NYO-3993-2
RADIOISOTOPE AND RADIATION
APPLICATIONS (TID-4500)

AUTOMATED PRIMARY PRODUCTIVITY MEASUREMENTS IN WATER BODIES

Phase II - Design, Fabrication and Test of Demonstration Instrument

Prepared for:

Division of Isotope Development
U. S. Atomic Energy Commission

Contract No. AT(30-1)-3993

Contract Period
March 21, 1969 - February 1, 1971

Contractor

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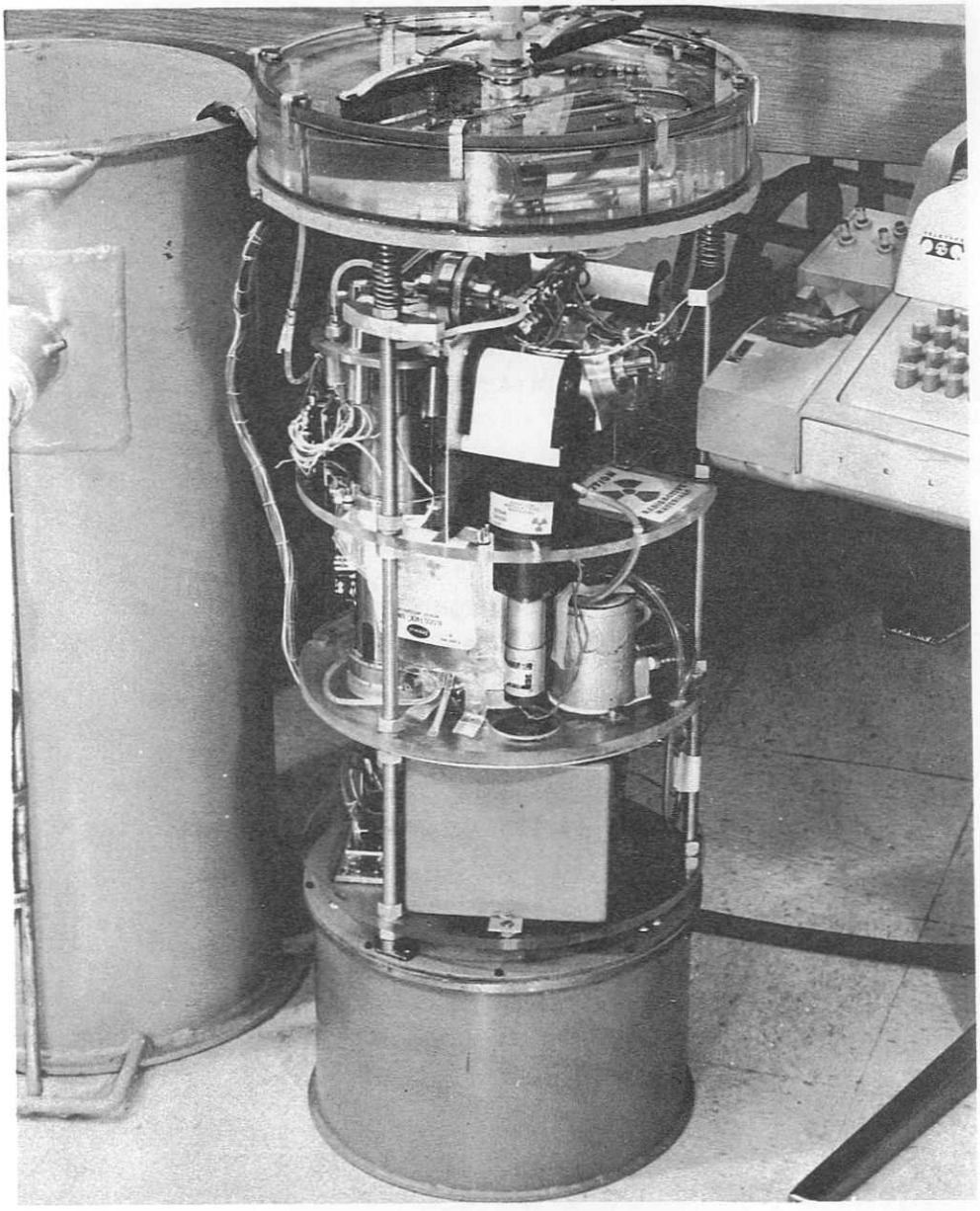

Engineering Test Model
of the Automated Primary Productivity Instrument

ACKNOWLEDGEMENTS

The authors wish to especially acknowledge the important effort of several of the many persons who contributed to this program. These include the following Biospherics' personnel:

Gilbert F. Pascal, Mechanical Design and Development

Michael Smisko, Fabrication and Testing

Margaret Schiffman, Biological Procedure and Testing

J. Rudolph Schrot, Ph. D., Microbiology

For the support of the Occoquan testing:

James Corbalis, Engineering Director, Fairfax County Water Authority

For the support of the Lake Lanier, Georgia testing:

Walter M. Sanders, III, Ph. D., Chief National Pollutants Fate Research Program

Patricia Kerr, Federal Water Quality Administration

For consultation and advice:

John Strickland, Ph. D., Scripps Institution of Oceanography

Alan Longhurst, Ph. D., National Marine Fisheries Science Laboratories

Robert Owen, National Marine Fisheries Science Laboratories

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TABLE OF CONTENTS

TABLE OF CONTENTS
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TABLE OF CONTENTS
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TABLE OF CONTENTS
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TABLE OF CONTENTS
(continued)

LIST OF FIGURES

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ABSTRACT

An automated instrument to make continual measurements of aquatic primary productivity by the radiocarbon method is under development. The instrument, based on the "light" and "dark" bottle technique, will permit oceanographers, limnologists, and pollution control workers to make greater use than hitherto possible of this fundamental ecological parameter. Automatic programming of the procedure will standardize the assay greatly, improving the comparability of data among different investigators. Another significant advantage of the instrument is that it conducts the assay in situ as opposed to the manual technique which requires that the sample be brought to the surface where it is exposed to physical and chemical changes which introduce error into the results.

An Engineering Test Model (ETM) of the Automated Primary Productivity Instrument (APPI) has now been designed, fabricated, and tested. The precise technique selected for automation was developed by extensive laboratory studies based on a search of the literature and direct discussions with leading authorities. Materials and systems for the instrument were selected and designed to minimize toxicity and mechanical damage to phytoplankton.

Laboratory and initial field tests showed a satisfactory degree of correlation between the APPI and results obtained by the manual procedure. However, a final field test produced APPI and manual results which did not correlate. Primary productivities determined by the APPI exceeded those obtained by the manual method by severalfold.

Efforts to assign instrument responsibility for the discrepancy have been unsuccessful. Several explanations suggesting possible inhibition of the manual samples as the result of environmental changes imposed on the particular water tested have been tentatively offered by various primary productivity authorities. Should such an effect be confirmed experimentally as the responsible cause, the advantage of the in situ aspect of the APPI would be strongly demonstrated. A clear resolution of this matter is required before proceeding to Phase III of the program, the development of a prototype APPI. Recommendations concerning clarification of data problems and the future course of the program are presented.

I. SUMMARY

The overall objective of this program is the development for the commercial market of an instrument to perform in situ, radiocarbon primary productivity tests for periods of several months of unattended, continual operation. The instrument will permit the routine determination of this fundamental parameter in the conduct of research and applied studies by oceanographers, limnologists, and pollution control workers. Advantages for the instrument are a reduction in the time and money expended in obtaining data, elimination of the variables introduced by the "art" of the manual procedure, and protection of the samples against the environmental shocks introduced by the manual assay. Phase II of this program, designed to demonstrate the feasibility of the Automated Primary Productivity Instrument (APPI), has now been completed. This work has resulted in the design, fabrication, and testing of an Engineering Test Model (ETM) of the APPI.

Phase II was carried out as a joint biological and engineering developed program. Building on the information gathered in Phase I, the laboratory effort carefully and quantitatively defined the procedure to be automated. The necessary subsystems and interconnections to mechanize the procedures in accordance with these design criteria were then engineered, integrated, and tested.

The ETM (shown in the frontispiece photograph), is contained in a hull 13 inches in diameter by 32 inches in length. The top end of the cylindrical hull is a transparent plate containing the photosynthesis incubation chamber. The hull contains all supplies, reagents, receptacles, mechanisms, electronics, logic system, and a programmer to perform a series of ten primary productivity determinations.

The program functions of the ETM consist of taking in the water sample through a protected inlet, adding a metered quantity of sterile $NaH^{14}CO_3$ solution to the sample, delivering 100 ml of the labeled sample into the photosynthesis chamber, retaining a duplicate 100 ml in a dark chamber, conducting a two-hour incubation period, sequentially filtering the samples through respective 47 mm diameter areas of a membrane filter tape, advancing the tape through a heating chamber to dry the filtered areas, and then advancing the filtered areas to a Geiger tube chamber for counting of radioactivity. The data are recovered in realtime on a shore or boat-based display. The tape is retained in the instrument for possible future examination of the organisms. Automated housekeeping operations include: continual wiping of the photosynthesis chamber optical window to prevent fouling or the accumulation of sediment, post-assay rinsing of the entire plumbing system to prevent fouling, onboard

storage of all waste streams, chemical scrubbing of $^{14}CO_2$ released (primarily in drying step) within the hull to suppress noise, and verification of each completed function.

The suitability of the design approach was demonstrated in a series of laboratory and field tests. The photosynthesis chamber, containing a free-floating piston, was found to fill easily and without air or gas entrapment. Silicone oil was used to reduce the refractive index mismatch of the water, glass, and plexiglass interfaces.

Although mechanical failures were experienced in the drive motor and piston lead-screw, these were relatively minor and the entire sample intake and preparation system, including the isotope metering pump and means for splitting the sample into "light" and "dark" portions, was found to be of good design concept.

It was recognized from the outset of the program that the most difficult problem would be that of preventing fouling of the photosynthesis chamber window, in particular, and other sensitive portions of the instrument as a result of growths occurring over weeks or months. A "windshield wiper" mounted on the exterior of the glass end-window of the ETM proved extremely effective over a test period of two months in a highly eutrophic portion of the Chesapeake Bay where silting and fouling were intense. Also, the wiping action created by an O-ring sealing the floating piston to the

inner wall of the chamber cleaned the internal surface of the photosynthesis chamber. In the same extended test, the internal plumbing pumps and chambers were maintained free from fouling by a system designed to apply repeated aliquots of a wash solution between test cycles.

The difficult problem of loading and storing the ^{14}C solution in sterile conditions was solved through the use of sterile blood bags which receive the isotope solution through a membrane filter for storage. Back contamination of the reservoir by the sample was also prevented in this manner.

One of the signal developments of the program was the creation of a miniature motor driven, anvil type valve used in the liquid transfer systems. A high torque motor drives a hammer against an anvil to pinch the flexible tubing sufficiently tight to ensure against leakage. In the open position, these valves present no head loss to the stream, nor do they come in contact with it. The valve tubing selected was tested through thousands of valve cycles with no evident deterioration of either.

Another difficult task successfully solved was that of effecting a filter tape train. This was accomplished through the use of nylon backed membrane filter tape. The tape is fed from a roll and the portion for use is held and supported

firmly in the jaws of the filter structure which prevents lateral movement of the water. Upon release of a detent to permit the tape to be advanced to the drying station, peripheral suction is maintained on the downstream side of the filter apparatus to preclude dripping from hang-up. The train has operated in leak-proof fashion.

A radioactivity counting station utilizing an end-window Geiger tube accommodating the 47 mm diameter filter spots on the tape was developed with a counting efficiency of 14%.

The logic sequence was designed in accordance with the optimized manual procedure selected by the laboratory effort. It is administered by a highly flexible punch tape programmer. Unit functions and elapsed times are isolated so that wide variations in program logic and timing can be introduced, if desired, through the preparation of new tapes. Not only is this approach highly desirable for an experimental model of the APPI, but it will permit users of the final instrument wide latitude in conducting the assay, should they desire to effect certain changes in specific study situations. These changes will be possible without requiring any reworking of mechanisms or electronics.

All of the supporting electromechanical and electronics subsystems were found to be adequate.

The power supply for the ETM was reduced to a simple 12-volt DC source. This consisted of shore or ship-based automobile storage batteries connected to the ETM through a waterproof cable. The same cable carries the radioactivity readings and housekeeping data from the instrument back to the base. In the ultimate APPI, the power supply and readout units will be housed within the data system buoy hull so that the entire operation will be self-contained.

One additional feature developed for the ETM was an electronic display unit which monitors and displays housekeeping data so that the otherwise undetectable functions of the submerged device can be followed.

Upon completion of the instrument, a laboratory test program was undertaken in which samples were split and run simultaneously under nearly identical conditions in the ETM and by the manual method. The two systems were found to correlate well. Field testing of the instrument was then undertaken with the first test run in the Occoquan Reservoir of the Fairfax County Water Authority. The instrument was deployed into the water from the dam face and manual samples were obtained near the ETM and processed simultaneous with the operation of the instrument. Satisfactory correlation was again achieved.

A final field test was performed at Lake Lanier, Georgia, in conjunction with the Southeast Water Laboratory of the Federal Water Quality Administration (FWQA), Department of the Interior. Several mechanical problems developed during this test. They involved the failure of the sample pump drive motor, stripping of the lead-screw within the pump, and minor leakage caused by slight corrosion-induced pitting of the hull head flange seal. These problems were relatively minor. However, a major question was raised by the data obtained in these tests.

The data produced by the APPI ETM in the Lake Lanier tests were significantly (up to severalfold) higher than the corresponding data from the manual samples. The effort available within the scope and cost of Phase II was expended to determine the cause of the data discrepancy. No design errors or malfunctions of the instruments which could have been responsible for these large differences were found. Nor were any flaws in the conduct of the manual technique detected.

Several possibilities were voiced or reported by experts in the field to indicate that the data discrepancy might be caused by stresses placed on the photosynthetic organisms by manipulations required in executing the manual primary

productivity assay. The phytoplankton encountered in Lake Lanier, a highly eutrophic and polluted body of water containing dense algal populations at the time of testing, may have been shocked by the sunlight or changes in oxygen tension when the samples were brought to the surface and manipulated in open glass vessels. If these hypotheses, detailed within the report, are supported, the data discrepancy would strongly endorse one of the key advantages seen for the APPI - that of conducting the primary productivity assay in situ.

The success of the APPI program hinges on the resolution of this major problem. Recommendations have been made for the necessary investigations. Should they prove successful in discovering some correctable malfunction of the ETM or in verifying the ETM data, it is recommended that Phase III, the development of a full prototype APPI, be undertaken.

II. INTRODUCTION

The determination of primary productivity is generally accepted by oceanographers and limnologists as a fundamental measurement of biological activity in a water body since it measures the rate at which inorganic carbon is being synthesized into living matter by photosynthesis. Routine primary productivity measurements in various water bodies would help to establish a baseline that would enable the assessment of trends in eutrophication and would also determine the effects of pollutants on phytoplankton. Despite the importance of this measurement in surface water technology, its current use has been confined mostly to research studies because the determination must be performed manually and the techniques are generally too complicated and time-consuming for routine use.

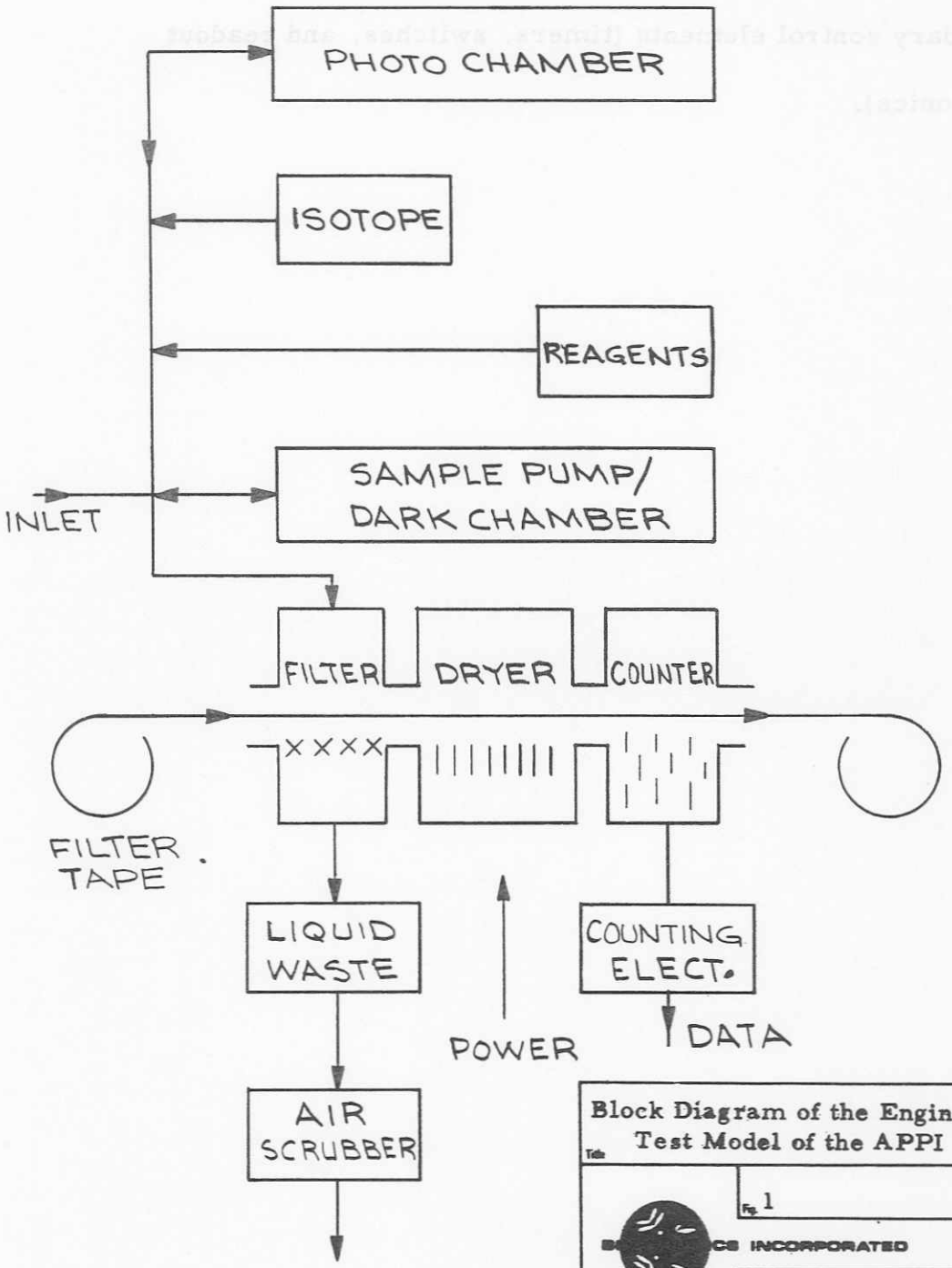
On the basis of a proposal submitted by Biospherics Incorporated, the U. S. Atomic Energy Commission (AEC) supported the development of the Automated Primary Productivity Instrument. The APPI was conceived to overcome the difficulties that exist with the manual, radiocarbon primary productivity assay and, also, to conduct the entire measurement process in situ, duplicating as closely as possible the actual environmental conditions surrounding the sample being assayed.

Phase I of this program - the Feasibility and Preliminary Design Study - has been reported (1) earlier. The study was planned to gather all applicable reference data

regarding the measurement of primary productivity and to use this material to perform a preliminary laboratory research program, to develop a conceptual design for the instrument.

The objective of the 18-month Phase II program herein reported was to develop the ETM of the APPI which could be used to test the biological and engineering feasibility of automating the primary productivity assay. The successful conclusion of the program was planned to be the operation of the instrument in a field demonstration. The standard manual technique would be run for comparative data.

A block diagram of the ETM is shown in Figure 1. In operation, the instrument takes in a sample of water and inoculates it with a small quantity of $NaH^{14}CO_3$. It then retains half of the sample in a dark chamber and places the other half into a photosynthesis chamber for a 2-hour incubation period. After inoculation, the "dark" and "light" samples are filtered separately, rinsed with acid, dried, and then assayed for ^{14}C. The resultant count rates from the sample assays are used in combination with an inorganic carbon determination from a separate sample to compute primary productivity. A program controller, which uses an endless loop program tape, automatically sequences the operations to effect the proper interconnection of the sample pump to the other functional elements (sample inlet, reagent



Block Diagram of the Engineering Test Model of the APPI

Fig. 1

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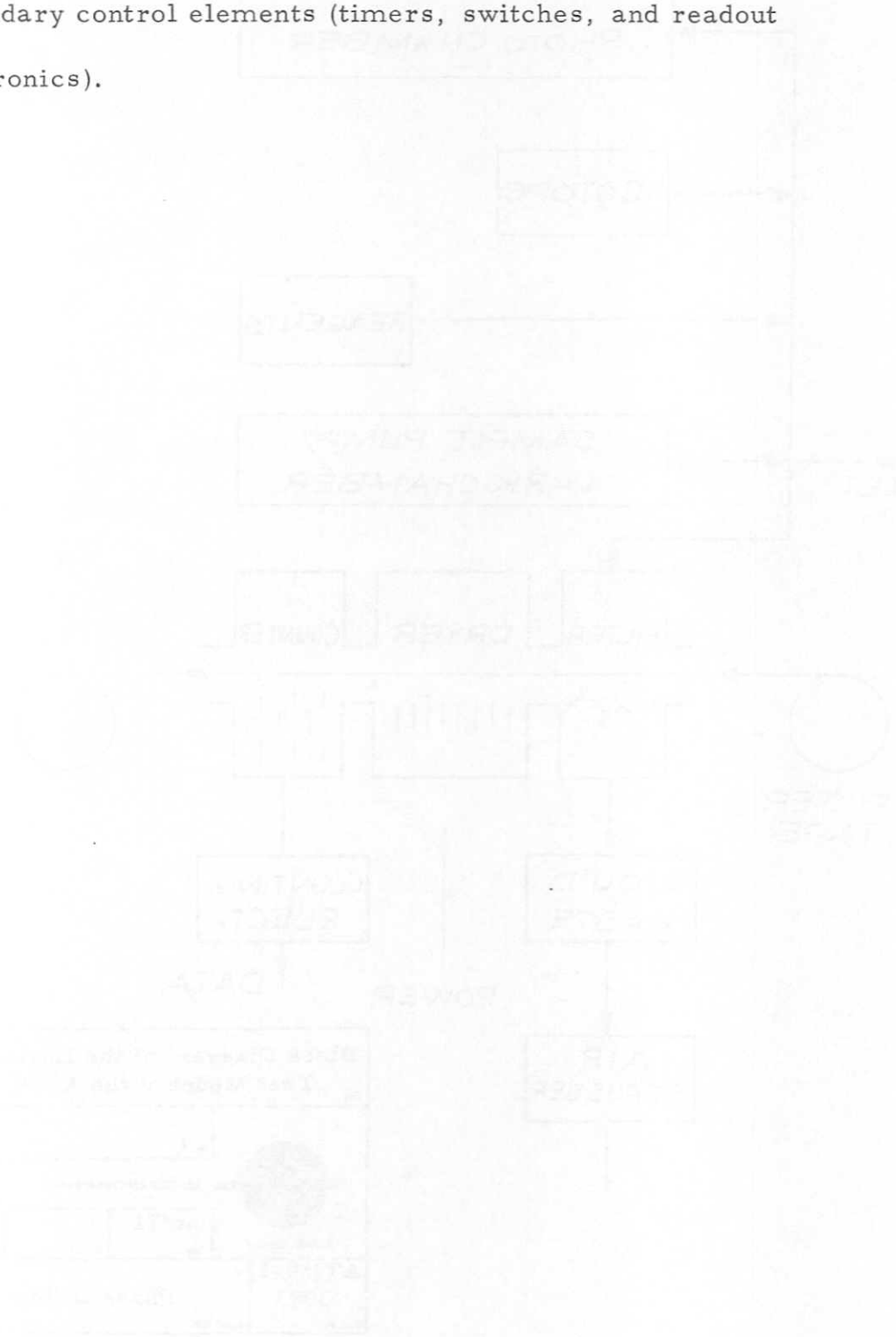
AT(30-1)-3993

Phase II Report

reservoirs, photosynthesis chamber, and filtration system) and secondary control elements (timers, switches, and readout electronics).



