpretation, and manual data reduction.

(4) MS-9

Some progress has been made on this project for direct computer acquisition of high resolution mass spectra. This included the installation of a remote data terminal (2704) of the ACME 360/50 adjacent to the MS-9 mass spectrometer in the Chemistry Department.

The ACME IBM 360/50 is a time-sharing computer located in the Medical Center and has a short cable connection to the Instrumentation Research Laboratory. The MS-9 is physically located in another building 1500 feet (cable length) away. The 270X-Y is a special IBM remote data access system designed to operate at these distances. The intent of the MS-9 development is to take the electrical output of MS-9 detector and, via suitable conversions and transmissions, sample it in real time with the ACME computer.

The required 270X-Y system has, in the last month of the reporting period, become usable from the ACME terminals. They still must be tested at the rate of data transmission required for the MS-9 operation. The ACME software system must make provision for allocation of the large data storage space required. Both of these requirements are within the specifications of the respective systems. The first, technical ability of the hardware to achieve the speed, is not expected to cause any problems. The software system necessary to accommodate this within the framework of time-shared computer use is not as straightforward. The requirements have been discussed with, and agreed to by the ACME programming staff.

During this reporting period the remainder of the "fast scan kit" purchased from the MS-9 manufacturer, Associated Electronics Industries, Ltd., was installed.

Physical facilities for drawing the 500 meter cable to connect the MS-9 and the ACME 360/50 were also completed during this reporting period. It was necessary to have a contractor install an electrical conduit and pull-boxes between the sites. This was accomplished and the conduit is ready for cable installation as of the 1st of October.

The electronics cabinet for the system has been started, power supplies, the 270Y, and Tektronic monitor scope have been installed. Other portions of electronics are under construction.

c. Computer Manipulation of Chemical Hypotheses

The work during this period is incorporated in Memo No. 54, Stanford Artificial Intelligence dated August 2, 1967. This will appear in "Symposium on Cognition", Carnegie Tech., John Wiley & Sons, (in press). The following is the abstract of that report.

"A computer program for formulating hypotheses in the area of organic chemistry is described from two standpoints: artificial intelligence and organic chemistry. The Dendral Algorithm for uniquely representing and ordering chemical structures defines the hypothesis-space; but heuristic search through the space is necessary because of its size. Both the algorithm and the heuristics are described explicitly but without reference to the LISP code in which these mechanisms are programmed. Within the program some use has been made of man-machine interaction, pattern recognition, learning, and tree-pruning heuristics as well as chemical heuristics which allow the program to focus its attention on a subproblem and to rank the hypotheses in order of plausibility. The current performance of the program is illustrated with selected examples of actual computer output showing both its algorithmic and heuristic aspects. In addition some of the more important planned modifications are discussed."

This research was supported by the Advanced Projects Agency of the Office of the Secretary of Defense (SD-183) as well as this NASA grant (NsG 81).

d. Particle or Cell Separation and Identification

(1) Cell Separator

We are continuing the development of the cell separator reported in the last report of this laboratory, IRL-1056, covering the period of October 1, 1967 to April 1, 1967.

As reported then, in order to achieve physical separation of cells according to their respective sizes, it is necessary to form a dynamically unstable, small, liquid jet which is excited ultrasonically to form uniform droplets.

Most of the electronic and mechanical parts of this instrument have

been completed and preliminary results indicate that this method of volumetric separation of cells has a 1 micron resolution with a minimum detection level of about 5 micron. The signal-to-noise ratio at this level of detection is approximately 5 to 1.

At the present time we are investigating the causes of clogging of the orifice or detection structure. Although filters of appropriate size have been incorporated, still the clogging of the orifice occurs much too often for practical use of the instrument. Besides the complete clogging of the orifice temporary disturbance of the stream has been observed by the presence of small particles in the vicinity of the interface of the two components of the orifice structure. This phenomenon upsets the stream velocity and direction and as a consequence the droplet formation point and size is affected. These two changes are absolutely objectionable since both the charging and deflection determine the size of the deflected cell.

Certain modifications have been carried out in the orifice assembly in order to minimize the clogging frequency and the turbulence in the liquid flow due to variations in orifice diameters. Figure 4 shows the orifice assembly. The two critical pieces, parts 9 and 8, are standard items and readily available. The metal orifice (8) is a platinum orifice having a 50 micron, polished hole. This part is available from the electron microscope manufacturers. The insulating orifice is a jewel having an 80 micron hole and is available from any jewelry store or watch repairing shop.

These modifications in the orifice structure, however, have not resulted in a trouble free operation. Clogging is still occurring very frequently and we are continuing to work on solving this problem.

Preliminary results are very promising. In Figures 5 to 8, pictures of the jet stream and formation of the drops are shown. From these experimental results we concluded that the pressure above a threshold is not a very critical parameter of the system. Figure 9 shows signals detected by suspending Paper Mullberry balls of 12-13 micron diameter into a normal saline solution. From the signal-to-noise ratio it is obvious that a much smaller diameter particle can be detected.

(2) Detection of Fluorescent Cells in a High Speed Flow System

The cell flow simulator system described in Technical Report No. IRL-1056 was used to evaluate electrical and optical conditions which would probably occur in a realistic high speed flow system. Results indicated the feasibility of a practical system for cell separation and work has proceeded accordingly.

In order to facilitate direct study of liquid droplet formation a test module was made which fitted the microscope stage (Fig. 10). Liquid under an adjustable air pressure flows through the stainless steel tubing at the left of the module and emerges from a glass micro nozzle as fine stream of circular cross section. The droplets which subsequently form are collected by a cup at the right of the module and drawn into the exit tube by a partial vacuum system. The module may be positioned in three axes to permit microscopic examination of any part of the liquid stream. At an air pressure of 10 PSI, turbulence was visible in the emerging stream some 3 mm from the nozzle and at 4 mm the stream separated into irregularly sized droplets of varied spacing. (Figures 11 and 12). The velocity of the stream at 10 PSI and with a 50 microns diameter nozzle was 10 meters per second; and the droplets ranged from 10 to 100 microns in diameter.

In order to regularize the droplet size and spacing means were provided to axially vibrate the nozzle at ultrasonic frequencies. Initially this was achieved by air coupling a small speaker diaphragm to a

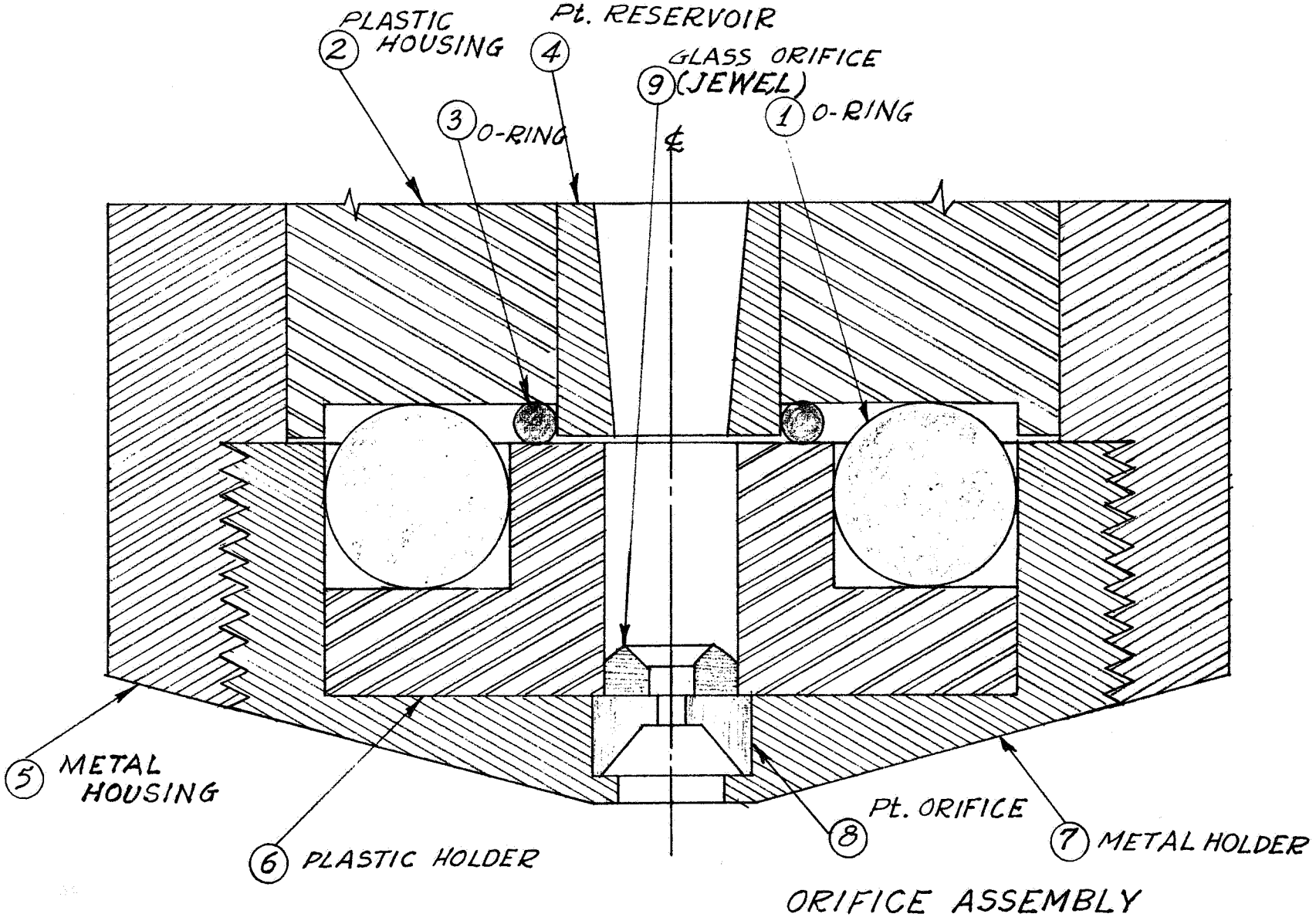
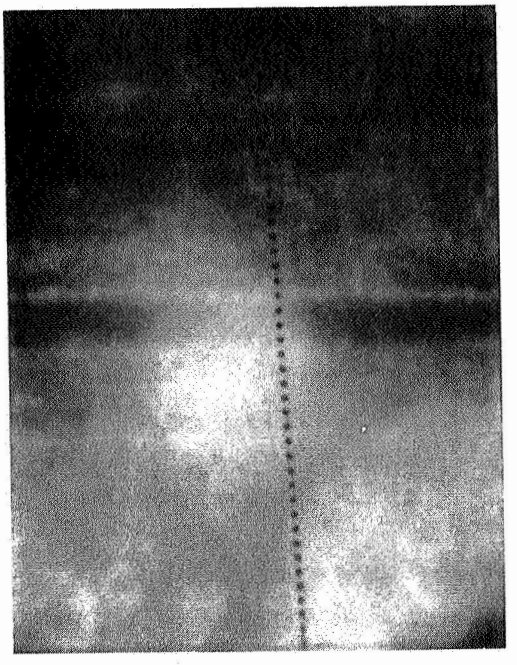
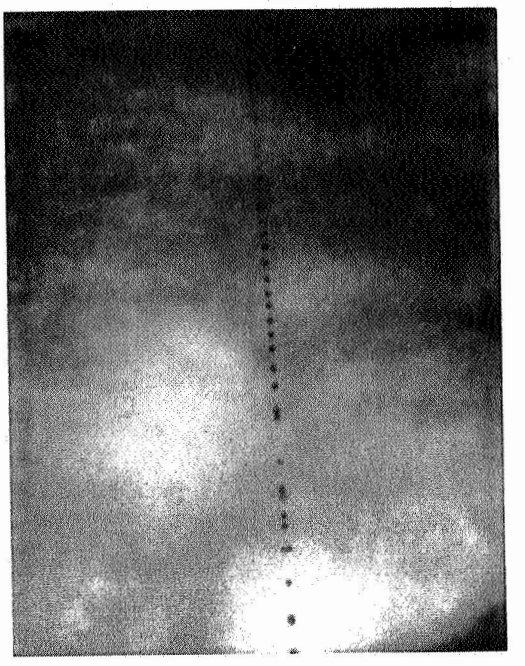