III. BACKGROUND

This program to develop an APPI was started in September 1968, after more than a year of critical examination of the technical foundation for the program's rationale, which led to the documentation of a specific need that could be met by the instrument. Until that time, automated biological instrumentation operating routinely in an ocean environment had not received serious consideration. However, since scientists currently make routine manual assays of primary productivity, it became apparent that an automated version could save many man-hours of labor and, thereby, enable much greater use of the assay. In addition, if such an instrument were to perform the assay in an in situ manner, a more realistic determination would result.

Since the AEC is not a user for this type of instrumentation, an expression of need from a potential "user" agency had to be obtained. One such "user" was the Bureau of Commercial Fisheries (now known as the National Marine Fisheries Science Laboratories), that currently performs many thousands of primary productivity assays each year in oceanographic studies. Relevancy of primary productivity to commercial fishing was discussed in the following excerpt from our proposal submitted to the AEC in March 1968:

> The potential use of the sea as a food resource for the World's burgeoning population is a major factor in the rapidly increasing interest

> in oceanography (2, 3). The President's Science Advisory Committee had stated (4) that "new mouths in the underdeveloped World will need some 300 million tons of additional grain annually by 1980" - an amount approaching the present total production of North America and Western Europe combined, and has called it imperative that full exploitation . . . for obtaining food from the sea, be attempted as rapidly as possible.
>
> If we are to become proficient in obtaining food from the sea, we must break with the primitive, inefficient practice of hunting for prey. The use of modern techniques (sonar, remote sensing) to hunt are improvements, but leave much to be desired. The next step on the path ultimately leading to farming of the sea should be means for predicting the quantities and locations of fish crops. The most fundamental measurement required for this prediction is primary productivity - the rate at which carbon is fixed photosynthetically in the seas to start the entire food chain. This classic measurement of biological oceanographers and limnologists integrates the effects of all factors upon which food production depends - such as light intensity, turbidity, temperature, nutrient concentrations, inhibitory substances, planktonic species and densities. To date, the measurement of primary productivity has been used only in limited research studies. Practical application to determining primary productivity of large areas of the sea has not been attempted because of the complexity and difficulties of current methods of making the many spatial and temporal measurements required.

The program was, therefore, originally designed to assist the fishermen by providing synoptic primary productivity information of use in finding the optimum fishing areas and times. However, as limnologists became aware of the prospect for an automated instrument to assay for primary productivity, they responded with considerable interest. One

of the strongest interests was expressed by the Southeast Water Laboratory, FWQA, which felt that the instrument had significant potential as a tool in their pollution control program.

Several specific recommendations for the instrumentation approach resulted from the Phase I effort. These were:

1. The instrument should automatically take in a sample of water for in situ testing in a sample chamber transparent to the ambient light at the sampling depth.
2. A second chamber, from which light is excluded, should be supplied with a similar sample for dark bottle measurements.
3. The instrument should have the capacity to measure the activity of at least 100 ml samples.
4. Approximately 1 ml of ^{14}C-sodium bicarbonate solution containing approximately 1 to 10 uCi should be injected into the sample.
5. An incubation period in the range of one to six hours should be provided.

6. Ancillary support determinations (temperature, pH, salinity) should be of secondary importance, but must be considered.
7. The instrument should be constructed to provide flushing and removal of any growths on the inside and outside of the sample chamber.
8. A conference of investigators actively at work in the appropriate fields should be organized during the performance of the program.

The automated instrument design concept resulting from the Phase I effort was subjected to a detailed review by the AEC technical staff and several consultants that were retained for this purpose. During the period of the review, one of us (G. V. L.) visited several authorities in the field and results of these meetings were reported to the AEC for their consideration. Meanwhile, the effort on the program was directed toward the research and development that appeared to have the highest probability of satisfying a "user." For three months of this review period, it appeared that an alternate version of the semi-automated design concept would be required to meet the needs of the Bureau of Commercial Fisheries. The alternate design of a "deployable concept" was developed during this period and is described in Appendix I.

Shortly after the preliminary design of the "deployable concept" had been submitted, the AEC review panel rejected it, as the system would not be compatible with the present-day methods being used in ocean studies. The concept required that the instruments be deployed during the normal grid-track operations and retrieved after at least one day, on a special voyage for this purpose. The added expense for this extra voyage could not be justified within the scope of measurement operations presently conceived. However, as the value of an in situ primary productivity assay is demonstrated, this evaluation must be reconsidered. At this point, the FWQA expressed considerable interest in the original concept, so the effort was again directed toward the Phase I conceptual design.

The technical approach for the performance of the Phase II program was planned as a parallel biological research and engineering development program. In this way, the research which was initiated during the Phase I effort was expanded to investigate more of the problems, in greater depth. Factors such as the optimization of the isotope activity and concentration in view of its effects upon precision, sensitivity, and dynamic measurement range were considered. Also, the filtration process, sample acquisition requirements, and instrument cleansing and rinsing requirements were included.

The result of these considerations was the development of a suitable assay program for both the instrument and supporting manual assay.

The conceptual engineering design from Phase I was used as a basis for a detailed design which was subsequently fabricated and integrated into the ETM of the APPI. Testing of the assembled unit was performed on both the laboratory level and in the field. The manually performed primary productivity assay was performed in parallel with the APPI assays.

Effort on the Phase II effort was performed within the framework of the following Task organization:

Task 300 - The overall Task for the Phase II Program

Task 305 - Development related to the "Deployable Concept"

Task 310 - Overall engineering design support

Task 320 - Biological and chemical research and support effort

Task 330 - Subsystem and component evaluation

Task 340 - Engineering test model fabrication

Task 350 - Engineering test model assembly and checkout

Task 360 - In-house testing and evaluation

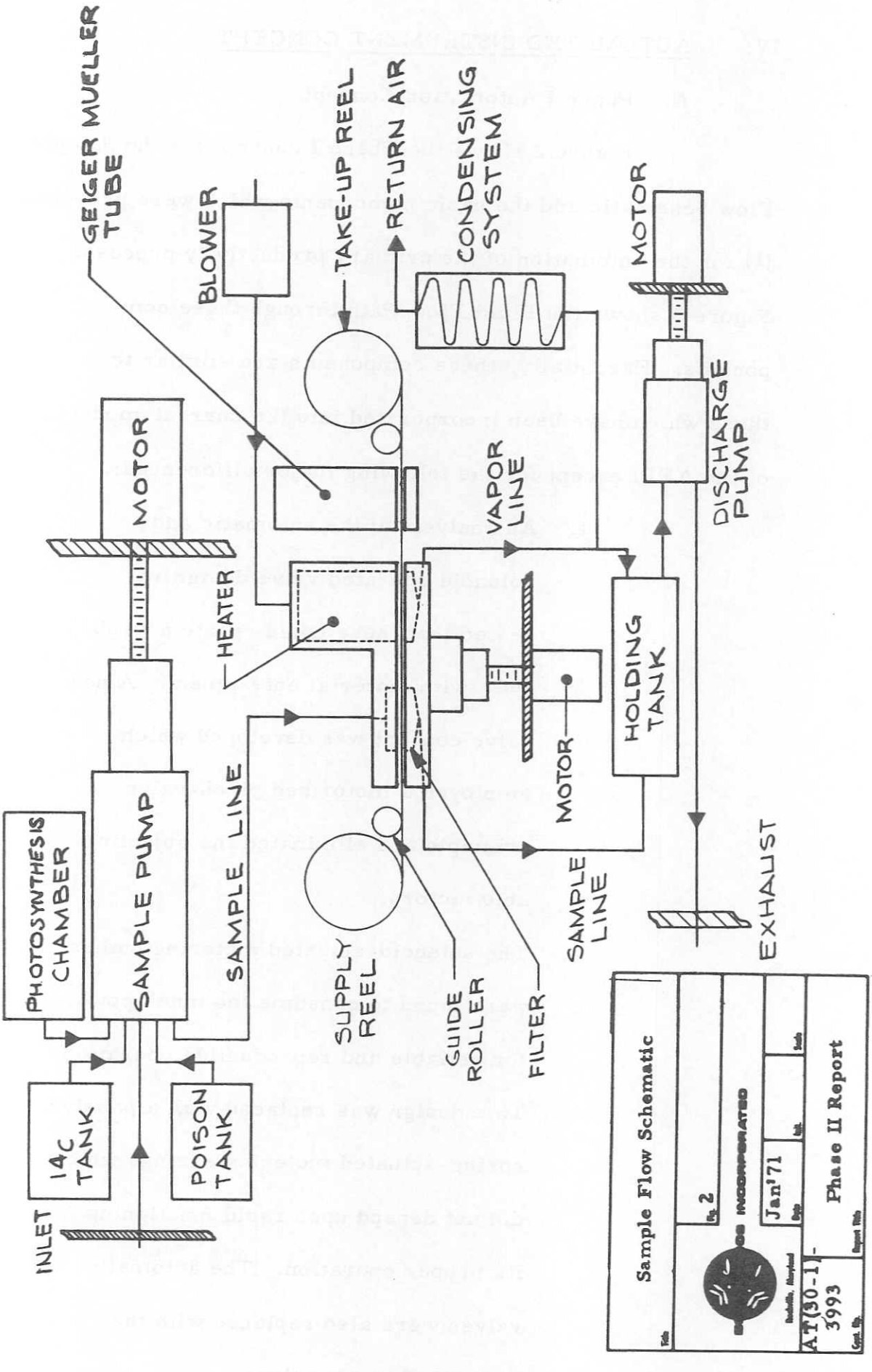
Task 370 - Field testing and evaluation

IV. AUTOMATED INSTRUMENT CONCEPT

A. Phase I Automation Concept

Figure 2 shows the Phase I concept for the Sample Flow Schematic and the basic components which were proposed (1) for the automation of the primary productivity process. Figure 3 shows the Fluid Flow Path through those components. Essentially, these components are similar to those which have been incorporated into the current model of the APPI except for the following major differences:

1. An analysis of the automatic and solenoid operated valve design revealed that these could create a problem with material entrapment. A new valve concept was developed which employed a motorized pinch-valve principle and eliminated the objectionable factors.
2. The solenoid-actuated metering pumps were found to consume too much power for reliable and reproducible operation. This design was replaced with a passive spring-actuated metering syringe which did not depend upon rapid functioning for its proper operation. The automatic valves were also replaced with the motorized pinch-valves.


GEIGER MUELLER TUBE
BLOWER
TAKE-UP REEL
RETURN AIR
CONDENSING SYSTEM
MOTOR
MOTOR
DISCHARGE PUMP
VAPOR LINE
HEATER
PHOTOSYNTHESIS CHAMBER
SAMPLE PUMP
SAMPLE LINE
SUPPLY REEL
GUIDE ROLLER
FILTER
MOTOR
HOLDING TANK
SAMPLE LINE
EXHAUST
INLET
14C TANK
POISON TANK
Sample Flow Schematic
Fig. 2
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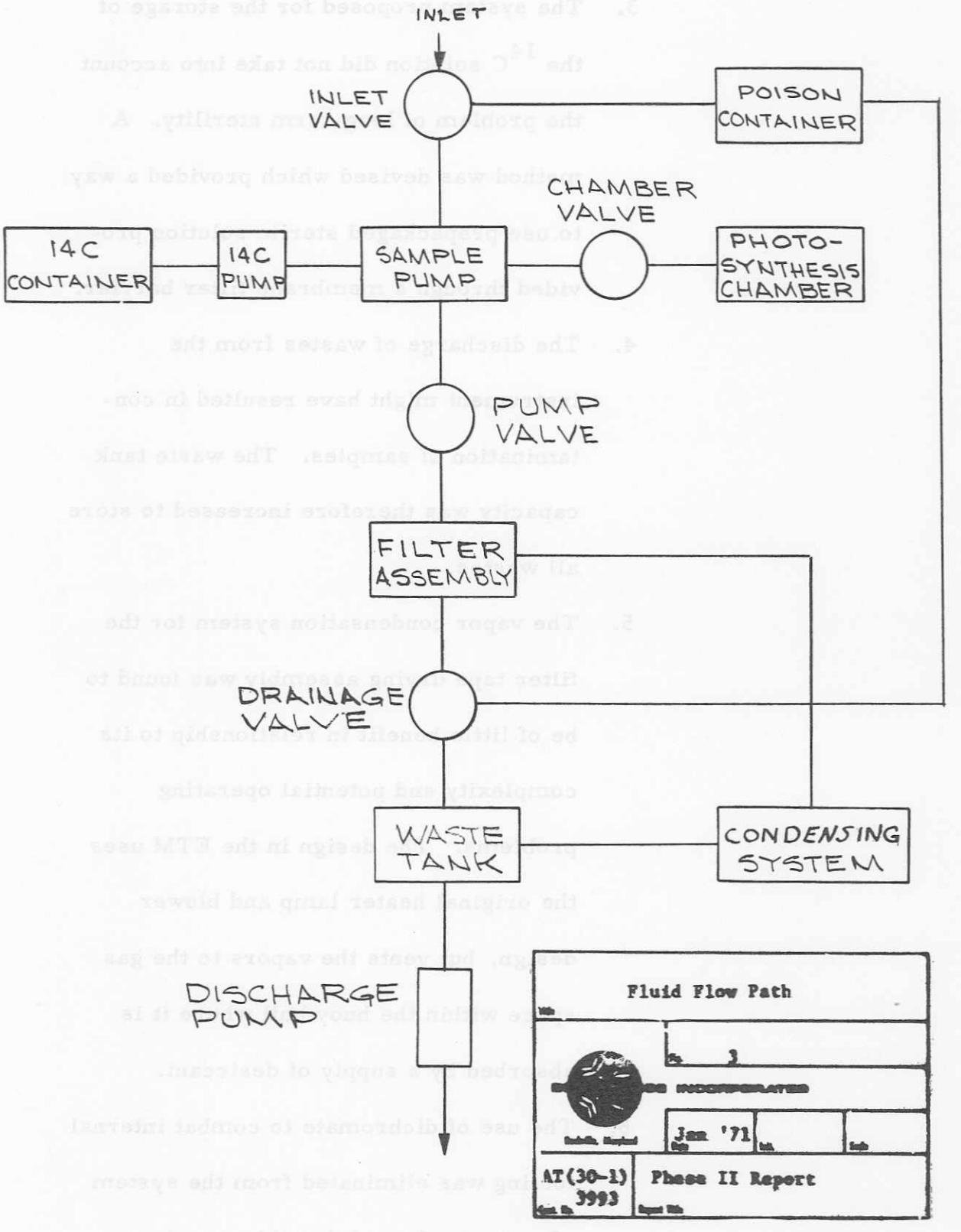
AT(30-1)-3993
Phase II Report


INLET
INLET VALVE
POISON CONTAINER
CHAMBER VALVE
14C CONTAINER
14C PUMP
SAMPLE PUMP
PHOTO-SYNTHESIS CHAMBER
PUMP VALVE
FILTER ASSEMBLY
DRAINAGE VALVE
WASTE TANK
CONDENSING SYSTEM
DISCHARGE PUMP
Fluid Flow Path
Jan '71
AT(30-1) 3993
Phase II Report

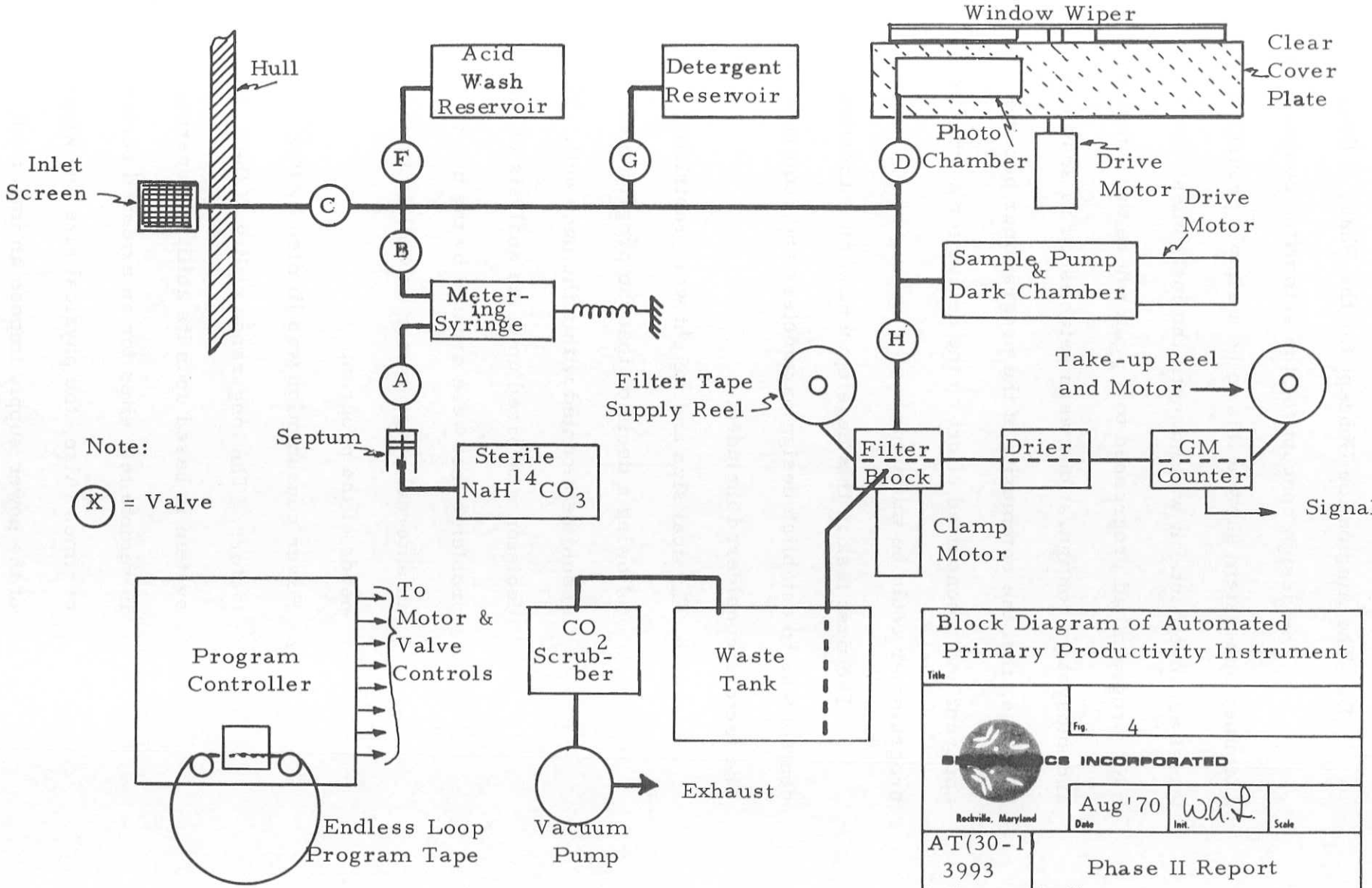
3. The system proposed for the storage of the ^{14}C solution did not take into account the problem of long-term sterility. A method was devised which provided a way to use prepackaged sterile solution provided through a membrane filter barrier.
4. The discharge of wastes from the instrument might have resulted in contamination of samples. The waste tank capacity was therefore increased to store all wastes.
5. The vapor condensation system for the filter tape drying assembly was found to be of little benefit in relationship to its complexity and potential operating problems. The design in the ETM uses the original heater lamp and blower design, but vents the vapors to the gas space within the buoy hull where it is absorbed by a supply of desiccant.
6. The use of dichromate to combat internal fouling was eliminated from the system after tests showed that this may be a potential toxicity problem through adsorption on the internal surfaces.

B. The Automation Concept for the Phase II Design

The design concept for the automated control programmer subsystem showed this to be a simple programmer wheel arrangement. It was not until the instrument development program had progressed to a point where the component and subsystem configurations were established as shown in Figure 4, that the complexity of the programmer became apparent and a concerted effort on the design for a suitable programmer could be initiated.

The first task in this development of an automated control was to establish design parameters and requirements. The factors considered included:

1. Physical size and weight were restricted following a decision that the programmer should be mounted within the buoy hull. The decision was based upon the analysis of the problems that were created by the use of an external programmer as well as future needs of the program.
2. Power consumption was limited by two factors. The long-range utility of the system is based upon its ability to operate in remote test sites for an extended period of time. Also, the physical size and weight of the power supply impose an important



Block Diagram of Automated Primary Productivity Instrument

Title

Fig. 4

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restriction upon the desired mobility of the system.

3. Reliability of the programmer must be high since it is the goal of this project to attain remote operation for extended periods without maintenance.
4. Complexity of the programmer can be reduced by restricting the possible number of subroutines required in the program scheme.
5. Flexibility in program format was desired to allow simple modification of the programmer's routine. Changes of the program sequence should be possible simply by replacing the formatting device. This is an important feature in this project, since the instrument is a developmental tool and will be used for the optimization of the processing.

The programmer which was designed for this project is described in detail in Section VI. Essentially, the system employs a perforated tape reader which provides a unique set of electrical signals for each operation of the process. The program tape is a standard 8-level paper or mylar tape that is prepared on a tape perforator such as is found on a Model ASR-33 Teletypewriter.