FIGURE 4

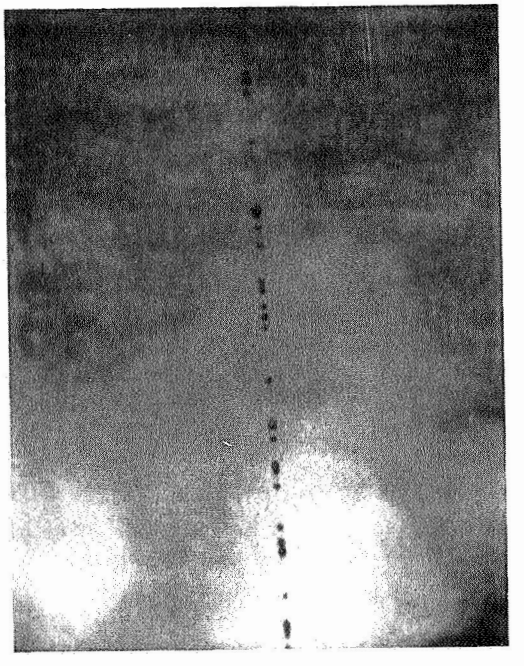
ORIFICE ASSEMBLY



PRESSURE : 3016
ULTRASOUND : ON



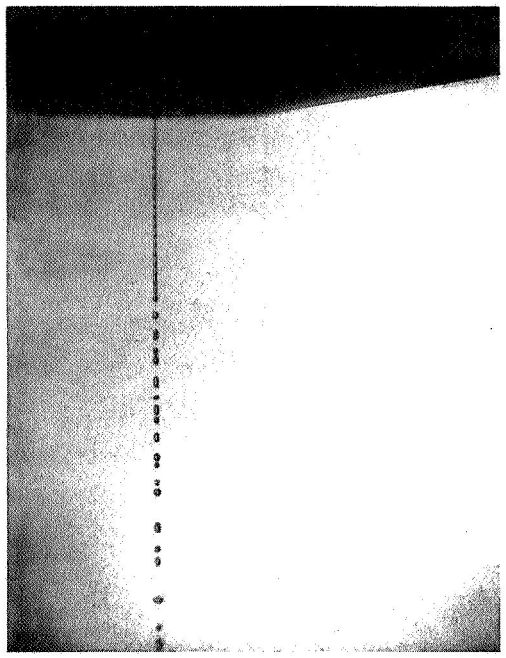
PRESSURE : 3016
ULTRASOUND : ON (MIN)



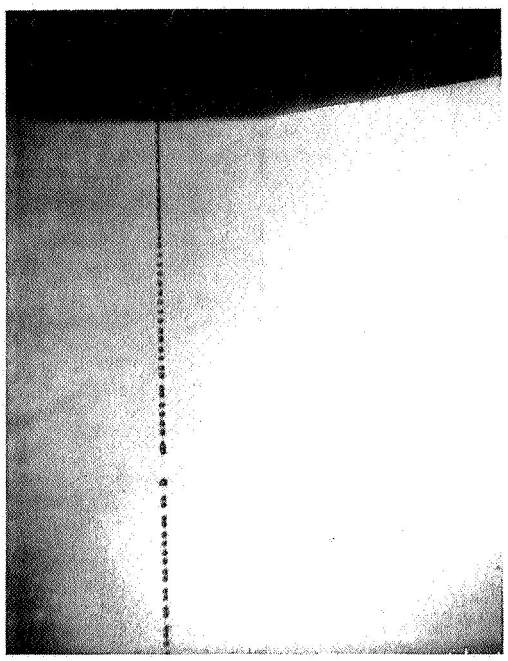
PRESSURE : 3016
ULTRASOUND : OFF

FIGURE 5

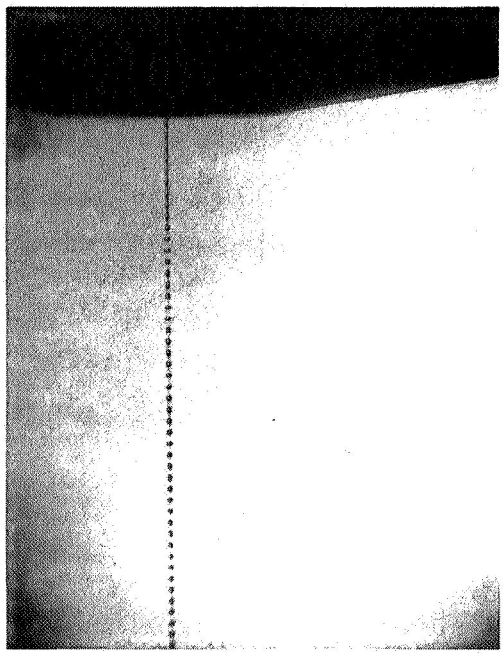
Down-Stream Pictures



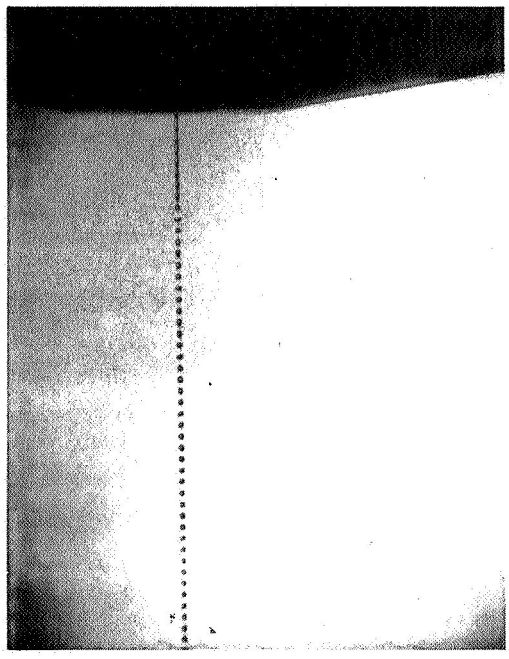
PRESSURE : 15\16
ULTRASOUND I = 2Amp
V = 47VDC } P.P.-16Ω



PRESSURE : 15\16
ULTRASOUND: I = 4Amp
V = 94V } PP-16Ω

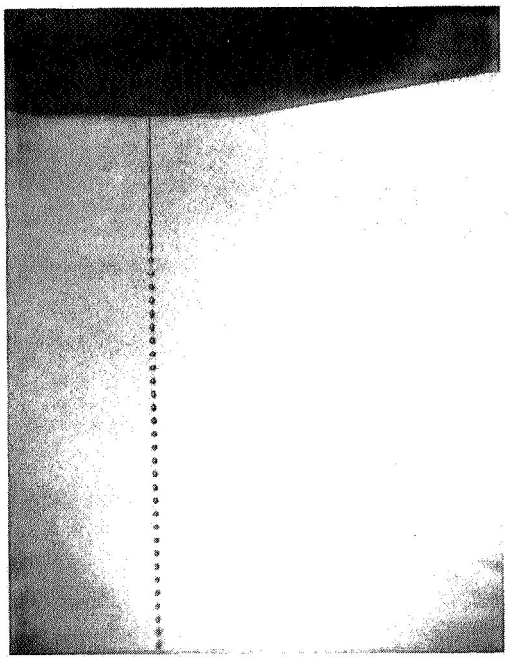


PRESSURE : 20\16
ULTRASOUND: I = 2Amp
V = 47V } P.P.-16Ω

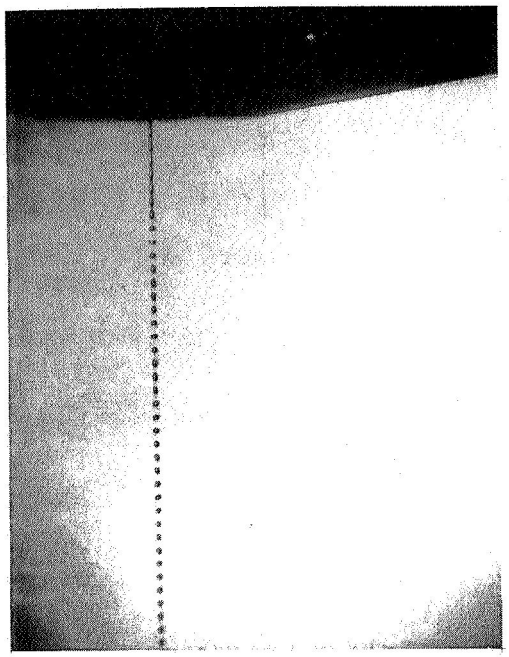


PRESSURE : 20\16
ULTRASOUND: I = 4Amp
V = 94v } P.P.-16Ω

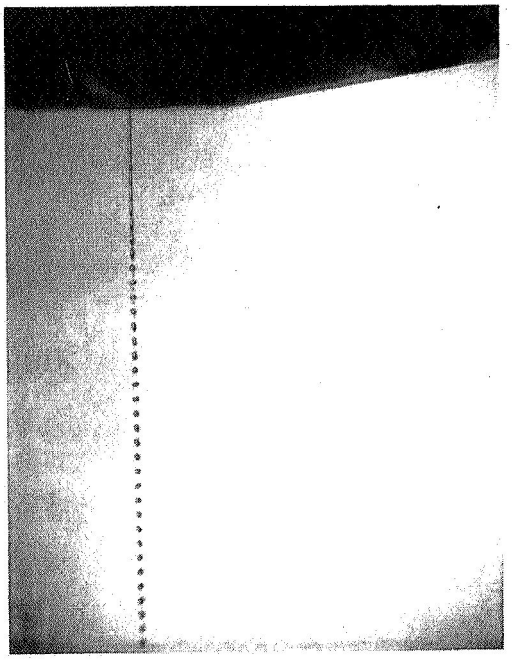
FIGURE 6



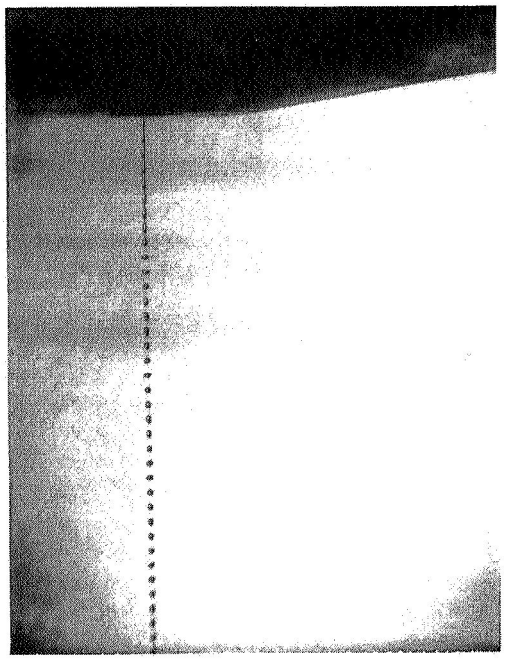
PRESSURE : 25 lb
ULTRASOUND : I = 2 Amp, V = 47 V } P.P.-16 %