Operational codes (letters and symbol characters) are organized on the tape to direct the programmer to perform the desired sequence of operations.

C. The Automated Instrument in the Environment

A serious problem area was identified early in this project, and discussed at the 28 February 1969 Technical Review. This problem stemmed from the recognition of the fact that the instrument must operate in a hostile environment for long periods of time. During this time, it must simulate, without degradation, the exact in situ lighting, temperature, pressure, and chemical composition of the water sample. Volumes of material have been written on this subject, and it is generally conceded that the most difficult problem to solve is that of marine fouling. For this reason, fouling problems received significant attention in the research and testing program as is discussed throughout this report and, in particular, in Section VII.

V. BIOLOGY DEVELOPMENT PROGRAM

A laboratory investigation was conducted as part of this program in order to study the requirements and make recommendations for automation of the primary productivity measurement using the ^{14}C uptake technique. Literature and laboratory studies were performed to enable the selection of suitable volumes, reagents, materials, subsystems, ^{14}C levels, and other parameters for the instrumental measurement. The biology development program is described in detail in the ensuing sections of this report.

A. Laboratory Procedure for the Determination of Primary Productivity

One of the initial objectives of the biology development program was to select a radioisotopic laboratory method for the determination of primary productivity and become proficient in it. This method was then used as a tool with which to conduct investigations of the effects of instrumental parameters upon the primary productivity measurement.

The method selected for use in this study was that described by Strickland and Parsons (5) for the measurement of biological uptake of radioactive carbon. The method in general has been subjected to careful scrutiny for a number of years until it is now a reliable, although somewhat cumbersome, laboratory procedure when performed by trained personnel.

These authors describe the method in great detail and discuss some of the limitations of the method as well as the precautions required to obtain consistent data. An outline of the laboratory procedure, with certain minor modifications which must be used under some circumstances, is presented below:

Apparatus:

Light Bottles - Clear glass bottles are used which have a capacity of 118 ml and are sealed with a polyethylene snap cap.

Dark Bottles - Similar bottles are used which have been painted black and are wrapped in aluminum foil to exclude light during the dark incubation period.

Light Box - A specially constructed box containing a bank of fluorescent lights is used, if desired, to incubate the samples under 400 to 500 foot-candles. Alternatively, ambient natural illumination is used.

Geiger Counter - A 2-inch end window, Geiger-Mueller tube is used to measure the radioactivity collected on the membrane filters.

Filtration Apparatus - The samples are filtered, using suction, through a 47-mm diameter, 0.45 u membrane filter mounted in a filtration apparatus.

Reagents:

0.01 N H_2SO_4

Chromic-sulfuric acid cleaning solution

$NaH^{14}CO_3$ solution - An unbuffered aqueous solution, pH 11, is prepared containing 100 uCi/ml of $NaH^{14}CO_3$ having a specific activity of from 20 to 50 uCi/mM.

Procedure:

1. Bottles and associated apparatus coming in contact with the sample or isotope solution are cleaned with a chromic-sulfuric acid solution.
2. The desired number of replicate light and dark bottles are filled with representative portions of the sample.
3. A 0.1 ml portion of $NaH^{14}CO_3$ solution containing 10 uCi is carefully added to each bottle. The isotope is added from a disposable hypodermic syringe submerged below the surface of the sample.
4. The bottles are capped and mixed thoroughly by shaking.
5. All sample bottles are placed under the desired illumination. The dark bottles are exposed with the light bottles to minimize temperature differences.
6. After the incubation period (generally two

hours), the bottles are removed for immediate filtration under vacuum. The bottles are placed in an ice bath if there is to be any significant delay between incubation and filtration. The walls of the filtration apparatus and the membrane filter are washed with 10 ml of 0.01 N H_2SO_4 after each sample has passed through the filter.

7. The filters are then dried under an infrared lamp.
8. Each filter is counted for radioactivity.
9. A separate portion of the water sample is analyzed for total inorganic carbon.

Calculations:

The equation for calculation of primary productivity is given below:

$$P = \frac{(L-D)}{R\,t} \times C \times f$$

Where P = primary productivity in mg $C/m^3/hr$

L-D = net radioactivity taken up in counts per minute (cpm), light bottle - dark bottle

R = total available radioactive carbon in cpm

t = incubation period in hours

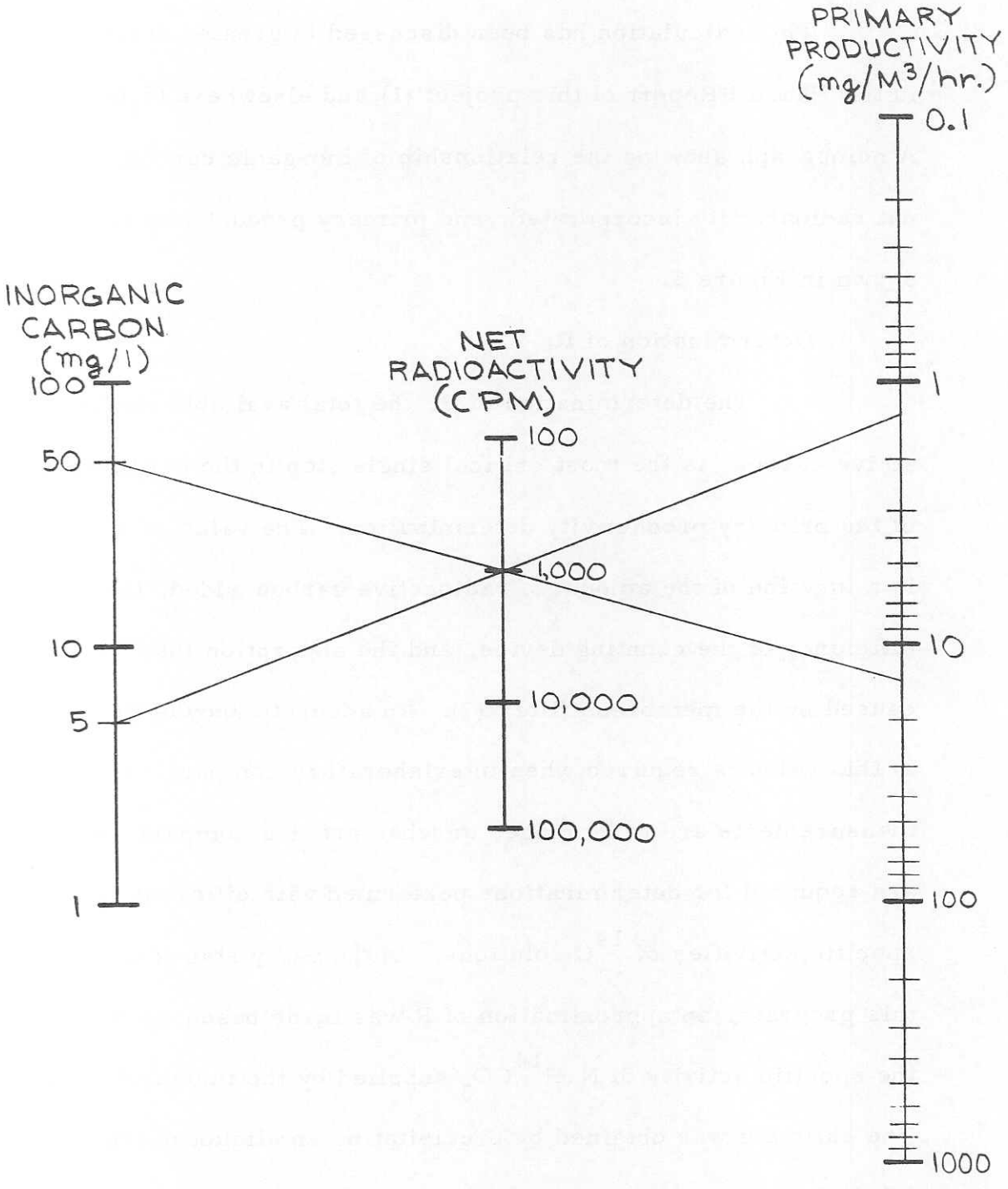
C = total inorganic carbon concentration in mg C/m^3

f = 1.06, the isotope correction factor to account for differences in uptake rates of ^{14}C and ^{12}C.

This calculation has been discussed in greater detail in the Phase I Report of this project (1) and elsewhere (5,6). A nomograph showing the relationship of inorganic carbon, net radioactivity incorporated, and primary productivity is shown in Figure 5.

Determination of R:

The determination of R, the total available radioactive carbon, is the most critical single step in the calibration of the primary productivity determination. The value of R is a function of the amount of radioactive carbon added, the efficiency of the counting device, and the absorption loss caused by the membrane filter (7). An accurate knowledge of this value is required when interlaboratory comparisons of measurements are to be made, or when precise comparisons are required for determinations performed with different specific activities of ^{14}C solutions. In the early stages of this program, an approximation of R was made based upon the specific activity of $NaH^{14}CO_3$ supplied by the manufacturer. The estimate was obtained by precipitating an aliquot of the labeled solution with barium ion which was then filtered through a 0.45 u membrane filter, dried, and counted. A value of 1.95×10^5 cpm/uCi was obtained. The error in this value resulted principally from self-absorption losses in the $BaCO_3$ and from errors in the manufacturer's measurement of specific activity. Later in the program, a more precise



Relationship of Primary Productivity Total Inorganic Carbon and Net Radioactivity

Fig. 5

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Jan '71

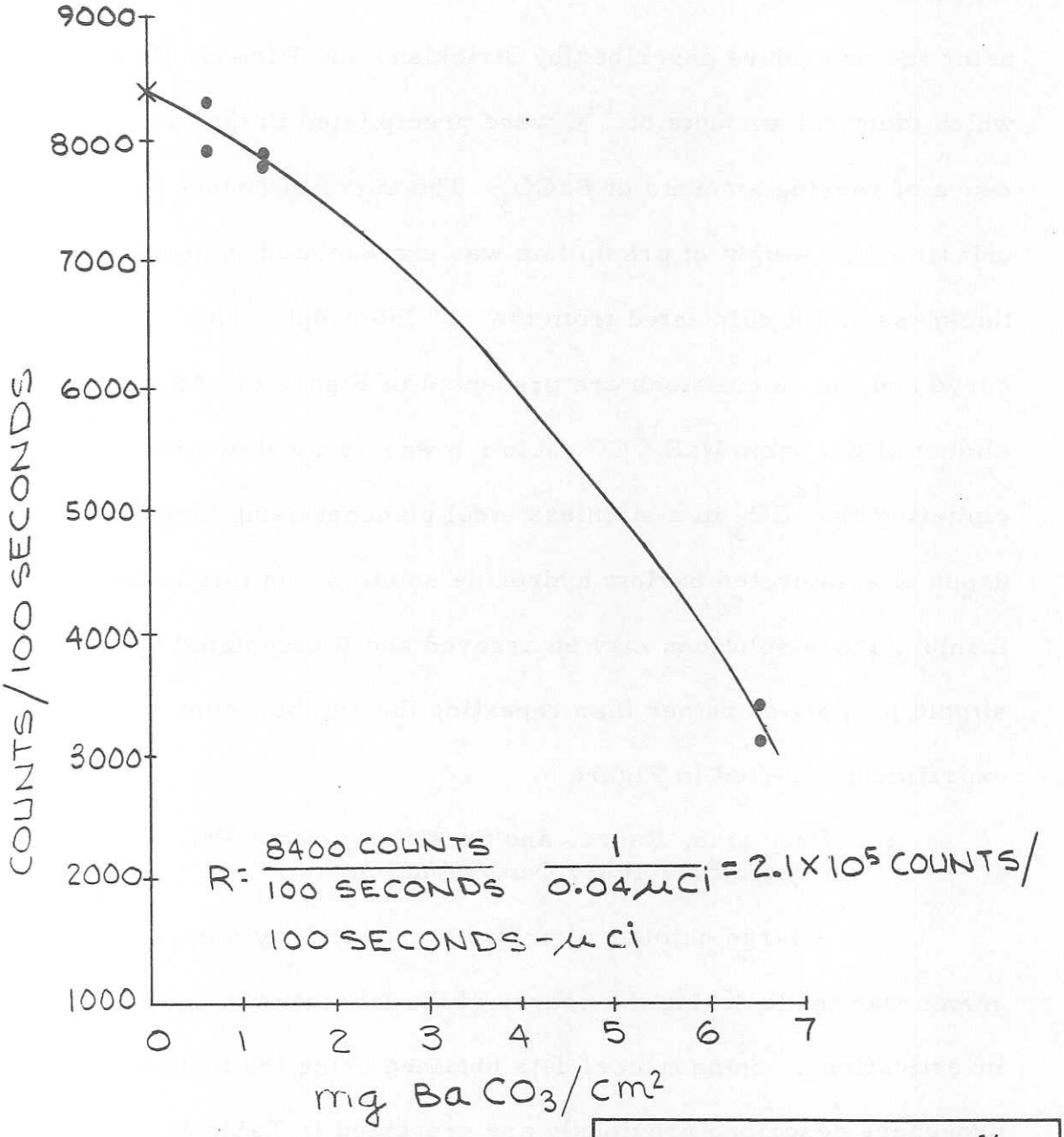
AT(30-1) 3993

Phase II Report

estimate of R was obtained and the determination was made using the procedure described by Strickland and Parsons (5) in which identical amounts of ^{14}C were precipitated in the presence of varying amounts of $BaCO_3$. The curve of counts per unit time vs. weight of precipitate was extrapolated to zero thickness and R calculated from the "Y" intercept. This curve and the calculations are presented in Figure 6. An aliquot of the same $NaH^{14}CO_3$ solution was assayed by precipitating $Ba^{14}CO_3$ in a stainless steel planchet using three drops of a saturated barium hydroxide solution. In this latter fashion, fresh solutions may be assayed and R calculated by simple proportion rather than repeating the cumbersome experiment depicted in Figure 6.

B. Precision, Range, and Sensitivity of the Primary Productivity Determination

A large number of primary productivity measurements was made during the course of the laboratory investigations. Some typical data obtained using the manual procedure described previously are presented in Table 1. A wide range of values was observed from a low of less than 0.1 mg $C/m^3/hr$, obtained from a local stream during the winter months, to a high of over 600 mg $C/m^3/hr$, obtained in the Potomac River during a period of heavy bloom. The ability to measure this range must be incorporated into the instrument to enable monitoring all types of conditions which exist throughout the various bodies of water of interest.



Title: Determination of R in $NaH^{14}CO_3$ Solutions	
	Fig. 6
BIOCS INCORPORATED, Rockville, Maryland	Date: Jan'71
Cont. No.: AT(30-1) 3993	Report Title: Phase II Report

Table 1

Typical Primary Productivity Data
Manual Determinations on Natural Water Samples

Sample	Primary Productivity ($mgC/m^3/hr$)	Inorg. Carbon (mgC/l)	L-D (cpm)	^{14}C-Added (uCi/bottle)	Incubation Period (Hours)
Rock Creek, Rockville, Maryland	0.1	13.9	57	10.0	3
Potomac River, Great Falls, Maryland	1	13.8	46	1.0	2
Occoquan Reservoir, Fairfax County, Virginia	1.0	26.8	383	10.0	2
Potomac River, Roaches Run, Virginia	2	17.5	0	0.5	3
Potomac River Boat Center, Washington, D. C.	2	17.5	40	0.5	3
Potomac River, Alexandria, City Dump	4	20.0	160	0.5	3
Potomac River, Chain Bridge	5	17.5	240	0.5	3
Occoquan Reservoir, Fairfax County, Virginia	5.8	20.9	293	10.0	2
Occoquan Reservoir, Fairfax County, Virginia	7.3	14.0	560	10.0	2
Potomac River, Chain Bridge	9	19.8	240	1.0	1

Table 1
(continued)

Typical Primary Productivity Data
Manual Determinations on Natural Water Samples

Sample	Primary Productivity ($mgC/m^3/hr$)	Inorg. Carbon (mgC/l)	L-D (cpm)	^{14}C-Added (uCi/bottle)	Incubation Period (Hours)
Lake Ontario, New York	10	28.6	180	0.5	2
Potomac River, Great Falls, Maryland	10	15.6	480	0.5	3
Potomac River Boat Center, Washington, D. C.	11	19.7	280	1.0	1
Potomac River, Alexandria City Dump	14	22.8	360	1.0	1
Atlantic Ocean, Southern Fla.	17	28.8	630	1.0	2
Potomac River Boat Center, Washington, D. C.	23	16.6	1458	1.0	2
Potomac River, Great Falls, Maryland	26	17.4	780	1.0	1
Rock Creek, Rockville, Md.	34	13.6	2660	1.0	2
Ohio River, Marietta, Ohio	63.6	10.0	46,601 *	10.0	4
Lake Ontario, New York	87	30.2	3020	1.0	2

Table 1
(continued)

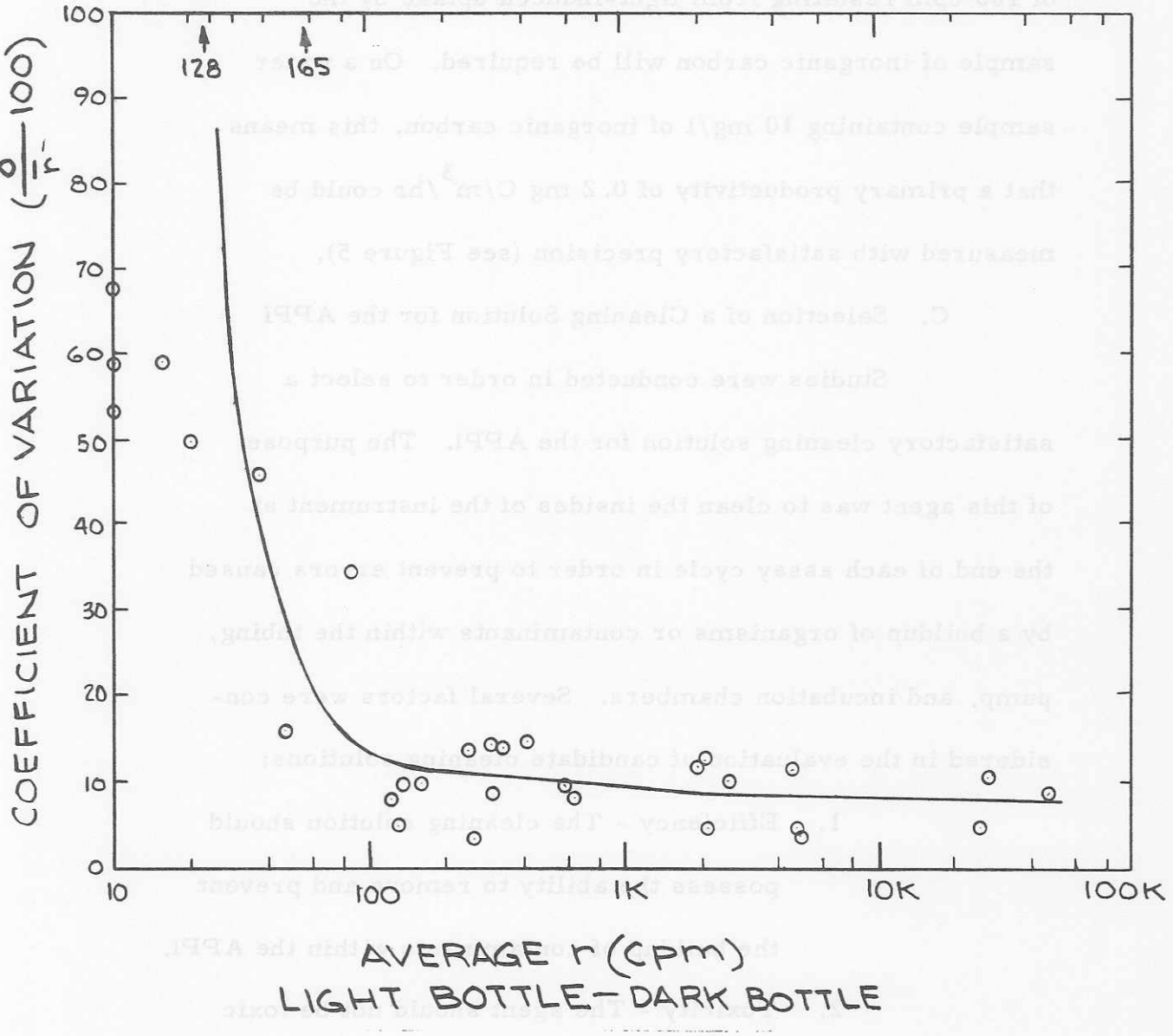
Typical Primary Productivity Data
Manual Determinations on Natural Water Samples

Sample	Primary Productivity ($mgC/m^3/hr$)	Inorg. Carbon (mgC/l)	L-D (cpm)	^{14}C-Added (uCi/bottle)	Incubation Period (Hours)
Potomac River, Roaches Run, Virginia	206	16.6	6560	1.0	1
Potomac River, Fort Washington, Maryland	605	23.8	46,725 *	10.0	1

* These samples were counted using a Tracerlab Type 11002 thin window Geiger-Mueller tube (R = 195.0 cpm/uCi). The other samples were counted using a Nuclear-Chicago Gas Flow Counter (R = 558.1 cpm/uCi).

The inorganic carbon concentrations measured in the natural water samples encountered varied from 3 to 30 mg/l. Carbonate determinations were made using the alkalinity titration procedure described by Saunders et al (7). This method involved measurements of the initial sample temperature, pH, and the volume of 0.02 N H_2SO_4 required to lower the pH of 100 ml of sample to the methyl orange end point (pH = 3.7). The titration procedure is subject to many interferences, but was selected because it is generally reproducible for sample-to-sample comparisons of a single water body. Also, this assay is rapid and easy to perform, both in the laboratory and in the field.

Replicate determinations of the light-induced uptake of inorganic carbon were made on a large number of natural and artificial water samples to obtain an estimate of the sensitivity and precision of the manual procedure for the measurement of primary productivity. These data were statistically analyzed and are summarized in Figure 7. The majority of the experimental points shown represent averages of ten replicate determinations plotted against the coefficient of variation (relative standard deviation). This analysis demonstrated that a coefficient of variation of 10% or better was obtained above a level of 1000 cpm net radioactivity incorporated. If a maximum acceptable coefficient



Title: Sensitivity and Precision of Laboratory Procedure for Measuring Primary Productivity	
	Fig. 7
Bioscience Incorporated, Rockville, Maryland	
	Date: Jan'71 / Init. / Scale
Cont. No.: AT(30-1) 3993	Report Title: Phase II Report

of variation of 15% is arbitrarily selected, then a minimum of 100 cpm resulting from light-induced uptake by the sample of inorganic carbon will be required. On a water sample containing 10 mg/l of inorganic carbon, this means that a primary productivity of 0.2 mg $C/m^3/hr$ could be measured with satisfactory precision (see Figure 5).