PRESSURE : 25 lb
ULTRASOUND : I = 4 Amp, V = 94 V } P.P.-16 %

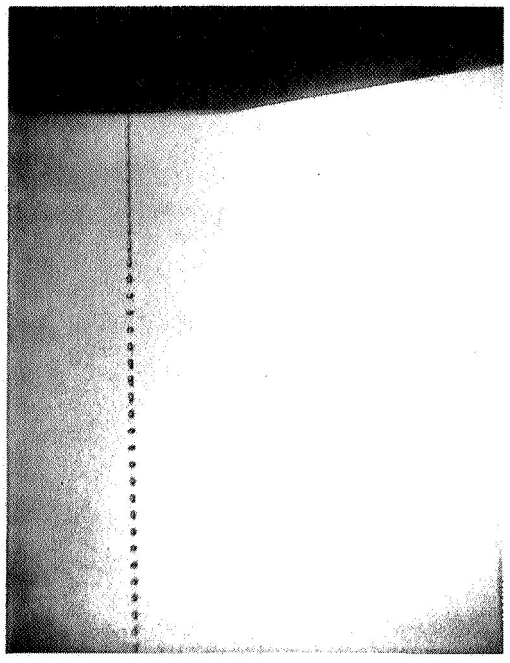


PRESSURE : 30 lb
ULTRASOUND : I = 2 Amp, V = 47 V } P.P.-16 %

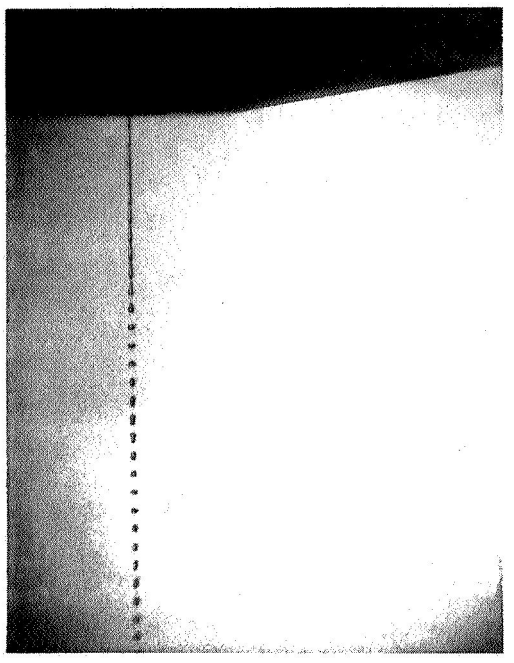


PRESSURE : 30 lb
ULTRASOUND : I = 4 Amp, V = 94 V } P.P.-16 %

FIGURE 7

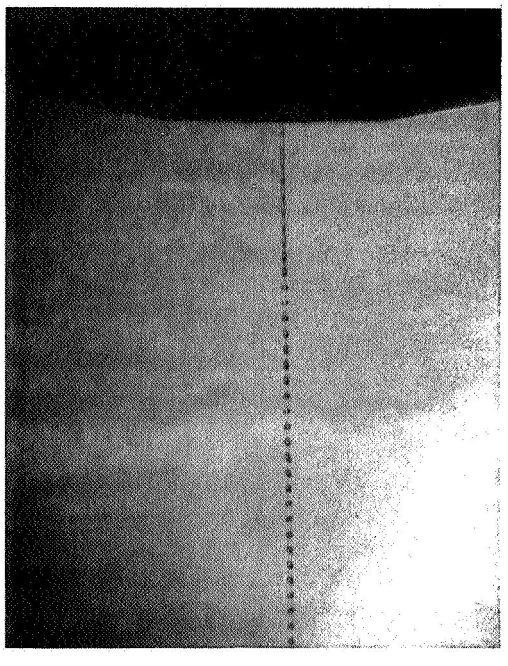


PRESSURE : 40lb



PRESSURE : 50lb.

ULTRASOUND : I = 4Amps, V = 94 V } P.P. - 16 Ω



ULTRASOUND : I = 64mA P.P.
V = 94 VDC P.P.
f = 75,500 CPS

PRESSURE : 30lb.