C. Selection of a Cleaning Solution for the APPI

Studies were conducted in order to select a satisfactory cleaning solution for the APPI. The purpose of this agent was to clean the insides of the instrument at the end of each assay cycle in order to prevent errors caused by a buildup of organisms or contaminants within the tubing, pump, and incubation chambers. Several factors were considered in the evaluation of candidate cleaning solutions:

1. Efficiency - The cleaning solution should possess the ability to remove and prevent the buildup of contaminants within the APPI.
2. Toxicity - The agent should not be toxic or inhibitory to organisms in the water samples. This property would ensure against erroneous results in the event of accidental contamination of the samples or the surrounding environment.
3. Reactivity - The agent should not react with or corrode the APPI materials.

The candidate cleaning solutions considered were dichromate-sulfuric acid, nitric acid, hydrochloric acid, isopropanol, Cidex (2% alkaline gluteraldehyde germicide) solution, and a "Woolite" (liquid detergent) solution.

Sulfuric acid-dichromate and 30% nitric acid solutions have been used in many laboratories as effective agents for cleaning glassware. The effects of these cleaning agents upon the primary productivity measurement were examined by conducting determinations in bottles cleaned with the agents and then incompletely rinsed. The results of this experiment are shown in Table 2. A total of 18 bottles were carefully cleaned, rinsed thoroughly, dried and then divided into three groups of six bottles each. A volume of 20 ml of acid-dichromate was placed in each of one group of bottles, 20 ml of 30% nitric acid in the second group, and 20 ml of distilled water in the third group. The bottles were tilted to wet the entire surface of the inner walls of the bottles, emptied, and then rinsed incompletely using two 50-ml portions of distilled water. The bottles were allowed to dry overnight in an inverted position and then used to measure the primary productivity of an algal suspension. Triplicate light and dark bottles were set up with each set of bottles. No toxic effects were noted from the residual acid-dichromate or 30% nitric acid in this experiment. A second experiment was conducted

Table 2

Effect of Cleaning Solutions Upon the Uptake of Inorganic Carbon by Algae

Cleaning Solution	^{14}C Uptake (cpm) * Light Bottle	Dark Bottle
Sulfuric Acid-Dichromate	315 ± 42	59 ± 3
30% Nitric Acid	273 ± 26	64 ± 6
Distilled Water	251 ± 5	53 ± 3

* Each value reported is the average of triplicate determinations ± the average deviation.

Assay Conditions:

The bottles were exposed to the various cleaning solutions above and then incompletely rinsed using two 50-ml portions of distilled water.

Incubation - 2 hours

Sample - 200 ml Chlorella sorokiniana (10^6/ml)

Illumination - 300 foot-candles

Isotope - 1 uCi $NaH^{14}CO_3$

with the acid-dichromate in order to study further the inhibitory action of hexavalent chromium. The literature contains many references to the toxic action of hexavalent chromium and several of the scientists contacted during this program recommended against its use in a biological system. A series of bottles was coated as before using 20 ml of acid-dichromate solution. One set was rinsed thoroughly with tap water and then distilled water, the second set was rinsed incompletely with one 50-ml portion of distilled water, and the third set rinsed with a 20-ml portion of distilled water. This procedure was repeated on a second day. The results of this experiment, presented in Table 3, again failed to demonstrate a serious problem with the toxicity of dichromate. No extreme inhibitory effects were noted even in the third set of bottles which had been rinsed with 20 ml of water and which contained visible amounts of residual cleaning solution. However, the acid-dichromate and the other acid cleaning solutions were dropped from further consideration at this point, because of their very high reactivity. It was felt that the materials proposed for use in the APPI would not be able to withstand these reagents over an extended period of time.

Cidex, isopropanol, and a cold water liquid detergent were the next group of candidate cleaning solutions examined. Two separate tests were made of the efficacy of these agents

Table 3

Effect of Acid-Dichromate Cleaning Solution
Upon Primary Productivity

Rinse Procedure	Primary Productivity ($mgC/m^3/hr$)	
	Test 1	Test 2
Thorough rinsing with tap and distilled water	15,600	17,100
Incomplete rinsing with one 50-ml portion of distilled water	14,600	18,500
Incomplete rinsing with one 20-ml portion of distilled water	8,700	17,500

Assay Conditions:

The bottles were exposed to 20-ml portions of acid-dichromate and then subjected to the indicated rinse solution. Bottles rinsed with a one 20-ml portion of distilled water contained residual amounts of dichromate which were visible to the naked eye.

Incubation - 2 hours

Sample - 200 ml Chlorella sorokiniana (10^6/ml)

Illumination - 300 foot-candles

Isotope - 1 uCi $NaH^{14}CO_3$

in the removal of fouling from glass. Glass materials were deployed in the Chesapeake Bay until they had acquired a substantial amount of surface fouling. The glass was then removed and the fouling, silt and organisms allowed to air dry into a hard coating. Individual pieces of the fouled glass were soaked for several hours in each of the three solutions. The glass pieces were then removed and rubbed carefully with a dry cloth in order to determine the relative cleansing power of the three solutions. The 0.5% solution of "Woolite" proved to be better than the Cidex, while the isopropanol had almost no effect upon the coating. It was principally on the basis of these two trials and a materials test which is discussed below, that isopropanol was removed from further consideration.

An exposure test was set up next in which the effects of these three cleansing solutions upon proposed APPI materials were studied. Buna-N and Silicone elastomers, stainless steel, polyvinylchloride (Tygon) tubing, plexiglass, and glass were the materials chosen for the exposure test. Isopropanol was found to leach plasticizer from the Tygon so that it became rigid and practically unusable after only a 24-hour period. This solvent did not affect the other materials tested over the 9-month period of the study with the exception of the elastomers, which swelled considerably

during their exposure. Cidex caused Tygon tubing to lose most of its elasticity in about one month. This solution also etched the surface of plexiglass, causing a marked decrease in its ability to transmit light. Cidex also formed a considerable amount of precipitate during the exposure period. The detergent solution had no noticeable effect on any of the materials with the exception of Tygon tubing. The Tygon had lost a little of its elasticity and clarity during the 9-month exposure to detergent.

It was on the basis of these experiments that a 0.5% "Woolite" solution was chosen for use as the cleaning solution for the APPI. The effect of various "Woolite" concentrations upon the primary productivity measurement was studied. The results of this experiment, presented in Table 4, show that this material has only a very slight inhibitory effect.

D. Study of the APPI Filtration Procedure

1. Membrane Filter Selection

Strickland and Parsons (5) recommend the use of plain white 25 mm diameter HA Millipore filters for the filtration of radioactive water suspensions in the determination of primary productivity. These authors also suggest that 47 mm diameter filters of the same type be used in the filtration of very dense suspensions in order that the filtration time be

Table 4

Effect of Detergent Solution Concentration
(0.5% "Woolite") Upon Primary Productivity

Detergent Solution Added (%)	Counts/100 Seconds	
	Light Bottle	Dark Bottle
0.0005	13,010	498
0.005	11,475	420
0.05	10,760	312
0.5	10,315	364
5.0	9,795	296

Assay Conditions:

A natural water sample from Flat Creek, Georgia, containing the indicated amounts (v/v) of detergent solution was studied.

Incubation - 2 hours

Sample - 118 ml

Illumination - 450 foot-candles

Isotope - 10 uCi $NaH^{14}CO_3$

kept below 10 minutes. The recommended filters have an average pore size of 0.45 u. This size was reported (1) to be small enough for complete retention of incorporated radioactivity if the suction applied to the filter flask does not exceed about one-third of an atmosphere. However, an experiment was conducted with Millipore filters to determine the effect of pore size upon the primary productivity determination. Replicate determinations were run on Chlorella sorokiniana suspensions on three separate days using 0.45 u and 5.0 u membrane filters. The results of this experiment, shown in Table 5, demonstrated that a marked loss in photosynthetic organisms occurs when the larger pore size filters are used. On the basis of these study results,"Acropor" AN-450 membrane filter tape, having an average pore size of 0.45 u, was selected for use in the APPI.

The proposed design of the APPI required a filter tape which would be processed through the filter block where sample filtration would take place, then advanced to a drier and to a counter, counted, and finally stored on a take-up reel. This system imposed a requirement for higher strength than used in conventional membrane filters. A review of filter materials showed that Gelman Instrument Company's "Acropor" AN-450, an acrylonitrile polyvinylchloride

Table 5

Effect of Membrane Filter Pore Size Upon the Primary Productivity Determination

Run No.	Filter Pore Size (u)	Primary Productivity * ($mgC/m^3/hr$)	Coefficient of Variation (%)
1	0.45	3.7	24
	5.0	2.6	28
2	0.45	13.5	14
	5.0	10.7	20
3	0.45	1.5	84
	5.0	1.8	22

Assay Conditions:

Samples - Chlorella sorokiniana suspensions, 100 ml

Incubation - 4 hours

Illumination - 500 foot-candles

Isotope - 10 uCi $NaH^{14}CO_3$

* Each figure is the average of five replicate determinations.

copolymer membrane was reinforced with nylon. It is available in tape form with the recommended 0.45 u pore size and the degree of physical strength required.

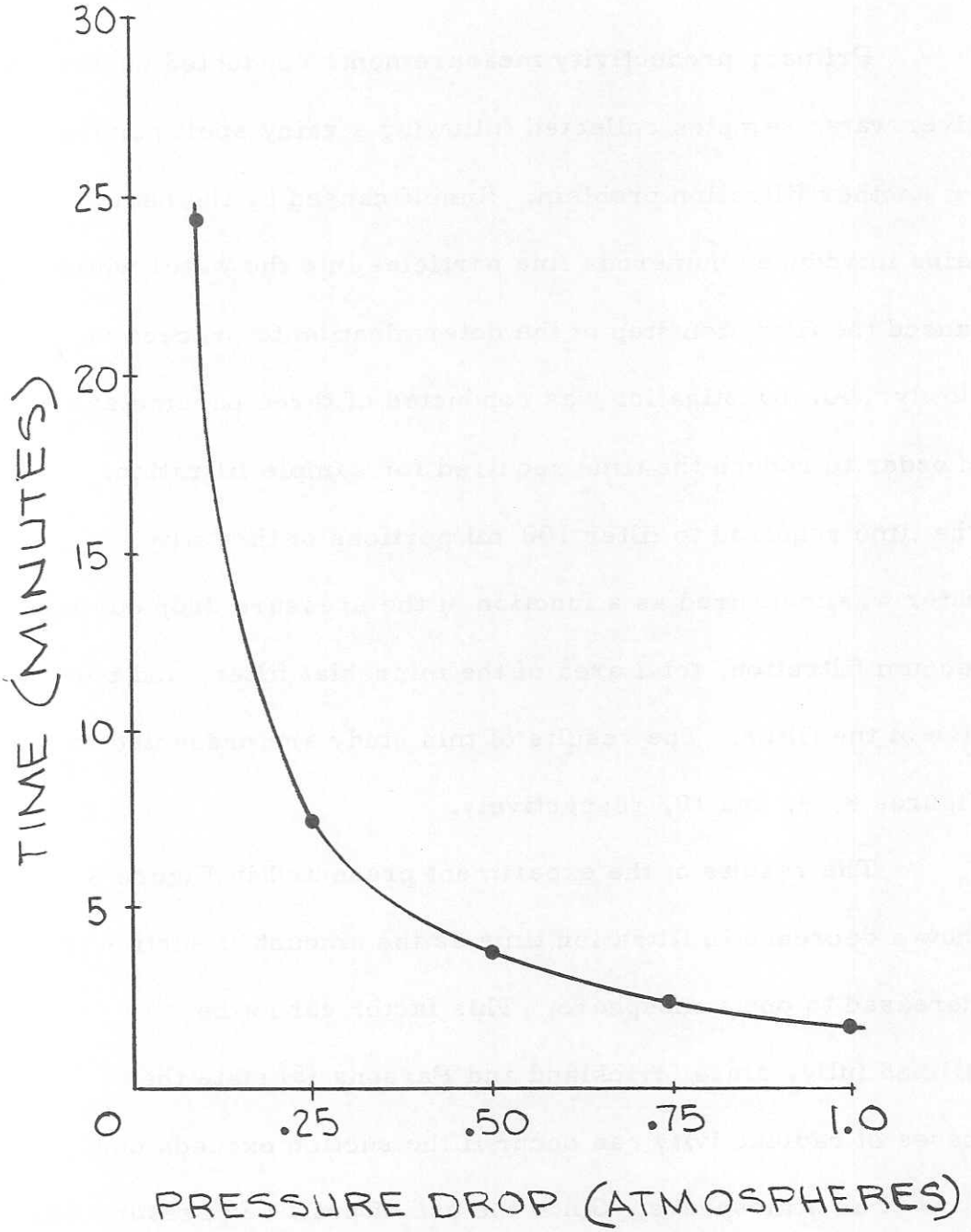
Two other types of membrane filters which were made from polytetrafluoroethylene (Teflon) were considered briefly. These filters possessed the desired strength and it was thought, based upon our previous studies with this material, that Teflon might retain less radioactivity through nonbiological mechanisms. An experiment conducted with these Teflon filters demonstrated no advantage in the degree of nonbiological retention of inorganic carbon over membrane filters prepared from conventional materials. Also, since the Teflon filters were not available in the desired pore size (0.45 u), they were dropped from further consideration.

2. Factors Affecting Filtration Time

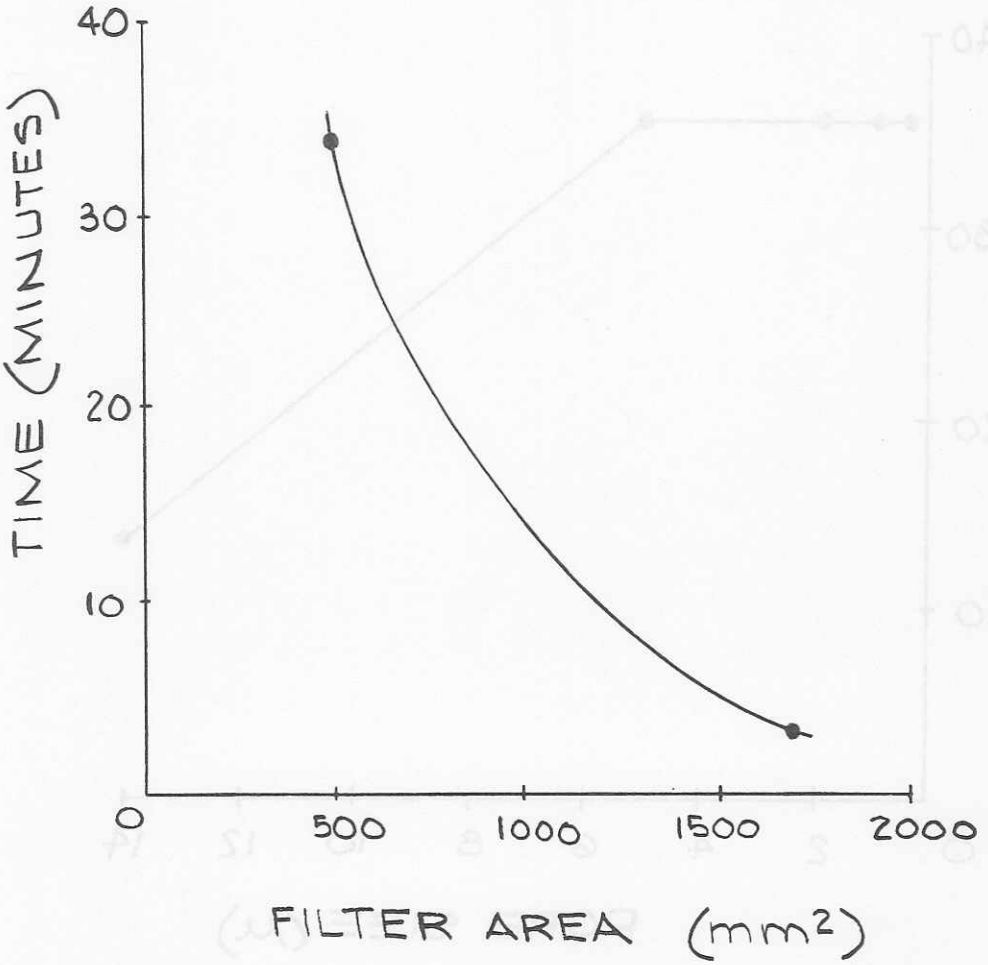
"Acropor" was found to have a slower filtration rate than a standard 0.45 u nitrocellulose membrane filter. A period of 5 minutes was required for the vacuum filtration of a sample through "Acropor" AN-450. This same suspension took only 2.5 minutes when filtered through nitrocellulose under similar conditions. Similar amounts of radioactivity were retained by both materials. The longer filtration period was not felt to be a serious problem in the development of an automated filtration system using "Acropor" tape.

Primary productivity measurements conducted on Potomac River water samples collected following a rainy spell pointed out another filtration problem. Runoff caused by the heavy rains introduced numerous fine particles into the water which caused the filtration step of the determination to proceed very slowly. An investigation was conducted of three parameters in order to reduce the time required for sample filtration. The time required to filter 100-ml portions of this silty water was measured as a function of the pressure drop during vacuum filtration, total area of the microbial filter, and pore size of the filter. The results of this study are presented in Figures 8, 9, and 10, respectively.

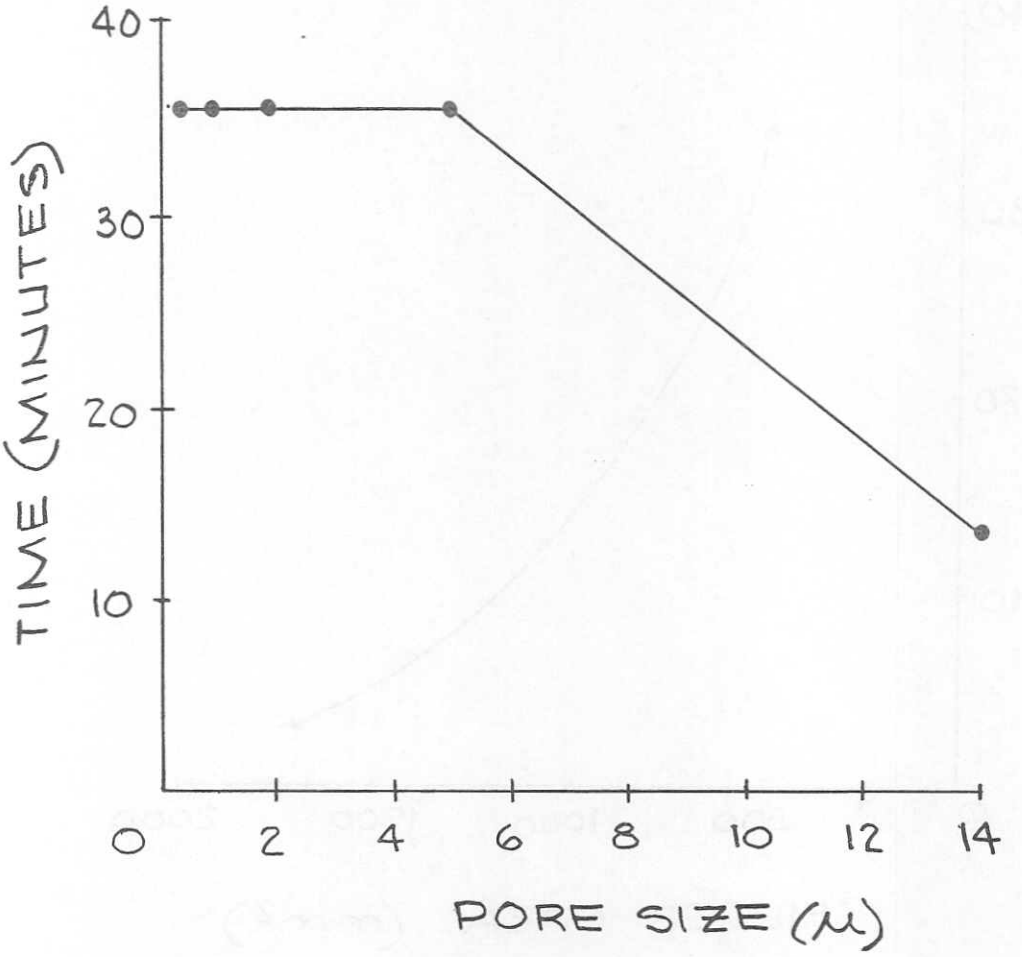
The results of the experiment presented in Figure 8 show a decrease in filtration time as the amount of suction is increased to one atmosphere. This factor cannot be utilized fully, since Strickland and Parsons (5) state that losses of radioactivity can occur if the suction exceeds one-third of an atmosphere. Since the use of positive pressure was contemplated for the APPI filtration step, an experiment was conducted in order to determine whether positive pressure could be substituted for vacuum. An average of 4700 cpm was retained by "Acropor" AN-450 membrane filters using vacuum filtration on six replicate portions of a labeled water suspension. An average of 4600 cpm was retained when nine



Title: Effect of Pressure Drop Upon Time Required for Vacuum Filtration	
	Fig. 8
Rockville, Maryland	CS INCORPORATED
	Jan'71 (Date) / Init. / Scale
AT(30-1) 3993 (Cont. No.)	Report Title: Phase II Report



Effect of Filter Area Upon Filtration Time (Title)	
Rockville, Maryland	Fig. 9
	Jan'71 (Date) / Init. / Scale
AT(30-1) 3993 (Cont. No.)	Phase II Report (Report Title)



Title: Variation of Filtration Rate With Membrane Filter Pore Size	
Rockville, Maryland	Fig. 10
	CS INCORPORATED
	Date: Jan'71 / Init. / Scale
Cont. No.: AT(30-1) 3993	Report Title: Phase II Report

replicate portions of this same labeled suspension were filtered using 7 psig of pressure. Although the time required for pressure filtration was slightly longer, filtration using 0.5 atmosphere of positive pressure was deemed to be acceptable for use in the APPI on the basis of this experiment.

Figure 9 shows a marked decrease in the time required for filtration as the diameter of the membrane filter is increased. This represented a significant finding and, on the basis of this experiment, the recommendation was made to use a 47 mm diameter filter tape in the APPI.

Figure 10 demonstrates no significant advantage in increased pore size of the membrane filter up to about 5.0 u. Previous experiments have shown, however, that membrane filter pore sizes of 5.0 u and larger were not usable due to substantial losses of radioactivity.

3. Filter Tape Storage

Experimentation was undertaken in order to investigate possible loss of radioactivity from the filter tapes after filtration of the labeled samples in the APPI. Strickland and Parsons (5) state that the dried filters may be stored for several weeks without significant loss of radioactivity. The results shown in Table 6 confirmed that dried filters can be stored for at least 5 days without loss of radioactivity.

Table 6

Influence of Time Upon Radioactivity Retained on Dried Membrane Filters

Time (days)	% of Original Radioactivity *	
	Light-Incubated Sample	Dark-Incubated Sample
0	100	100
	107	99
1	98	99
2	99	99
3	102	105
	97	108
5	105	100

Assay Conditions:

Sample -	100-ml portions of Chlorella sorokiniana suspensions
Radioactivity -	1 uCi of $NaH^{14}CO_3$ per 100 ml
Illumination -	2 hours at about 500 foot-candles
Filters -	0.45 u membrane filters dried immediately after sample filtration and stored in the dark in petri dishes between counting intervals

* Values reported are the averages of triplicate determinations.

These experiments were performed by filtering three replicate portions of a labeled Chlorella suspension which had been incubated in the light and one which had been incubated in the dark. The six membrane filters from each experiment were immediately dried and then monitored periodically for radioactivity. The filters were stored in the dark in petri dishes between counting intervals.

The effect of time delay, after sample filtration and prior to drying of the membrane filter, upon the amount of radioactivity on the filter was evaluated in the next phase of this study. Experiments similar to those described above were performed in which replicate aliquots of labeled cell suspensions were filtered, stored while still moist, dried, and then counted. The results shown in Table 7 are those from experiments in which filters were exposed to ambient air prior to complete drying under an infrared lamp. No significant loss of radioactivity from the filters was observed as a result of exposure periods up to 24 hours in duration. These results demonstrate that it is not necessary to dry the filters immediately after filtration of the labeled cell suspensions.

Since the moisture content of the air within the APPI is likely to be higher that that of the ambient laboratory atmosphere, a set of experiments was conducted in which

Table 7

Loss of Radioactivity from Moist Filters
Upon Exposure to Ambient Air

Time (Hours)	% of Original Radioactivity *
0	100
0.25	103
0.5	108
1	100
2	99
3	101
4	100
24	103

Assay Conditions:

Sample - 100-ml portions of Chlorella sorokiniana suspensions

Radioactivity - 1 uCi of $NaH^{14}CO_3$ per 100 ml

Illumination - 2 hours at about 500 foot-candles

Filters - 0.45 u membrane filters were exposed to laboratory air after filtration for the indicated time periods, dried, and then counted

* Values reported are the averages of duplicate or triplicate determinations.

moist filters were exposed to 100% relative humidity, dried and the attendant loss in radioactivity measured. Replicate aliquots of a labeled suspension of Chlorella were filtered and the moist filters stored for varying time intervals in two sets of petri dishes. One set of petri dishes contained a ball of moist cotton to maintain an atmosphere near 100% relative humidity. The results of this experiment are shown in Table 8. No significant loss of radioactivity from the moist membrane filters occurred during the 4-hour exposure period prior to drying.

These experiments have shown that a considerable amount of leeway is available in devising instrumental procedures for handling the APPI filter tape after sample filtration. The tape may be held for considerable periods of time, either in the moist or dry state, without significant change in the level of radioactivity on the filter.

E. APPI Sample Acquisition System

The preliminary concept of the APPI sample acquisition system called for an external screen or prefilter to prevent the entry of large, foreign particles into the sample intake port. The sample was to be taken up through 1/16 in. i.d. stainless steel tubing by the action of a syringe or piston type of pump and moved through a manifold into the incubation chambers. The effects of various aspects of the

Table 8

Loss of Radioactivity from Moist Filters
Upon Exposure to High Humidity

Time (Hours)	% of Original Radioactivity * 100% RH
0	100
0.25	99
0.5	104
0.75	102
1	100
2	98
3	97
4	98

Assay Conditions:

Sample -	100-ml portions of Chlorella sorokiniana suspensions
Radioactivity -	1 uCi of $NaH^{14}CO_3$ per 100 ml
Illumination -	2 hours at about 500 foot-candles
Filters -	0.45 u membrane filters exposed to 100% relative humidity, dried and then counted

* Values reported are average of four replicate determinations.

acquisition system upon the primary productivity measurement were evaluated through a series of laboratory studies.

1. Prefilter Selection

A number of eminent oceanographers were contacted during the course of this program in order to obtain opinions and advice relative to certain critical parameters in the primary productivity measurement. The use of a screen or prefilter was recommended. One such recommendation suggested the use of a 250 u prefilter and another called for the use of a plankton net to prevent passage of large particles into the interior of the APPI.

A plankton net (20 mesh, nylon, 173 threads/inch) was obtained from the Wildlife Supply Company of Saginaw, Michigan, and the effect of sample prescreening with this net upon the primary productivity was measured. Triplicate determinations of primary productivity of a water sample taken from Rock Creek in Washington, D. C., averaged 27 mg $C/m^3/hr$. This same water sample gave an average value of only 7 mg $C/m^3/hr$ after passage through the plankton net. A more extensive testing was conducted with this net in which samples taken from Rock Creek on three separate days were studied. These results, shown in Table 9, indicated that use of the net, and certainly the 250 u prefilter,

Table 9

Effect of Plankton Net as Prefilter for Natural Fresh Water Sample on the Determination of Primary Productivity

Samples Prefiltered Through Plankton Net

Sample No.	Light (cpm)	Dark (cpm)	PP (mg $C/m^3/hr$)	Coefficient of Variation (%)
1	220	49	22	22
2	84	38	6	16
3	141	74	7	20

No Prefiltration

Sample No.	Light (cpm)	Dark (cpm)	PP (mg $C/m^3/hr$)	Coefficient of Variation (%)
1	160	35	17	8
2	155	38	16	5
3	316	51	27	3

Assay Conditions:

Isotope - 1.0 uCi $NaH^{14}CO_3$/118 ml

Samples	No. of Replicates	Condition
1 Rock Creek - 9/3/69	3	rich in algae
2 Rock Creek - 9/4/69	3	rich in algae
3 Rock Creek - 8/28/69	3	rich in algae

Incubation -	4 hours
Illumination -	500 foot-candles
Membrane Filter -	0.45 u Cellulose Acetate
Prefilter -	20 mesh, nylon plankton net

would severely attenuate the apparent photosynthetic activity of a water sample and thus could not be used in the APPI. An additional effect was noted in that use of the prefilter greatly increased the degree of scatter in the data.

In an experiment conducted with an analog of the sample acquisition system, to be described later, no attenuation of photosynthetic activity was noted when water was taken into the incubation chambers through a length of 1/16 in. i.d. stainless steel tubing. A 10 mesh stainless steel wire cloth, having openings slightly smaller than 1/16 in., was selected for the APPI prefilter.

2. Analog of Sample Acquisition System

An analog of the sample acquisition system, beyond the prefilter, was assembled in order to evaluate the effects of moving the sample through stainless steel tubing. This particular analog consisted of a glass 100-cc capacity syringe fitted with a 15-gauge needle slightly over 4 inches in length. The photosynthetic uptake of ^{14}C was measured on stream samples drawn up into the syringe and expelled into standard light and dark bottles. Replicate measurements were then made on the same water sample handled in the conventional manner. A mean of 196 cpm was obtained from 10 light-dark bottles when the water sample

was drawn up with the syringe, while the control bottles, which were filled by pouring, gave a net count of 191 cpm. It was concluded, on the basis of this experiment, that this portion of the APPI sample acquisition system would have no effect upon the determination of primary productivity.

A determination was made of the hang-up or sample-to-sample cross contamination which will occur in the APPI. A second analog of the sample acquisition system was assembled from the actual manifold of the instrument with added polyvinylchloride tubing and a 100-cc syringe to act as the pump and sample chamber. A photograph of this setup is shown in Figure 11. A solution containing approximately 1 uCi/ml of ^{14}C-sodium bicarbonate was prepared in pH 11 distilled water. A 100-ml portion of this solution was drawn into the syringe through the intake tube of the manifold, held for 3 minutes, and then expelled. Two subsequent washes were made with 100-ml portions of distilled water by slowly drawing in and then expelling the wash water. A third 100-ml volume of distilled water was introduced into the syringe and retained 3 minutes. The last 100-ml portion simulated the second sample, but contained no added ^{14}C. The first and second sample solutions, as well as the two washes, were collected in flasks. Ten 1-ml aliquots were drawn from each

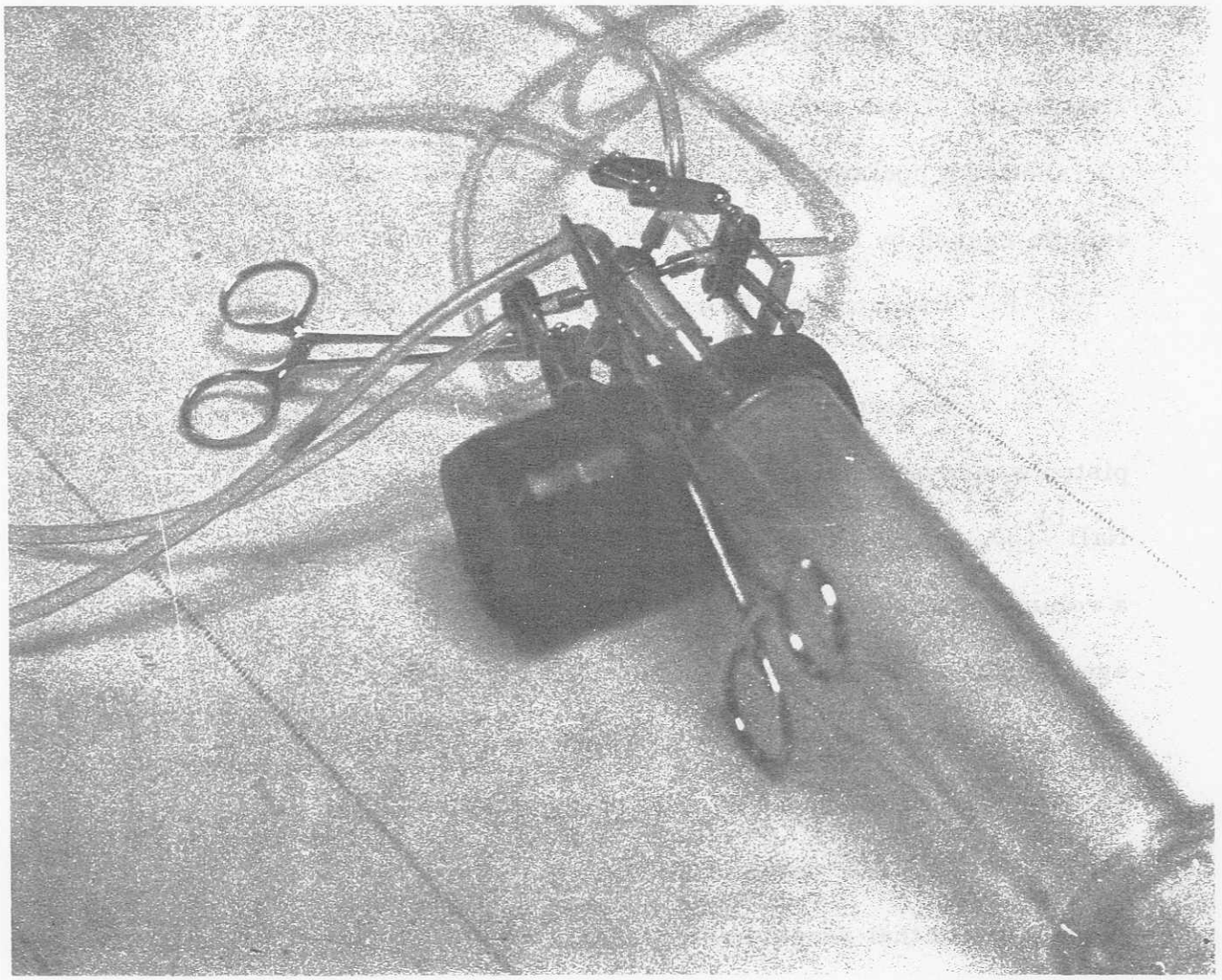


Figure 11

Analog of Sample Acquisition System

flask and placed in stainless steel planchets containing a few drops of a saturated barium hydroxide solution. The planchets were dried and then counted for one minute. The results of this experiment, which are presented in Table 10, showed a contamination level of 0.11% in the second sample caused by hang-up in the instrument.

F. Preparation, Storage, and Use of Radioisotope Solution

A stainless steel chamber containing a spring-loaded piston was originally considered for storing and dispensing $NaH^{14}CO_3$ reagent solution in the APPI. The chamber had a volume of about 250-cc and was designed to store the reagent under slight pressure with no vapor space over the liquid. Mechanical problems with this device were noted in early tests. In addition, the chamber was found to be difficult to load and maintain sterile. Laboratory tests demonstrated that reagent solutions stored under non-sterile conditions rapidly lost their radioactive carbon. For these reasons, use of the stainless steel chamber system was discontinued and another system instituted which made use of sterile, plastic blood bags. The plastic blood bags, Code No. JE-1, were obtained from Fenwall Laboratories of Morton Grove, Illinois. These were sterile, pyrogen-free bags having a capacity of 600-cc. The bags were filled under sterile conditions with $NaH^{14}CO_3$ solution, pH 11.0, containing

Table 10

Determination of Sample-to-Sample Contamination
Due to Hang-Up

Radioactivity from 1 ml Aliquots of Sample and Wash Solution (cpm/ml)

1st Sample	1st Wash	2nd Wash	2nd Sample
370,071	42,473	936	417
365,264	45,695	941	393
326,584	45,272	913	399
353,479	45,164	914	384
362,491	44,051	1,014	429
359,497	41,321	946	373
331,961	40,768	904	375
352,264	44,618	907	416
359,131	44,848	965	396
340,880 / 352,162	45,103 / 43,931	964 / 940	401 / 398

Sample-to-Sample Contamination Due to Hang-Up =

$$\frac{398}{352,162} \times 100 = 0.11\%$$

and will not load the filter as severely in eutrophic waters, those most likely to be sampled by the APPI. The sample is still large enough for high sensitivity. Further, the sample-to-sample cross contamination caused by hang-up and incomplete washing in the APPI will not be excessive if this sample volume is taken.

3. Incubation

An incubation period of two hours, without stirring of the sample, is recommended. Stirring of the sample during incubation was not recommended in the manuals (5, 6, 7) which detailed procedures for measuring primary productivity. A scientist contacted during this program recommended that the samples should not be agitated. A relatively short incubation period is desirable in order that changes in rate of photosynthesis which occur during the day can be studied. However, the minimum quantity of primary productivity which may be determined with an acceptable precision is inversely proportional to the length of the incubation period. A time period of two hours represents a compromise between these factors. An additional factor which was considered was that activity within the bottles is not indicative of the conditions extant in the sample area during the later portions of extended incubation periods. Furthermore, the two-hour period is believed short enough to preclude significant

secretion of labeled metabolic products from the plankton in most cases.

4. Radioisotope Solution

Storage of an unbuffered aqueous solution of $NaH^{14}CO_3$ at pH 11 is recommended. The labeled carbonate solution should have a specific activity of at least 20 uCi/mM and precautions should be taken during preparation and filter sterilization to ensure that significant amounts of cold carbonate are not picked up. A level between 5 and 10 uCi of $NaH^{14}CO_3$ per 100-ml of sample should be added in a relatively small volume to leave the sample essentially unaltered by the addition of isotope.

The plastic blood bag storage system, described in a previous section, is recommended for the APPI. Vapor space above the radioisotope solution should be eliminated or minimized. Additional study of the stability of this solution will be required if tests lasting longer than about one week are to be conducted.

5. Filtration

Filtration under 0.5 atmosphere pressure through 47 mm diameter "Acropor" AN-450 membrane filters is recommended. The filters should be washed with at least 10 ml of 0.01 N H_2SO_4. The filters should then be dried

under an infrared lamp. The length of the post-drying period prior to counting is not critical.

6. Counting Time

Studies have shown that net uptake of radioactivity as low as 100 cpm could be measured with satisfactory precision. Coefficients of variation at this low level of uptake were found to be from 5 to 15%. For these low count rates, a counting time of 5 minutes is recommended in order that the error due to counting statistics alone be no greater than 5%.

7. Cleaning Solution

The use of a nontoxic, noncorrosive aqueous cleaning solution, such as 0.5% "Woolite" detergent solution, is recommended for use in the APPI. (Diagnostic testing after the Lake Lanier demonstration added a further recommendation: The solution should be loaded and maintained under sterile conditions, either by membrane filtration or by disposal after use).

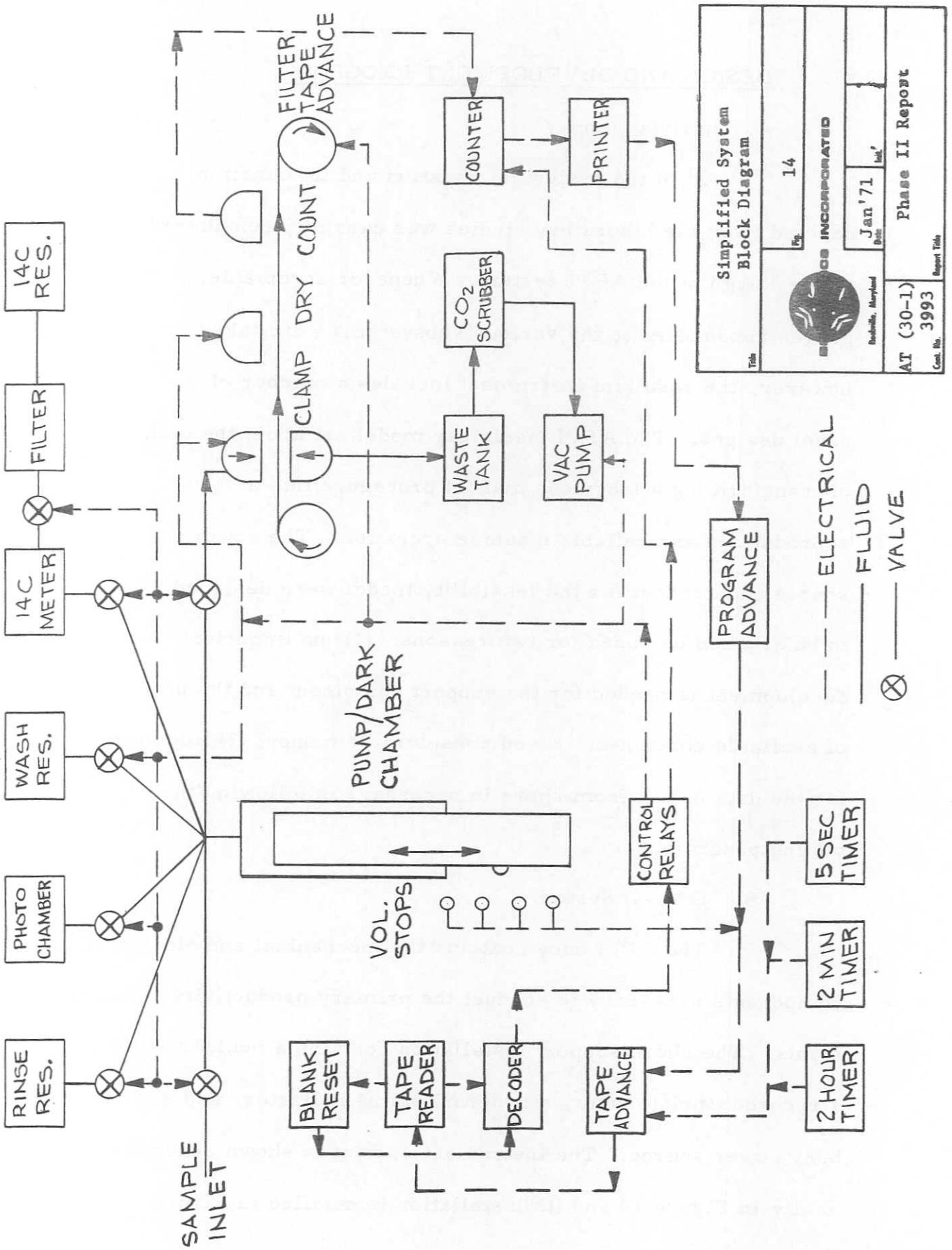
VI. DESIGN AND DEVELOPMENT PROGRAM

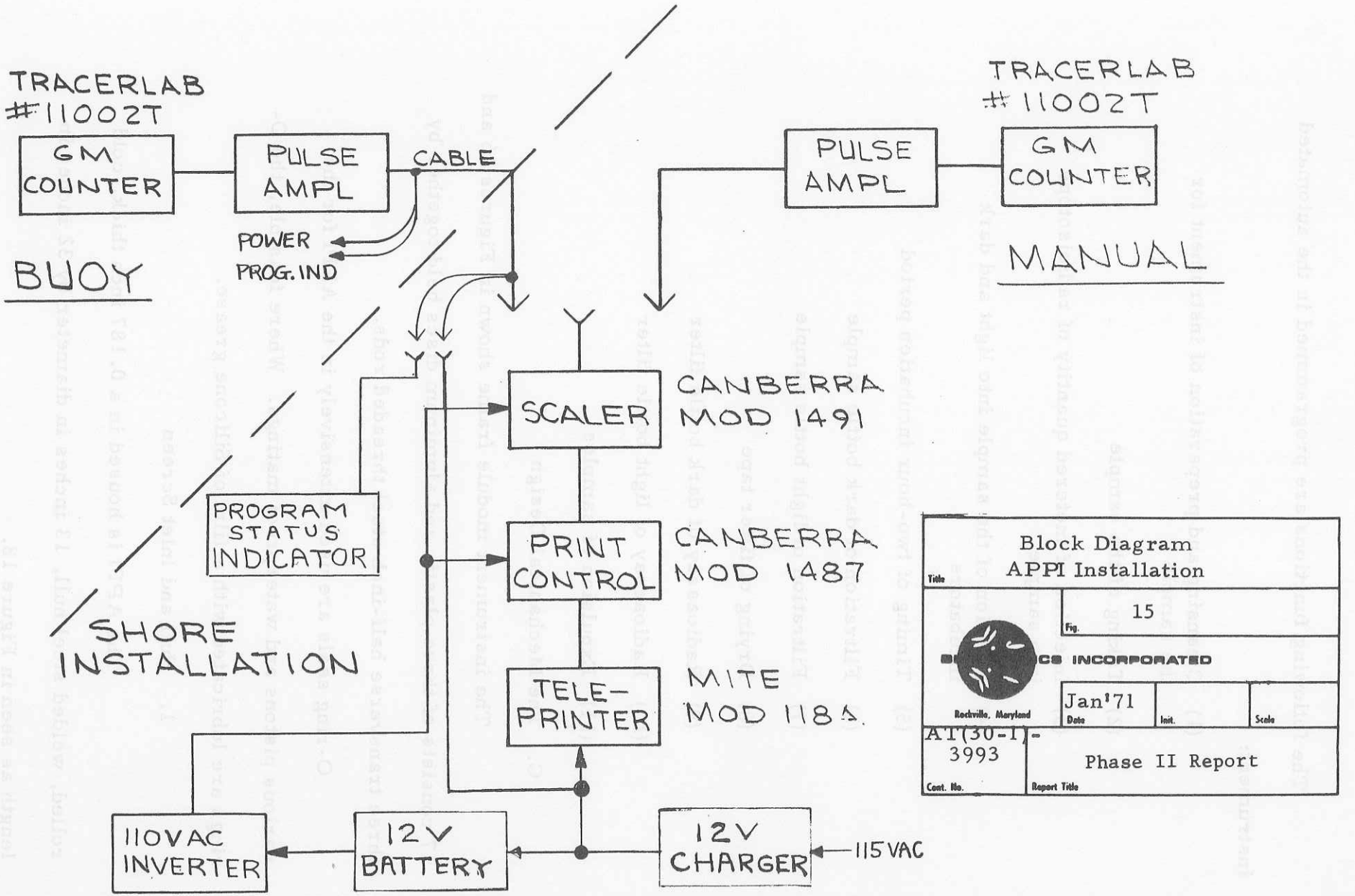
A. Introduction

All of the review information and information derived from the laboratory studies was carefully considered in the design of the APPI system. Whenever acceptable, proven approaches to the various subsystems were taken; however, the resultant instrument includes a number of novel designs. The APPI feasibility model set about the task of transforming a laborious manual procedure into a reproducible and reliable machine operation. The power source and readout for the feasibility model were designed to be situated on shore for two reasons: (1) no important development is needed for the support equipment and the use of available components saved considerable money; (2) monitoring the data output from shore is necessary in following the testing program.