FIGURE 8

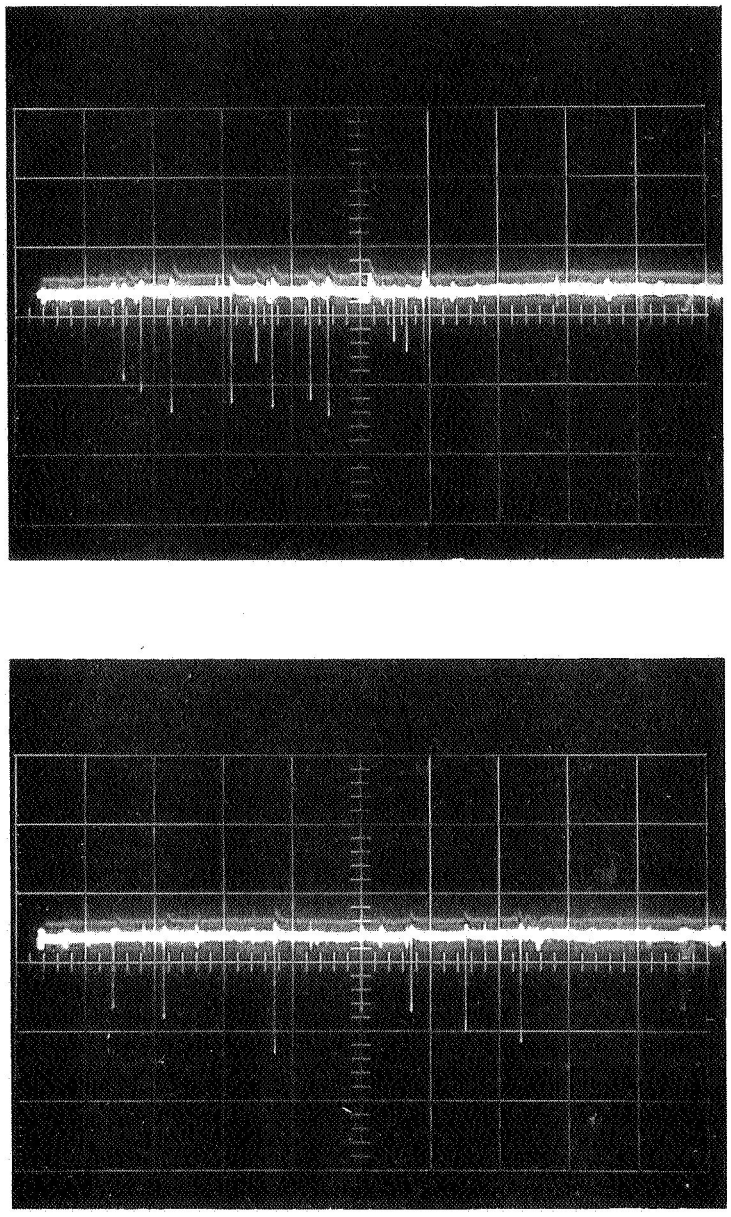


FIGURE 9

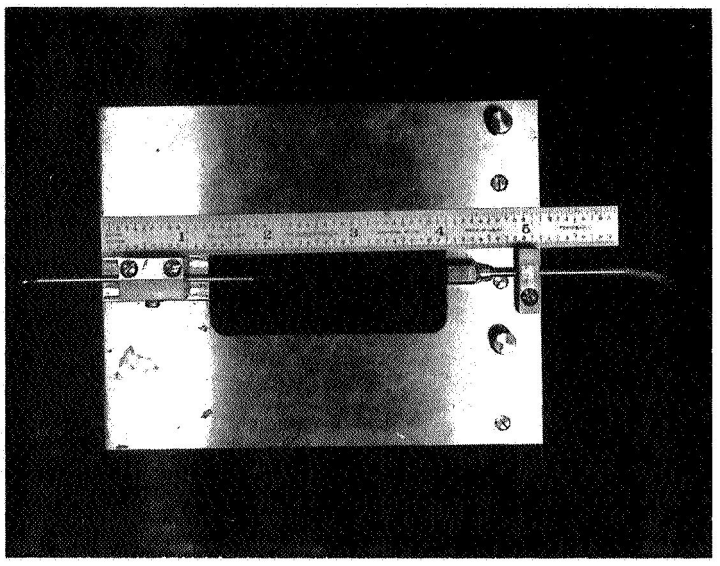


FIGURE 10

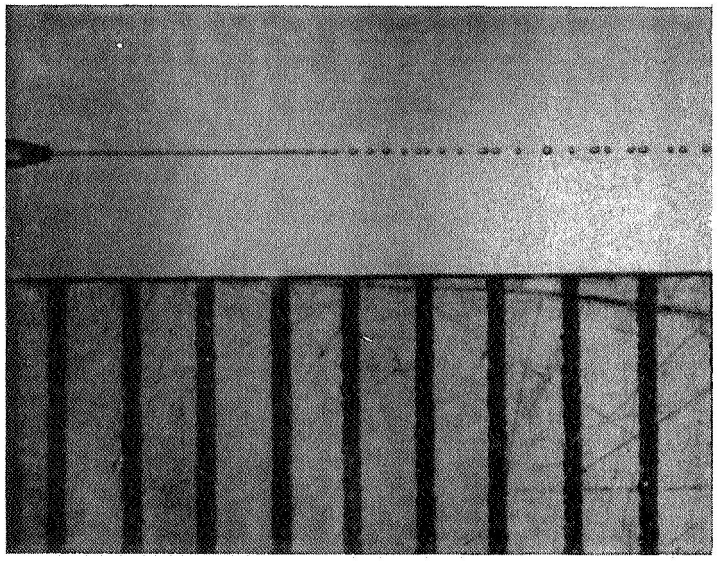


FIGURE 11

co-axial disk on the stainless steel tubing. A further modification led to the arrangement shown in Figure 13 in which the nozzle is vibrated by mechanical coupling to a barium titanate transducer. Figures 14, 15 and 16 demonstrate the uniformity of droplet generation so obtained.

Figure 14 shows the tip of the glass nozzle, the emerging liquid stream, and the separation of the first droplet at approximately 1.6 mm from the nozzle.

Figure 15 shows longitudinal oscillation of the droplets which continues for a distance of a few mm following separation.

Figure 16 shows the uniformly sized and spaced droplets of 88 microns diameter at 10 mm from the nozzle. Some image deterioration is noted, the result of random air fluctuations on the stream.

Some work was carried out with a 100 micron diameter nozzle to establish the sensitivity of the system. The liquid reservoir supplying the nozzle was alternately filled with distilled water and selected dilutions of fluorescein. The formed droplets, in this case 180 microns in diameter, were observed in dark field illumination using a Zeiss 0.95 - 0.8 NA condenser, a 0.8 NA objective and various light sources. The primary filter was BG 12 glass, the secondary Wratten 15A gelatin. At a droplet formation rate of 4×10^4 per second the 1P21 phototube used could discriminate between distilled water and a 10^{-7} molar concentration of fluorescein, providing typically a signal of 80 millivolts for the 10^{-7} molar fluorescein and 45 millivolts for the distilled water. The signals appearing across the 1P21 phototube load resistor represents a photocathode current of 2×10^{-11} amps for the 10^{-7} molar fluorescein and one of 1×10^{-11} amp for distilled water. The droplet size of 180 micron diameter at 10^{-7} molar concentration demonstrates that 2×10^8 fluorescein molecules can be detected in 4×10^{-4} seconds.