B. Overall System

The APPI buoy contains the mechanical and electronic components necessary to conduct the primary productivity measurements. The shore support installation contains a nuclear scaler, a recorder/printer unit, a program status indicator, and a buoy power source. The instrument system is shown schematically in Figure 14 and its installation is detailed in Figure 15.


SAMPLE INLET
RINSE RES.
PHOTO CHAMBER
WASH RES.
14C METER
FILTER
14C RES.
PUMP/DARK CHAMBER
VOL. STOPS
CLAMP
DRY COUNT
FILTER TAPE ADVANCE
WASTE TANK
CO_2 SCRUBBER
VAC PUMP
COUNTER
PRINTER
PROGRAM ADVANCE
CONTROL RELAYS
BLANK RESET
TAPE READER
DECODER
TAPE ADVANCE
2 HOUR TIMER
2 MIN TIMER
5 SEC TIMER
ELECTRICAL
FLUID
VALVE
Simplified System Block Diagram
14
Jan'71
Phase II Report
AT (30-1) 3993


TRACERLAB
#11002T
GM COUNTER
PULSE AMPL
CABLE
POWER
PROG. IND
BUOY
TRACERLAB
#11002T
PULSE AMPL
GM COUNTER
MANUAL
SCALER
CANBERRA MOD 1491
PROGRAM STATUS INDICATOR
PRINT CONTROL
CANBERRA MOD 1487
SHORE INSTALLATION
TELE-PRINTER
MITE MOD 1185
110VAC INVERTER
12V BATTERY
12V CHARGER
115VAC
Block Diagram
APPI Installation
Title
15
Fig.
BCS INCORPORATED
Rockville, Maryland
Jan'71
Date
Init.
Scale
AT(30-1)-3993
Cont. No.
Phase II Report
Report Title

The following functions are programmed in the automated instrument:

(1) Cleansing and preparation of instrument for the sample

(2) Taking of the sample

(3) Injection of metered quantity of radioisotope into sample

(4) Division of the sample into light and dark incubators

(5) Timing of two-hour incubation period

(6) Filtration of dark bottle sample

(7) Filtration of light bottle sample

(8) Drying of filter tape

(9) Radioassay of dark bottle filter

(10) Radioassay of light bottle filter

(11) Expulsion of samples

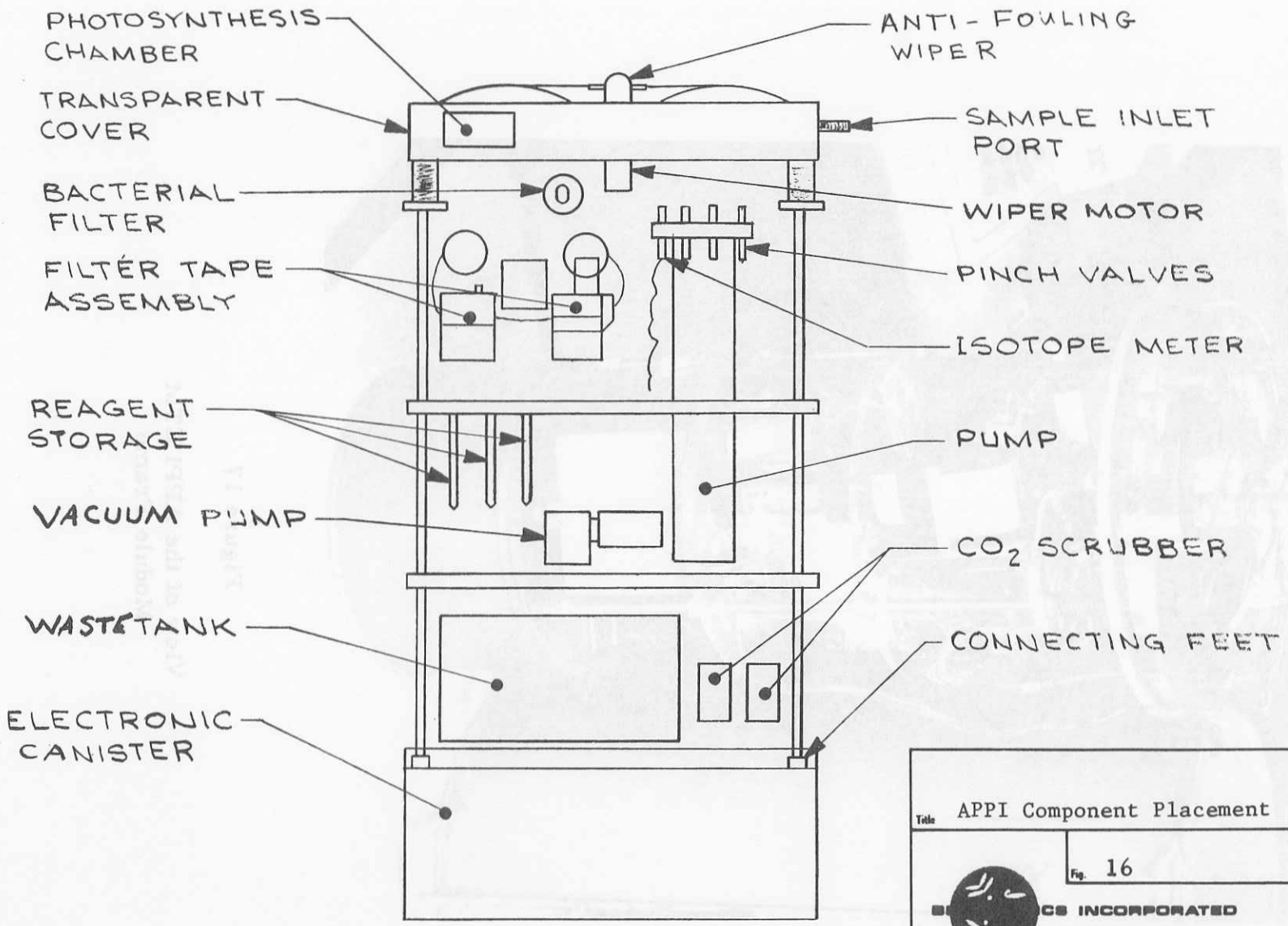
C. The Mechanical Design

The instrument module frame shown in Figures 16 and 17 consists of three plastic and aluminum discs held together by three transverse half-inch steel threaded rods.

O-ring seals are used extensively in the APPI for the various pistons and waterproof matings. Where feasible, the O-rings are lubricated with a film of Silicone grease.

1. Hull and Inlet Screen

The APPI is housed in a 0.187 inch thick, cold rolled, welded steel hull, 13 inches in diameter by 32 inches in length as seen in Figure 18.



Title: APPI Component Placement	
	Fig. 16
BIOCS INCORPORATED, Rockville, Maryland	Date: Jan'71 / Init. / Scale
AT(30-1) Cont. No. 3993	Report Title: Phase II Report

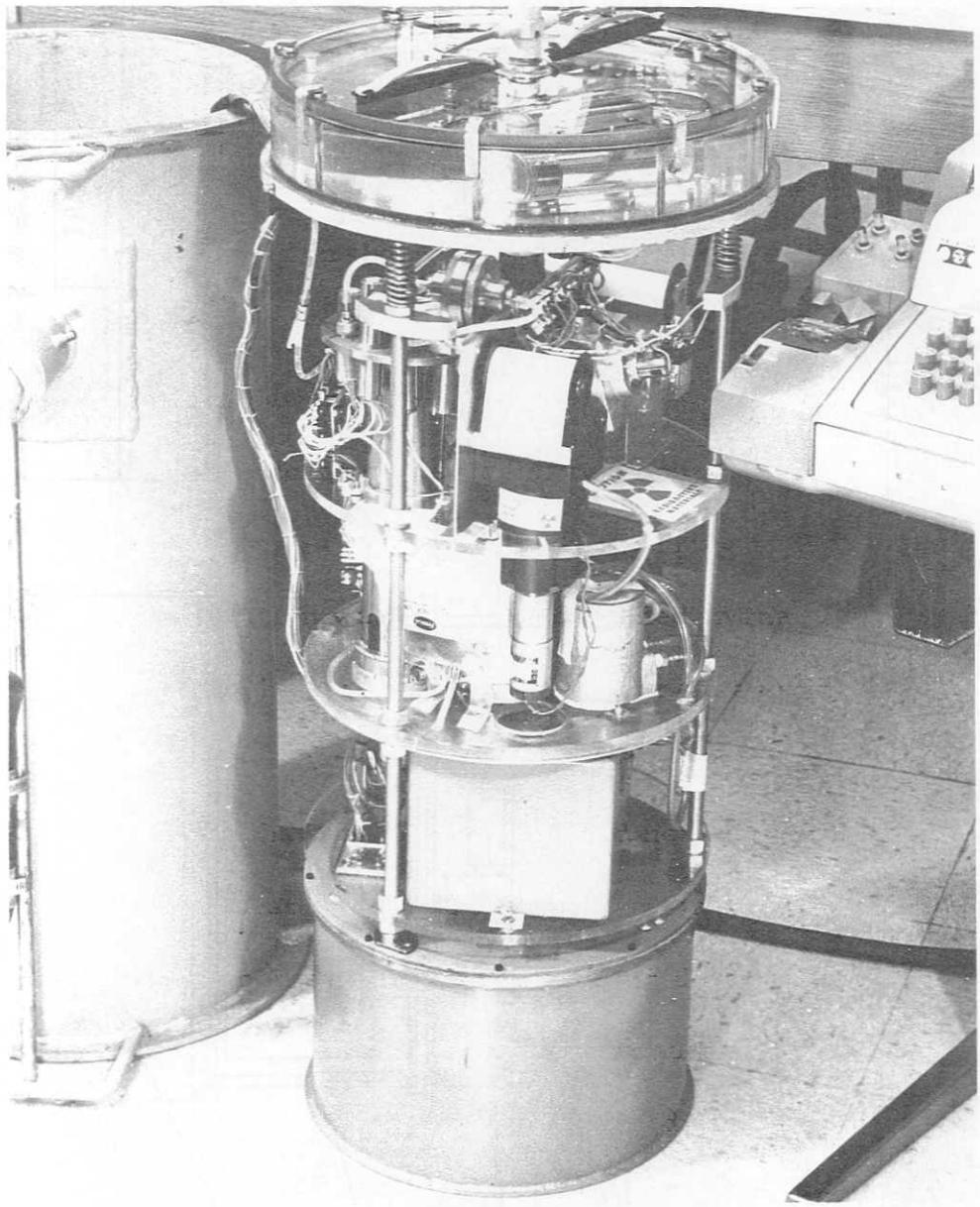
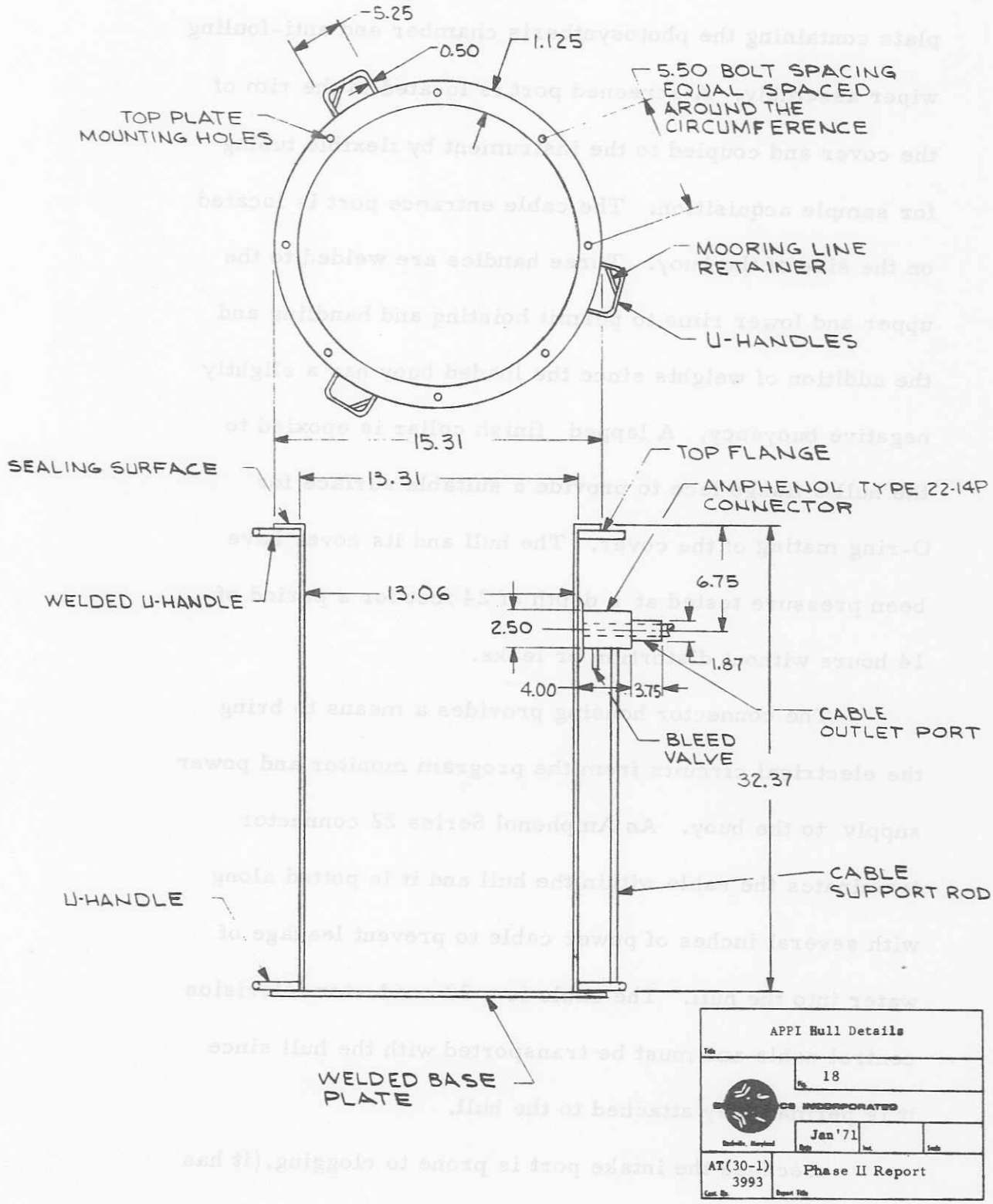


Figure 17

View of the APPI ETM
Module Frame


5.25
0.50
1.125
5.50 BOLT SPACING EQUAL SPACED AROUND THE CIRCUMFERENCE
TOP PLATE MOUNTING HOLES
MOORING LINE RETAINER
U-HANDLES
15.31
SEALING SURFACE
13.31
TOP FLANGE
AMPHENOL TYPE 22-14P CONNECTOR
WELDED U-HANDLE
13.06
6.75
2.50
1.87
4.00
3.75
CABLE OUTLET PORT
BLEED VALVE
32.37
CABLE SUPPORT ROD
U-HANDLE
WELDED BASE PLATE
APPI Hull Details
18
INCORPORATED
Jan'71
AT(30-1) 3993
Phase II Report

The hull is covered with a 2 inch thick transparent plate containing the photosynthesis chamber and anti-fouling wiper assembly. A screened port is located in the rim of the cover and coupled to the instrument by flexible tubing for sample acquisition. The cable entrance port is located on the side of the buoy. Three handles are welded to the upper and lower rims to permit hoisting and handling and the addition of weights since the loaded buoy has a slightly negative buoyancy. A lapped finish collar is epoxied to the hull's flange face to provide a suitable surface for O-ring mating of the cover. The hull and its cover have been pressure tested at a depth of 24 feet for a period of 14 hours without distortion or leaks.

The connector housing provides a means to bring the electrical circuits from the program monitor and power supply to the buoy. An Amphenol Series 22 connector terminates the cable within the hull and it is potted along with several inches of power cable to prevent leakage of water into the hull. The cable is a 28 conductor television control cable and must be transported with the hull since it is permanently attached to the hull.

Because the intake port is prone to clogging, (it has an inside diameter of 1/16 inch) it is caged by a large

cylindrical surface screen of number 10 mesh with an area of 9 square inches.

2. Sample Pump

The pump assembly (shown in Figures 19 and 20) represents one of the major components in the APPI. A cylinder of polished stainless steel is used for the housing and contains a removable head plate, a double O-ring piston with a long threaded arbor, a lead-screw, fractional horse-power drive motor (Globe Model 319, 300 RPM miniature motor), and a detent/microswitch assembly for controlling the volumes to be pumped.

The APPI programmer provides the power to operate the sample pump at high speed forward and reverse or slow speed forward. The motor powers a 1/8 inch diameter lead-screw which engages five threads of the threaded arbor attached to the piston. Rotation of the piston is prevented by a cylindrical key and groove attached to the piston. The piston can be driven from a spring-loaded jam position at the head plate to a position beyond the maximum volume of 200-ml.

The four microswitches are positioned to signal when the volumes of 0, 10, 100 and 200 ml have been reached. Each time a microswitch is energized, it stops the programmer

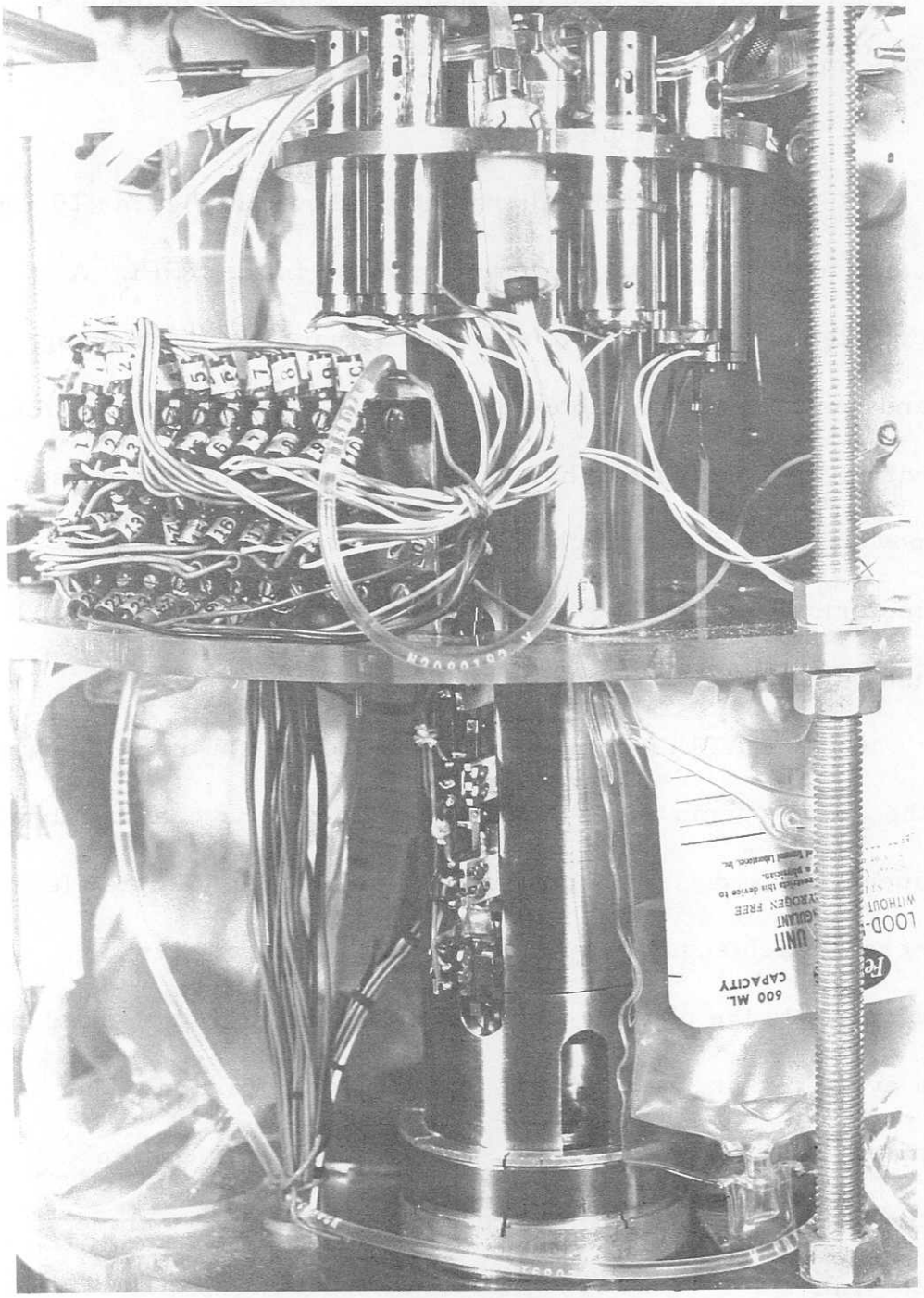
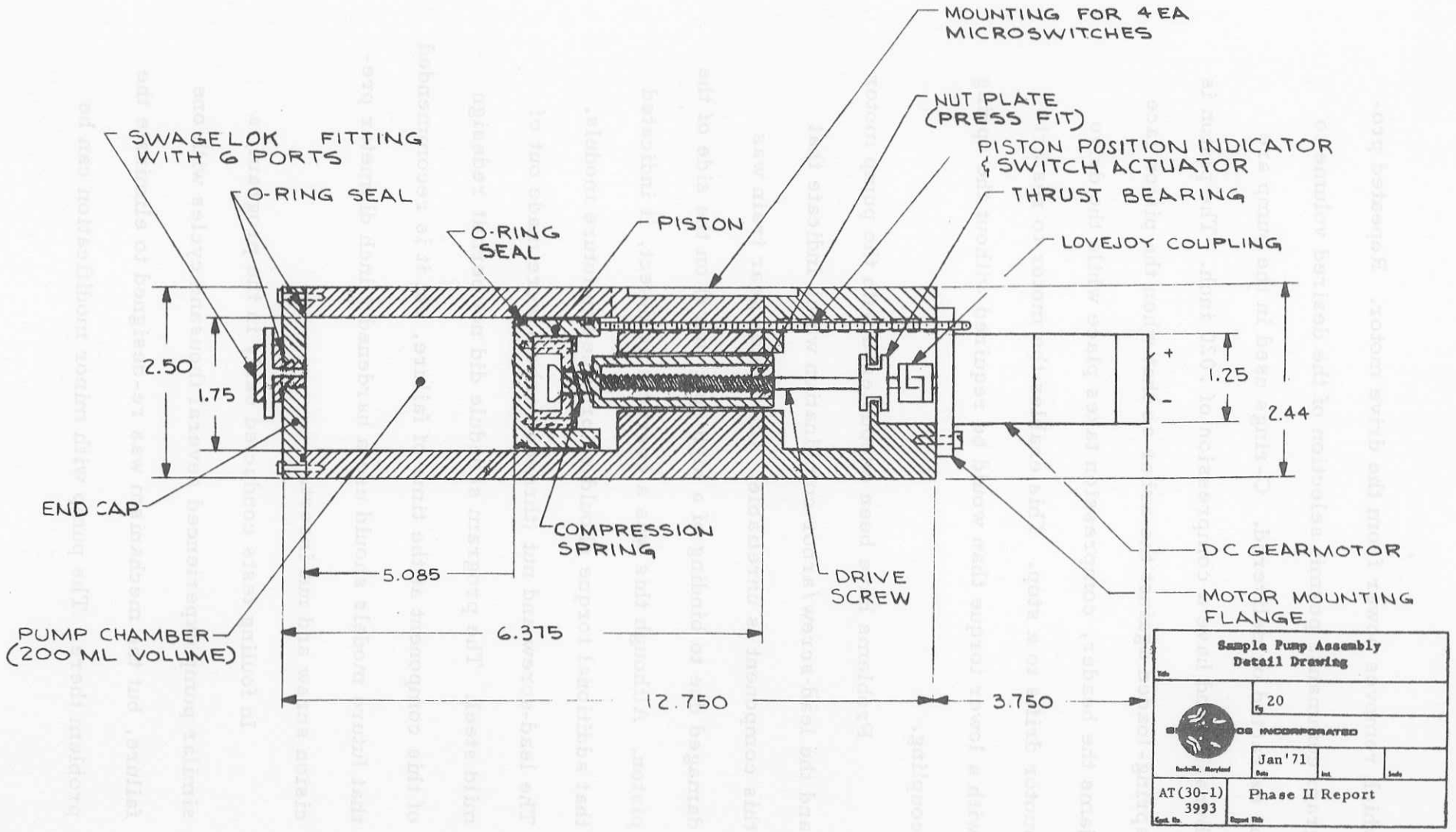


Figure 19

View of Sample Pump
with its Volume Switches


MOUNTING FOR 4 EA MICROSWITCHES
NUT PLATE (PRESS FIT)
PISTON POSITION INDICATOR & SWITCH ACTUATOR
THRUST BEARING
LOVEJOY COUPLING
SWAGELOK FITTING WITH 6 PORTS
O-RING SEAL
O-RING SEAL
PISTON
2.50
1.75
1.25
2.44
+
-
END CAP
COMPRESSION SPRING
5.085
DRIVE SCREW
DC GEARMOTOR
MOTOR MOUNTING FLANGE
PUMP CHAMBER (200 ML VOLUME)
6.375
12.750
3.750
Sample Pump Assembly Detail Drawing
Fig 20
OS INCORPORATED
Rockville, Maryland
Jan'71
AT(30-1) 3993
Phase II Report

which removes power from the drive motor. Repeated program commands permit selection of the desired volume to be extracted or delivered. O-rings used in the pump are Buna-N, and have a compression of .020 inch. The piston is spring-loaded against the arbor so that when the piston face jams the header, compression takes place while the drive motor drifts to a stop. This enables the motor to restart with a lower torque than would be required without the spring coupling.

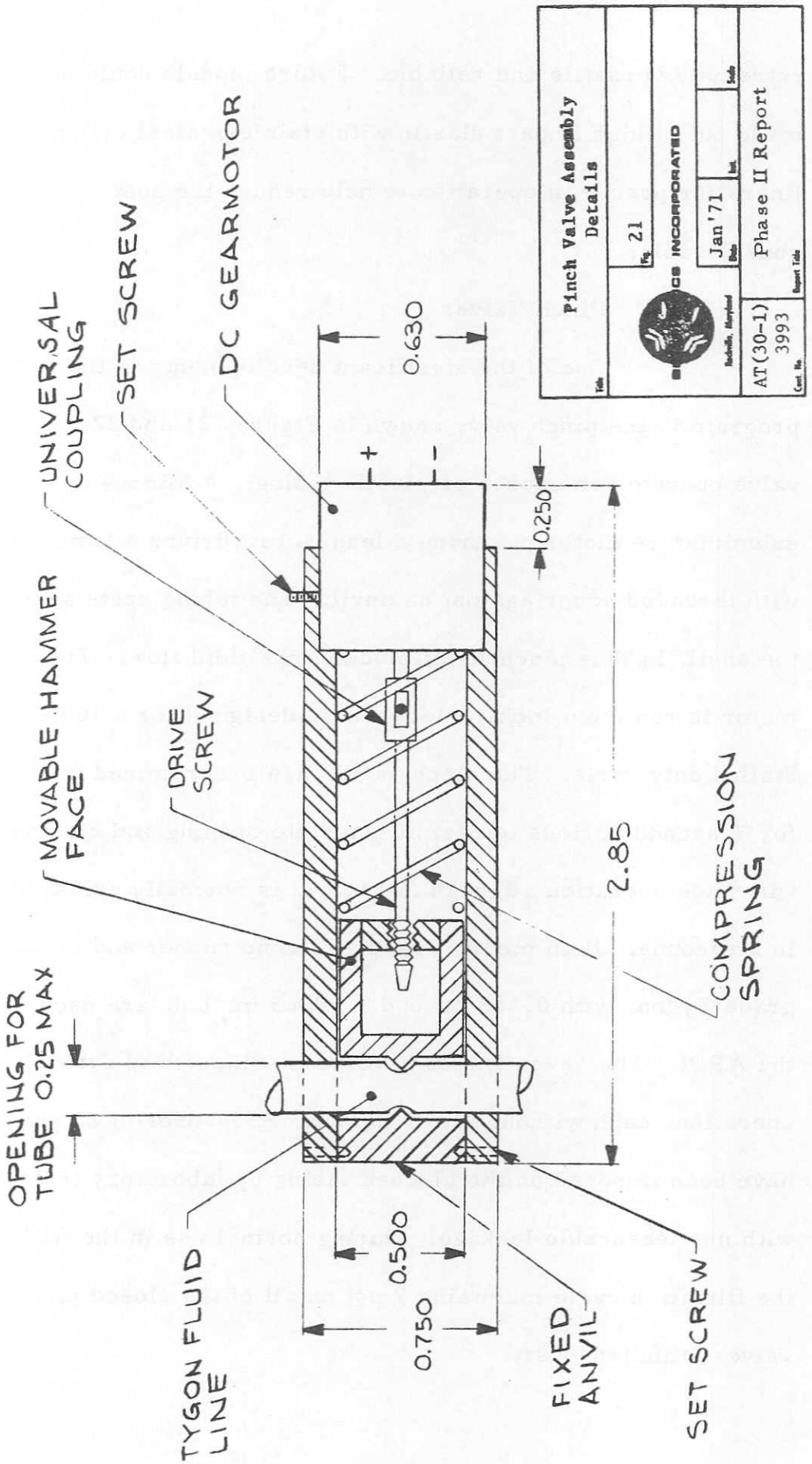
Problems have been encountered with the pump motor and the lead-screw/arbor combination which indicate that this component is unreliable. One motor gear train was damaged due to binding of a high spot (burr) on the side of the piston. Although this was a correctable defect, it indicated that additional torque should be provided in future models. The lead-screw and nut (threaded arbor) were made out of mild steel. The program schedule did not permit redesign of this component at the time of failure, but it is recommended that future models should use a hardened $\frac{1}{4}$ inch diameter precision screw and nut insert.

In fouling tests conducted early in the program, a similar pump experienced several thousand cycles with one failure, but the mechanism was re-designed to eliminate the problem there. The pump with minor modification can be

extremely versatile and reliable. Future models could be made out of high impact plastic with stainless steel cylinder liners for precision operation to help reduce the cost considerably.

3. Pinch Valves

One of the significant developments in the APPI program is the pinch valve shown in Figures 21 and 22. This valve operates on rubber or plastic tubing. A Micro-mo subminiature motor powering a lead-screw drives a hammer with threaded arbor against an anvil. The tubing rests against the anvil, is thus pinched-off, blocking the fluid flow. The motor is run from lock-to-lock and is designed for a 100% stalled duty cycle. The pinch valves are programmed to run for 7-second periods to permit complete opening and closing with each operation although full travel is normally achieved in 5 seconds. Both medical grade Silicone rubber and medical grade Tygon, with 0.188 in. o.d., .0625 in. i.d. are used in the APPI. The seven valves have been subjected to over 1,000 operations each without any difficulty. Pressures of 20 psi have been imposed on the pinched tubing by laboratory tests with no measurable leakage. During normal use in the APPI, the filtration cycle maintains 7 psi on all of the closed pinch valves simultaneously.


SET SCREW
DC GEARMOTOR
UNIVERSAL COUPLING
MOVABLE HAMMER FACE
DRIVE SCREW
OPENING FOR TUBE 0.25 MAX
TYGON FLUID LINE
FIXED ANVIL
SET SCREW
COMPRESSION SPRING
0.630
0.250
2.85
0.500
0.750
+
-
Pinch Valve Assembly Details
21
GS INCORPORATED
Rockville, Maryland
Jan'71
AT(30-1) 3993
Phase II Report

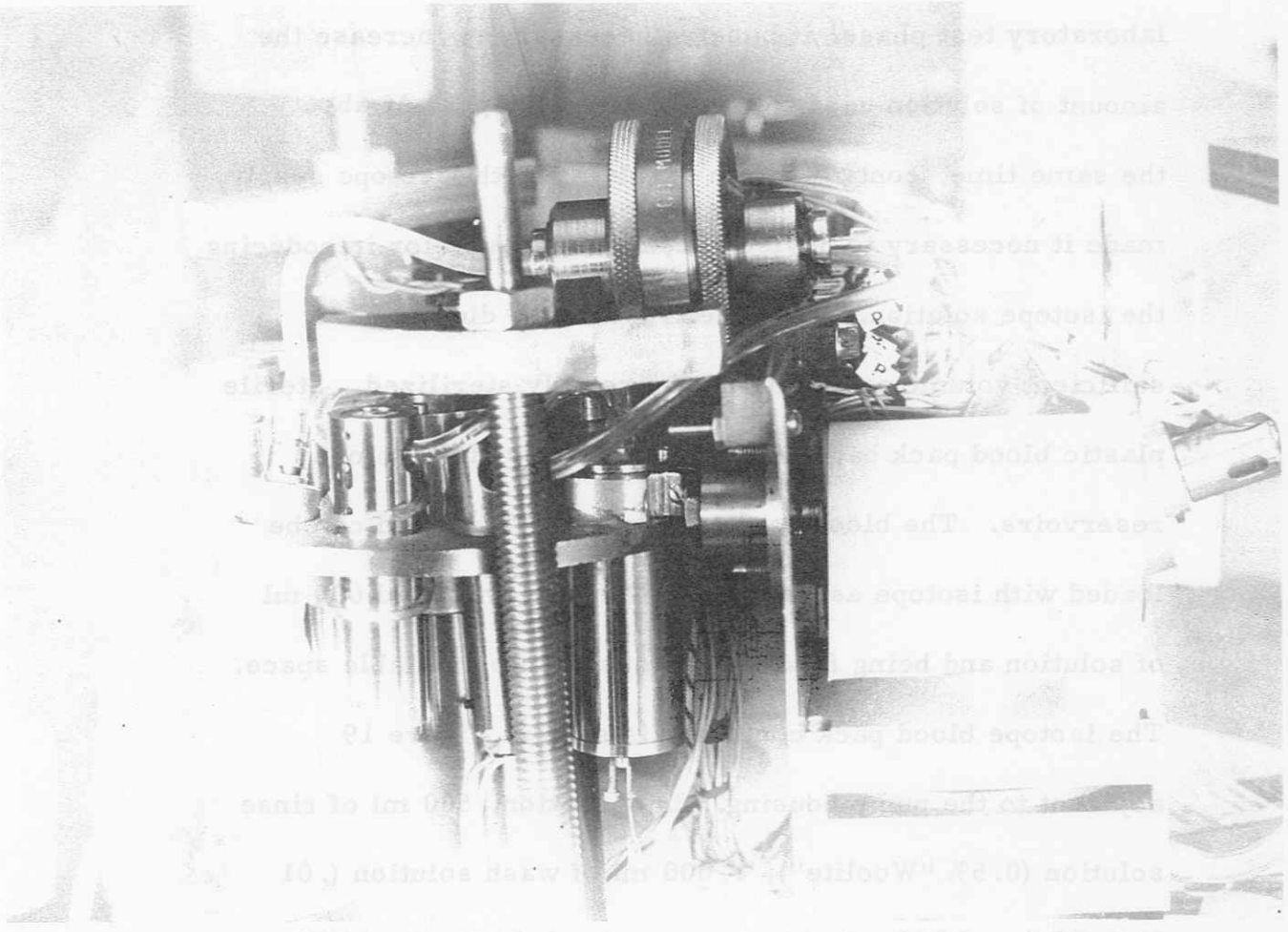


Figure 22

View of Pinch Valve Cluster, Metering Syringe and Isotope Filter

4. Reagent Reservoirs

The reagent reservoirs originally used in the APPI were stainless steel cylindrical containers. During the laboratory test phase, it became necessary to increase the amount of solution used to wash the filter tape. At about the same time, contamination problems in the isotope supply made it necessary to devise aseptic techniques for introducing the isotope solution. The steel reservoirs did not have sufficient volume and could not be readily sterilized. Sterile plastic blood pack bags were substituted for the isotope reservoirs. The blood packs are presterilized and can be loaded with isotope aseptically. The bags will hold 600 ml of solution and being flexible, fit easily into available space. The isotope blood pack container is seen in Figure 19 adjacent to the pump housing. In operation, 500 ml of rinse solution (0.5% "Woolite"), 1,000 ml of wash solution (.01 N H_2SO_4) and 250 ml of isotope are loaded into the APPI, providing reagents for 20 cycles of operation. The isotope and wash solutions are used once and passed to the waste storage tank. The rinse solution is reused. Future versions of the APPI should use a system to filter the rinse solution as it is returned to the storage reservoir to prevent bacterial contamination from being reintroduced into the chamber. While this is not a major problem at present, it is possible that it would be during long-term operation.

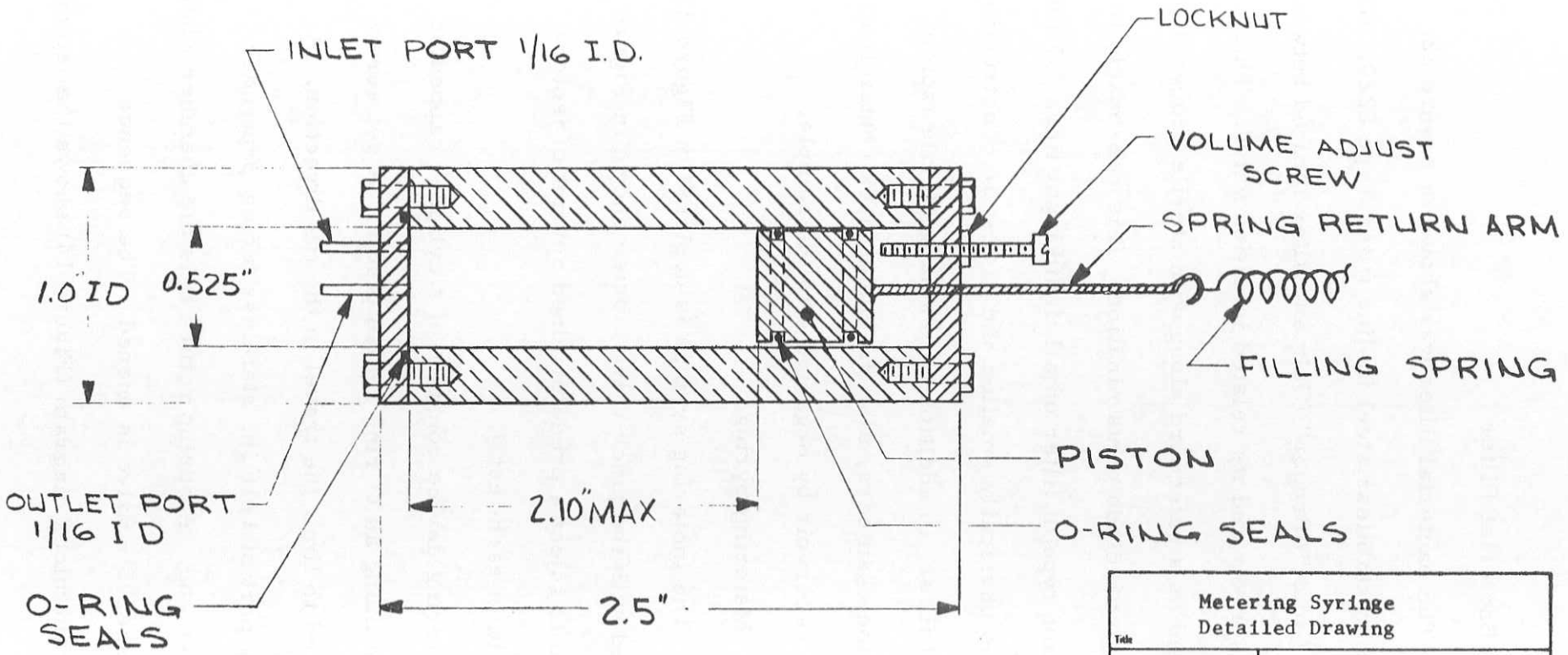
5. Bacterial Filter

The bacterial filter was shown in Figure 22. This is a Gelman stainless steel in-line filter Type 2220, fitted with a .45 u,47 mm "Acropor" (Gelman) filter located between the isotope reservoir and its related isolation valve. This filter was added as a safeguard along with sterile processing of the isotope to preclude contamination. It is reasonable to install this same type of filter on all liquid input lines. Future filters could be physically smaller and of plastic construction. The biological filter, in addition to assuring sterile isotope to the incubation chambers, also precludes back contamination of the isotope reservoir by organisms in the sample.

6. Metering Syringe

The metering syringe is depicted in Figure 23 and is mounted with the pinch valve cluster shown in Figure 22. It is used to inject a predetermined amount of isotope into the sample in each test.

The metering device consists of a cylinder capped at both ends containing an O-ring sealed piston. A set screw stop is adjusted to limit the travel in the fill direction. A fill and empty port of 1/16 in. stainless tubing protrudes from the active end. A spring return fills the chamber when the isotope reservoir valve is opened. The sequence illustrated in the block diagram (Figure 14) shows the isotope



Title: Metering Syringe Detailed Drawing	
	Fig. 23
BIOTECS INCORPORATED, Rockville, Maryland	Date: Jan'71
Cont. No.: AT(30-1) 3993	Report Title: Phase II Report

reservoir, biological filter, pinch valve, meter, and pinch valve all in series to the pump. In operation, the valve to the isotope reservoir is opened with adequate time allowed for the spring to draw back the piston, filling the chamber. This valve is then closed and the valve to the pump opened.

The pump is operated to the 10 ml stop, drawing 6 mls of isotope solution from the metering syringe, leaving the pump under a slight vacuum. The valve is then closed for program continuance. The valve to the isotope reservoir is opened only when programmed for the refilling of the metering syringe. The amount of isotope injected can be adjusted from 4 to 6 ml per 200 ml sample (less the isotope). The APPI as now operated utilizes 6 ml of isotope plus a 194 ml sample, with an accuracy of about 0.1 ml in isotope metering.