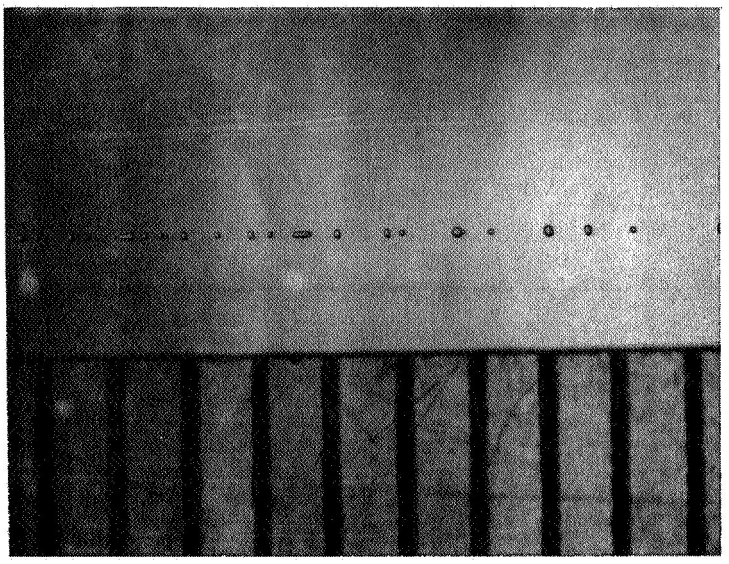


FIGURE 12

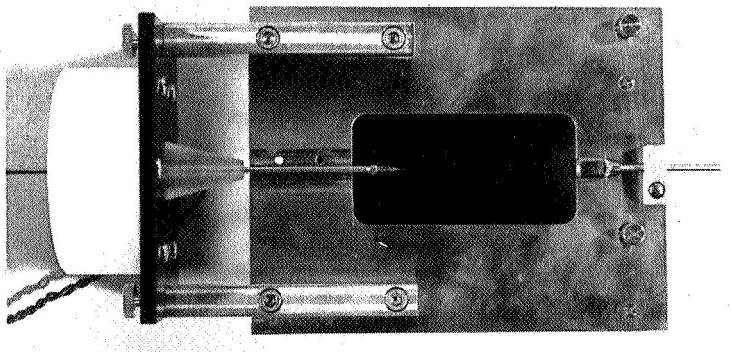


FIGURE 13

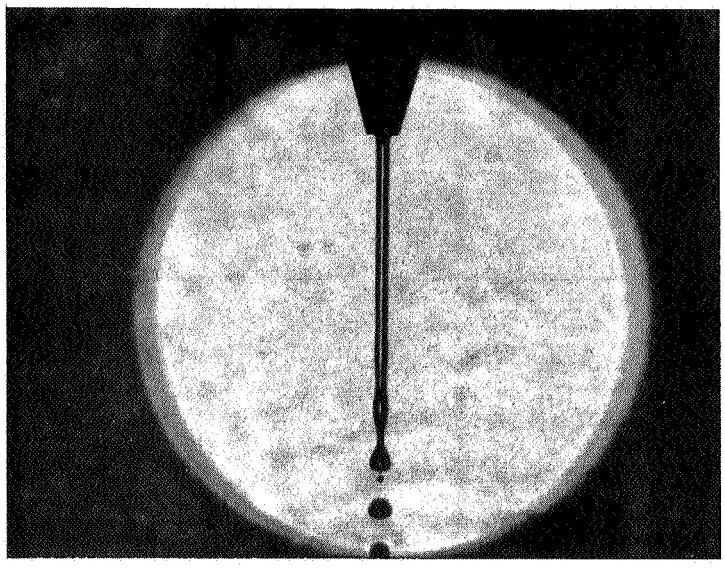


FIGURE 14

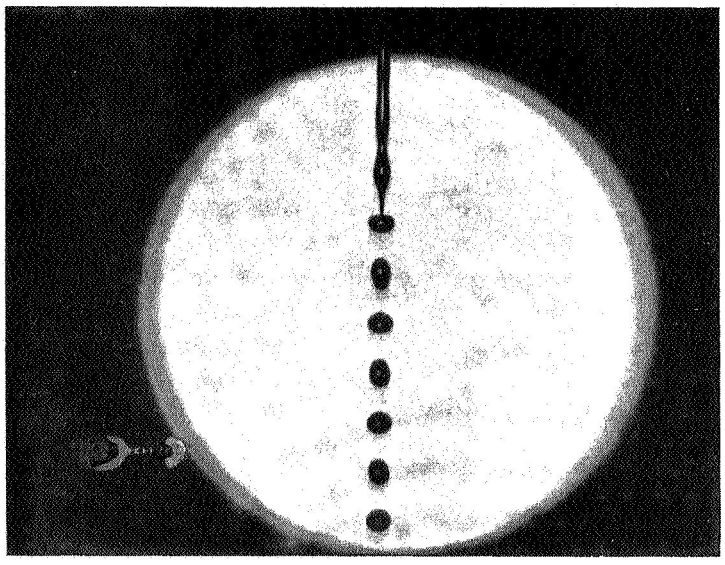


FIGURE 15

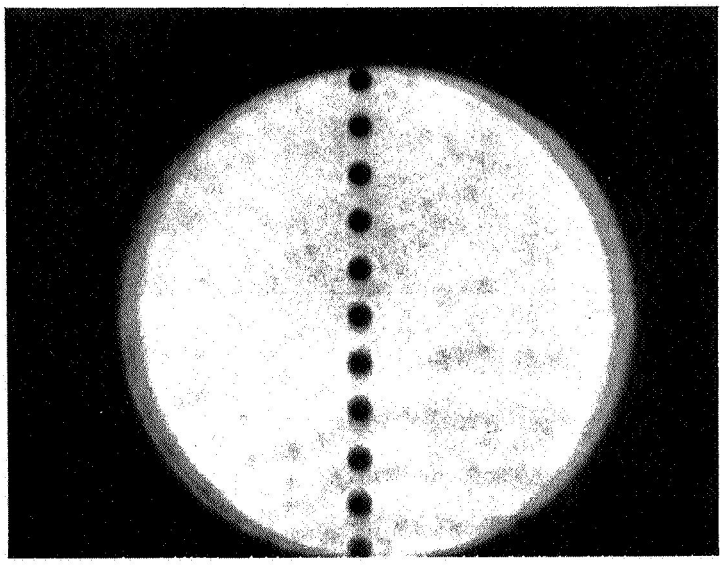


FIGURE 16

Further work is necessary to optimize droplet illumination conditions, and to improve the positional stability of the liquid stream. After this is carried out attention will be directed to the deflection and collection of selected droplets.

d. Thermal Chromatography

This work in this area during this period was confined to attempts to renature specific fractions from thermal chromatography runs on hydroxyapatite columns. No evidence of renaturation was observed in tests for the restoration of transformation activity on B. subtillis mutants requiring uracil and tryptophane.

IV. Prebiological Evolution

Various theories on origin of life have postulated primitive oceans reasonably rich in organic precursors of biological molecules. An investigation has been started into the probable concentration of such precursors in the primitive oceans. This investigation is described in detail in a paper entitled "Thermodynamics and the Origin of Life" submitted for publication to Science.

Most of the experimental activity in this field has been concerned with mechanisms for synthesis of compounds of interest. Once a mechanism has been established it is possible to arrive at some conclusions about possible rates of the synthetic reactions. In order to reach conclusions about concentration, it is necessary to know the rates of any possible degradative reactions as well. A literature search turned up some of the desired information but much is still lacking. One of the chief degradative processes in water solution is hydrolysis. A preliminary experimental investigation into the rates of hydrolysis of some of these biological compounds has accordingly been initiated.

The first compounds investigated were adenylic acid (AMP, ribose deocyadylicacid (d AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP), both in distilled water and in sea water. Hydrolysis rates were determined at temperatures between 55°C and 95°C, the activation energy determined from the differences in rates, using the equation

$$\ln \frac{K_2}{K_1} = \frac{E}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$

Where K_a represents the reaction rate at T_a, E the activation energy and R the gas constant.

The data indicated that activation energies for all the compounds in these solutions were in the range between 23,000 and 27,000 calories/mole, that the reactions were first order in the reactant hydrolyzed and that the lives of the compounds were in the range of several months to several years at 25°C and several weeks to several months at 37°C. Such relatively short lives on a geological scale would severely limit the concentrations of these phosphate compounds in the primitive oceans.

V. Pasteur Probe

An announcement of "Opportunities for Participation in Space Flight Investigations" concerning 1973 Voyager experiments was received from NASA Headquarters. After a series of discussions with personnel of the Life Detection Branch of the Ames Research Center, NASA, a decision was reached to submit a joint proposal with that group concerning the determination of molecular asymmetry for extraterrestrial life detection.

A notice of intent to propose was submitted, listing Glenn E. Pollock of Ames as the principal investigator, and Ralph Donaldson of Ames, Bert Halpern of the IRL and H. R. Hulett of the IRL as coinvestigators. The proposal for the instrument was nearly finished when notice of the suspension of the AFO was received. It will be completed and submitted to NASA Headquarters when required.

C. PAPERS AND REPORTS

April 1, 1967 to October 1, 1967

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1. W. E. Reynolds, J. C. Bridges, T. B. Coburn and R. B. Tucker, "A Computer Operated Mass Spectrometer System". Technical Report No. IRL-1062, (1967).

2. R. B. Tucker, T. Coburn, W. Reynolds, and J. Bridges, "Software for the LINC Computer", Technical Report No. IRL-1055, (1967).

PUBLICATIONS

1. J. Lederberg and E. A. Feigenbaum, "Mechanization of Inductive Inference in Organic Chemistry", Symposium on Cognition, Carnegie Institute, John Wiley & Sons, in press, (1967).

2. H. R. Hulett, "Thermodynamics and the Origin of Life", Science, in press (1967), IRL-1065.

3. H. R. Hulett, "Turbulence Limitations in Photographic Resolution of Planet Surfaces", J. Opt. Soc. Am., 57, 1335 (1967), IRL-1064.

4. B. Halpern, D. Nitecki, and B. Weinstein, "The Steric Purity of Model Peptides by N.M.R. Spectroscopy", Tetrahedron Letters, 3075 (1967), IRL-1066.

5. B. Halpern and D. Nitecki, "The Deblocking of t-BOC Peptides with Formic Acid", Tetrahedron Letters, 3031-33 (1967), IRL-1067.

6. B. Halpern, L. Chew and B. Weinstein, "Measurement of Racemization in Peptide Synthesis by N.M.R. Spectroscopy", J. Am. Chem. Soc., 89, 5051 (1967).

7. J. W. Westley, P. J. Anderson, V. A. Close, B. Halpern and E. M. Lederberg, "Aminopeptidase Profiles of Various Bacteria", J. Appl. Microbiol., 15(4), 822-825 (1967), IRL-1059.

8. J. W. Westley and B. Weinstein, "Magnetic Nonequivalence of the Methylene Group in Glycyl Dipeptides", Chem. Comm., in press (1967), IRL-1069.

9. O. Reynolds, E. Levinthal, G. Soffen, "The Role of the Scientist in Automated Laboratory Systems," AIAA Paper No. 67-632, (1967), IRL-1070.

10. E. C. Levinthal, J. Lederberg and C. Sagan, "Relationship of Planetary Quarantine to Biological Search Strategy", presented at COSPAR Meeting (Committee on Space Research), London, (1967), IRL-1071.

11. C. Sagan, E. C. Levinthal and J. Lederberg, "Contamination of Mars", Science, (accepted for publication), (1968), IRL-1072.