7. Photosynthesis Chamber

The requirement for a photochamber would appear to be a simple one to meet. It proved to be quite complex due to problems such as:

a. Optical coupling in materials

b. Optical coupling fluid seals

c. Both biological and sedimentary fouling

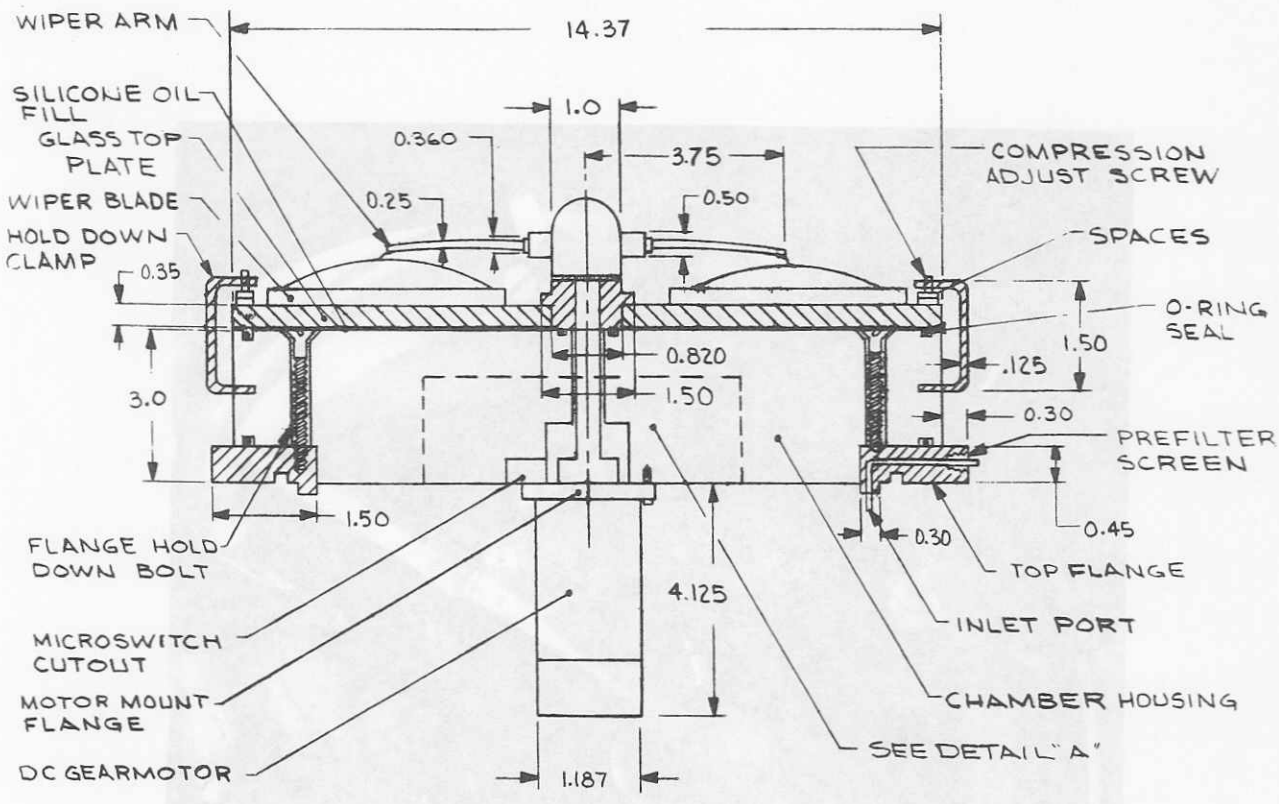
d. Configuration into the cover

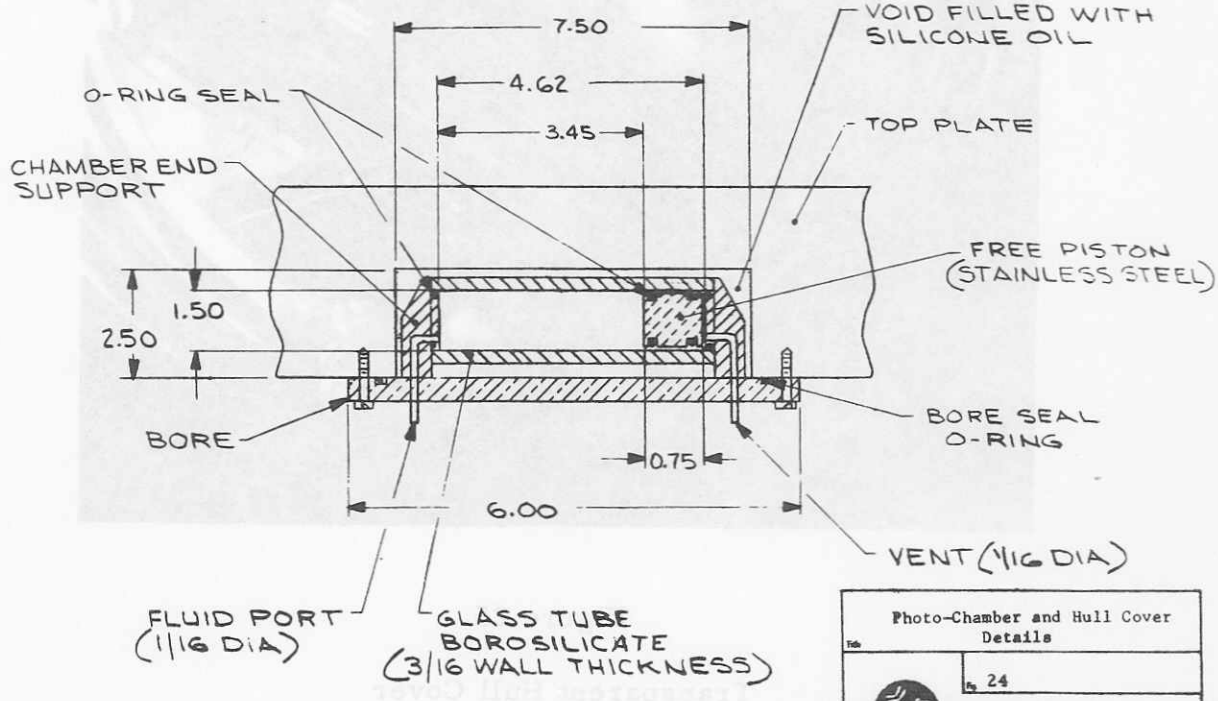
The materials selected for the optical path are Lucite, Silicone oil, plate glass, and precision glass tubing.

The range of index of refraction of these materials extends from 1.49 to 1.51. This is an extremely small range and causes an estimated light loss of less than 10%. Jars suspended in water for manual ^{14}C primary productivity measurements generally show a similar loss.

The APPI light chamber is a precision glass cylinder, capped at both ends with O-ring end plates. A floating piston follows the entrance and exit of sample, thus preventing air binding and incomplete filtering. The volume behind the piston is vented to the hull. The light chamber holds 100 ml when full. This chamber is fitted into a 2 inch thick Lucite cover as shown in the drawing in Figure 24 and the photograph in Figure 25. The void between the glass cylinder and the plastic is filled with Dow 550 Silicone fluid. The same fluid is used to couple the plastic cover to a plate glass cover plate. Seals in all cases are either Silicone or Buna O-rings. A waterproof bearing and shaft is installed through the center of the cover, coupled to a 4 RPM Globe Industries Model 319 motor. This motor operates on program for 2 minute periods once during each analysis cycle, driving a rubber wiper blade to remove any accumulated fouling.

Studies described elsewhere dictated the use of this technique to assist in the prevention of fouling. A self-completing micro-switch circuit is included so that the wiper blade will always





DETAIL "A"

Photo-Chamber and Hull Cover Details	
Fig. 24	
EG&G INCORPORATED	
Jan'71	
AT(30-1) 3993	Phase II Report

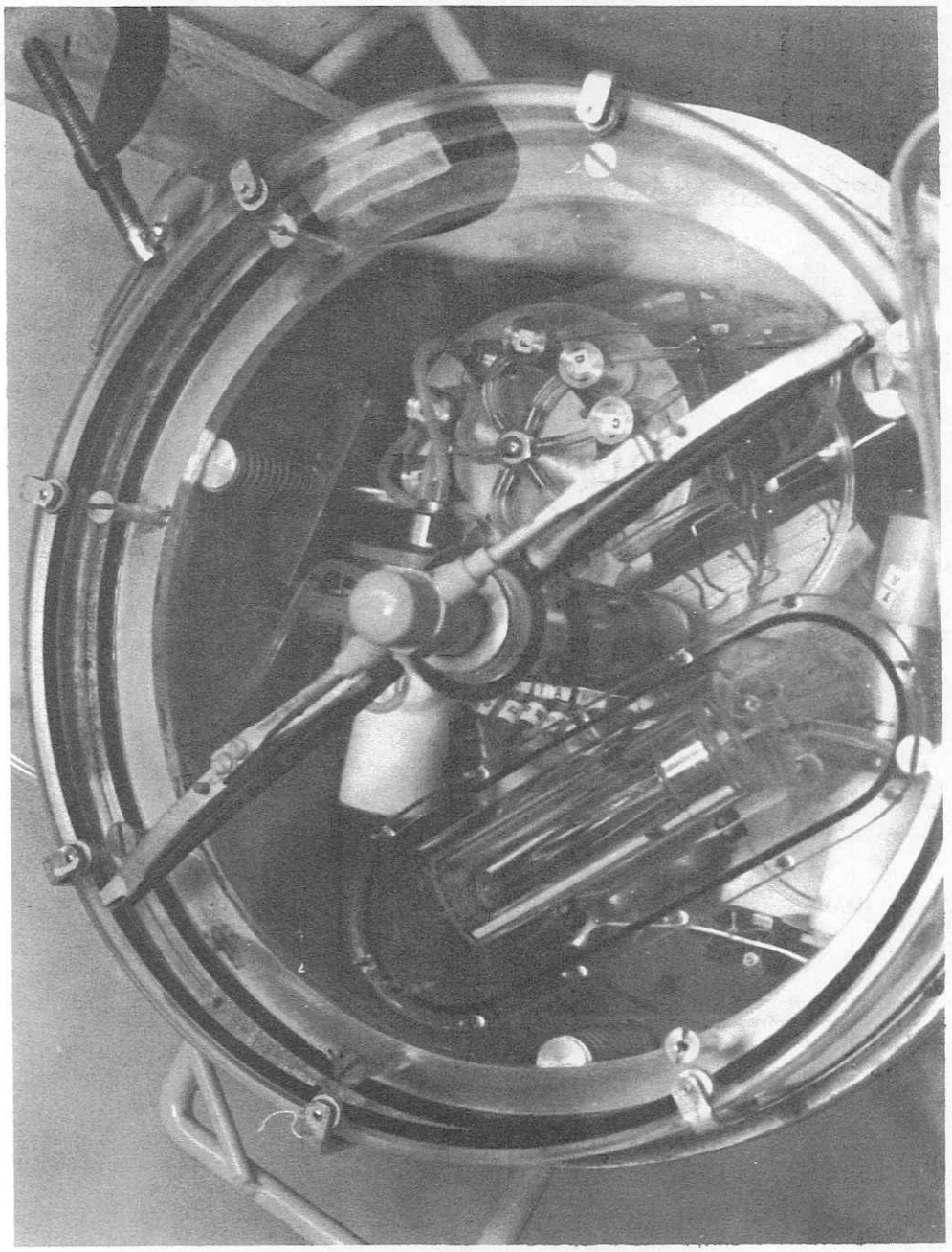


Figure 25

Transparent Hull Cover
with Photosynthesis Chamber

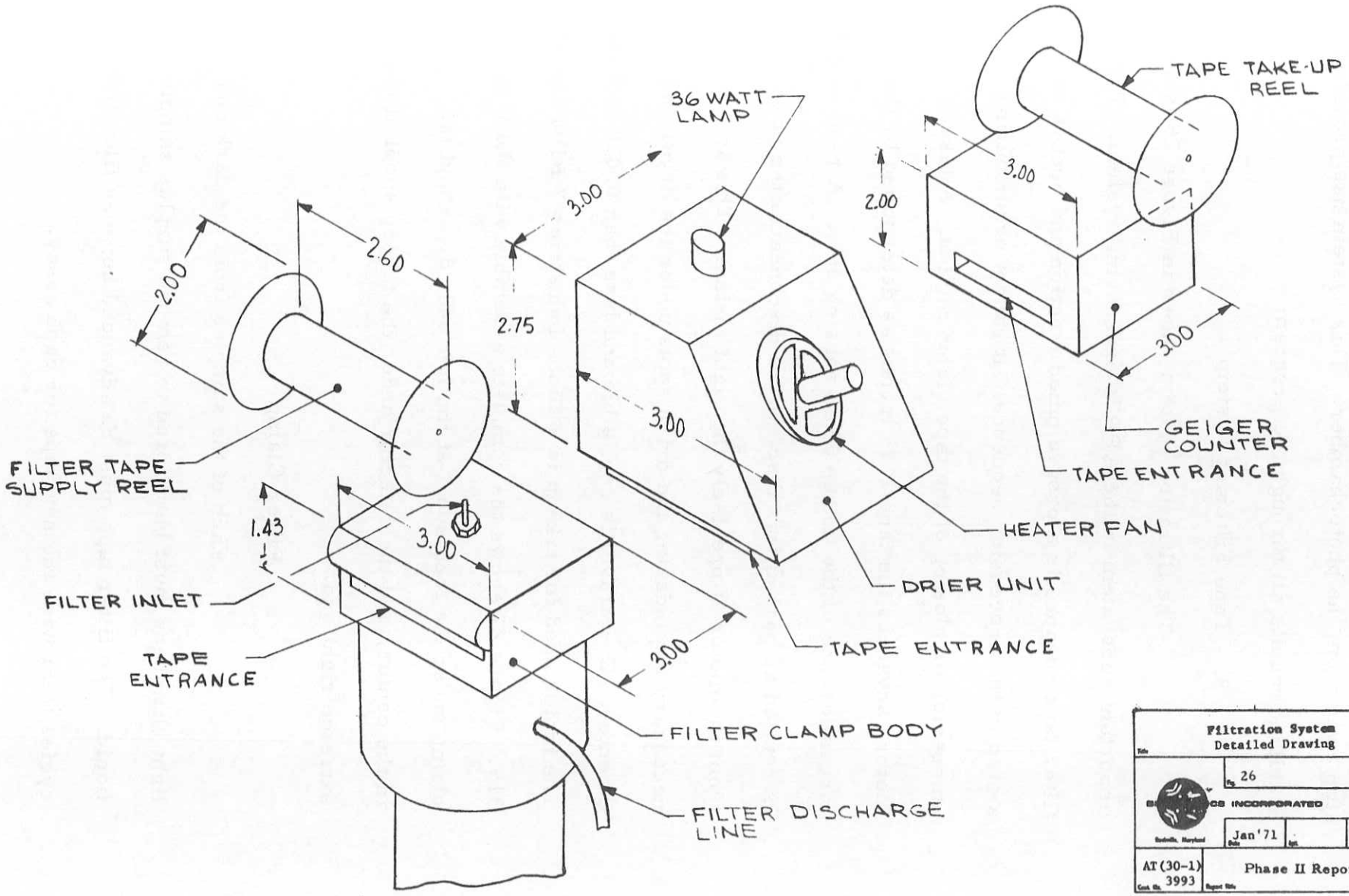
stop away from the photochamber. This system has provided excellent results all through the program.

8. Tape Filtering System

The filtration system shown in Figure 26 is an important subsystem in the APPI's processing system. The filter tape advance is a programmed function and occurs as a step in the processing sequence. It cannot occur during an opening or closing of the tape clamp motion. A takeup motor advances the tape 1.75 inches as determined by the circumference of the takeup drum sensing disc. A reed switch is rotated as part of this disc, which disconnects the power from the motor at precisely the right instant. This is necessary for indexing the dried spots under the dryer and detector. Overtravel is negligible and less than 0.050 inches. The motor used for takeup is a Globe Industries 7 RPM Model 319. Figure 27 shows the complete assembly with the tape clamp under the feed spool at the left end, drier and fan in the center, and the detector under the takeup spool at the extreme right end.

a. Filter Clamp

Each of the samples from the dark and light chambers must be filtered to remove surplus sample liquid. The filter tape must be advanced between filtration cycles to provide the new tape for each assay.



Title	Filtration System Detailed Drawing
Fig.	26
	INCORPORATED
Date	Jan'71
Cont. No.	AT(30-1) 3993
Report Title	Phase II Report

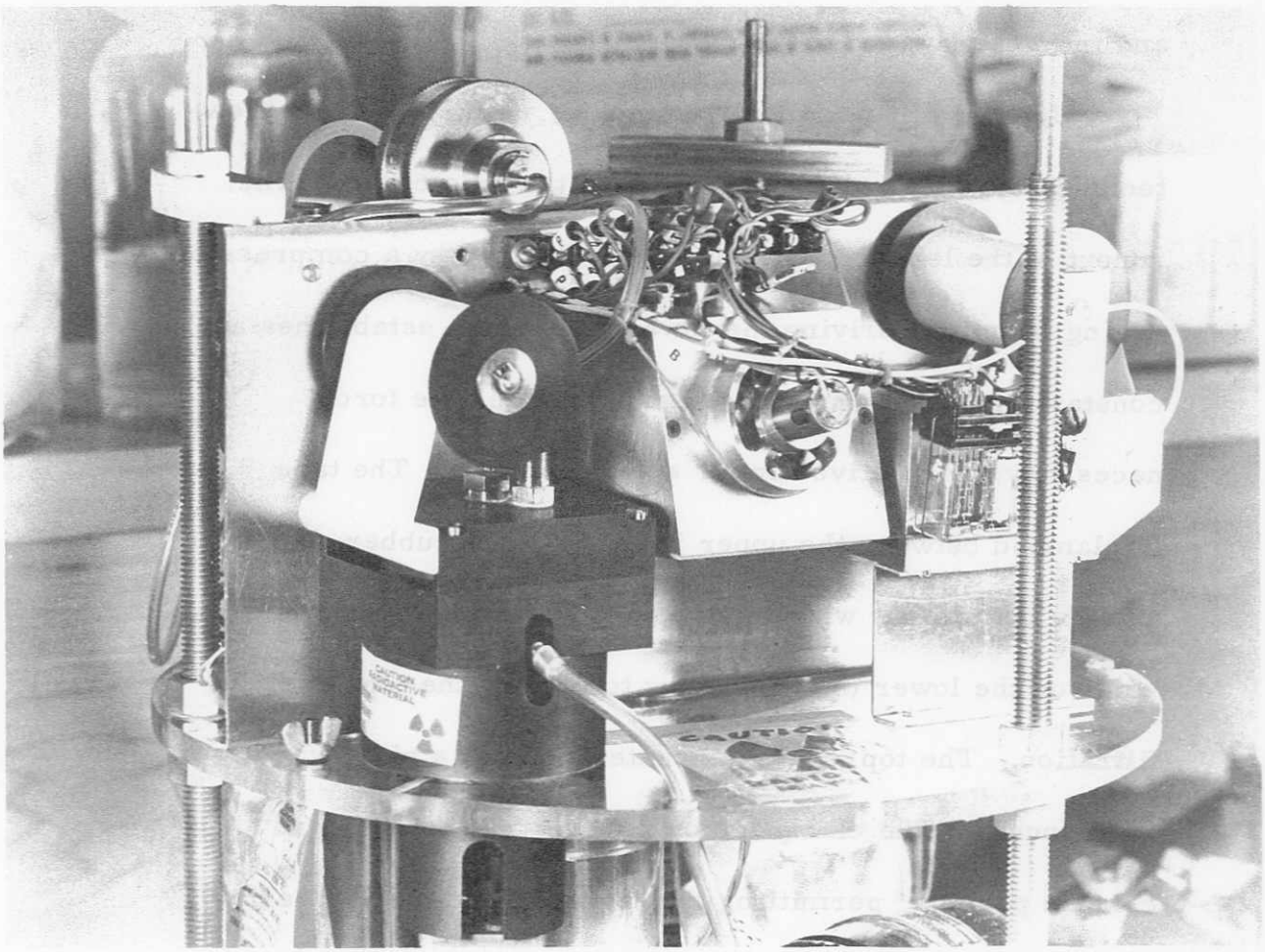


Figure 27

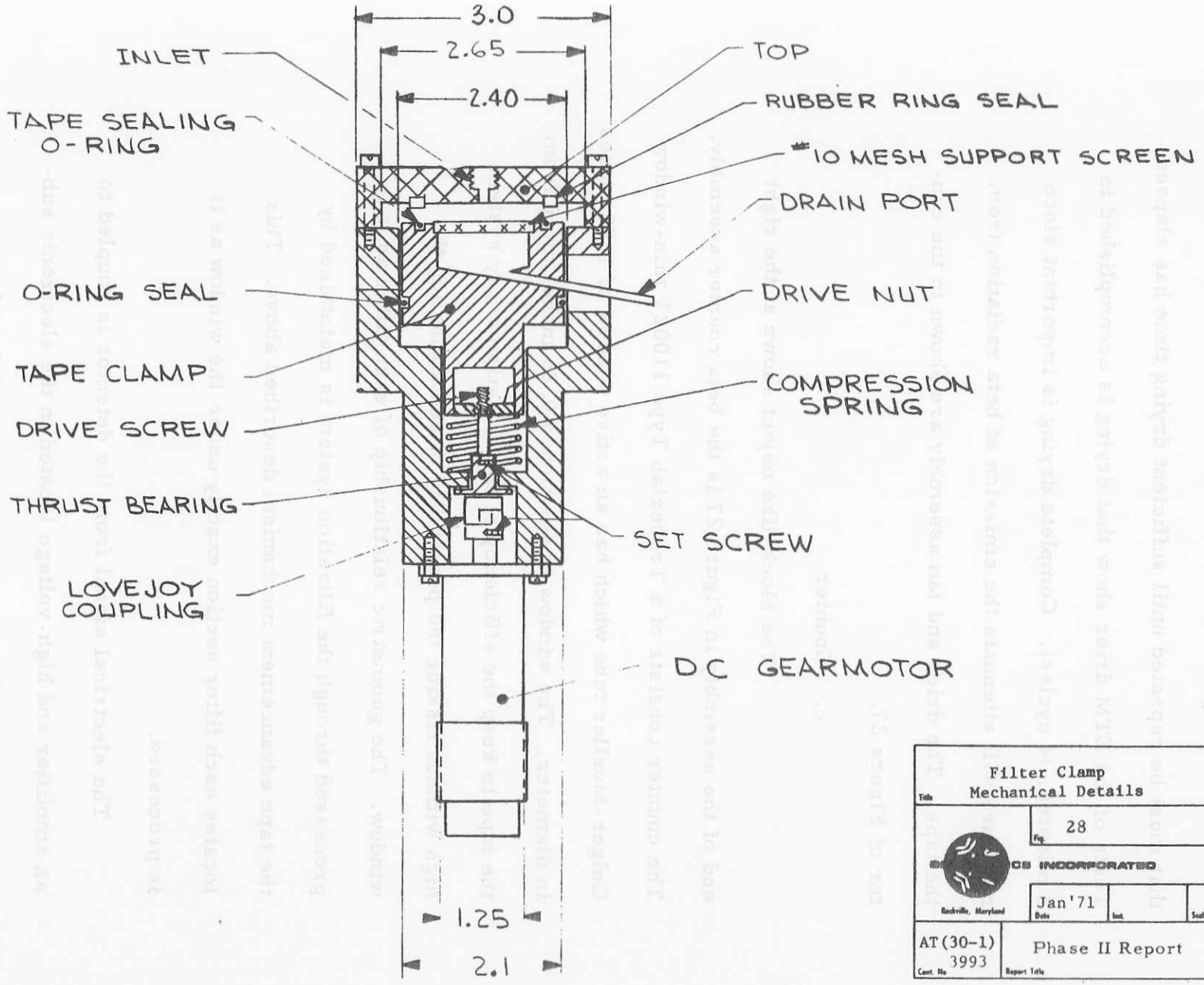
Tape Filtration System

The filter clamp assembly (Figure 28) includes a spool of filter material which is fed through the filter clamp to a takeup spool. The filter clamp is comprised of a stationary and movable jaw and drive motor.

Upon command, the drive motor, using the lead-screw technique, opens or closes the jaws of the tape clamp. The runout of the lead-screw is further enhanced by a compression spring coupling, driving the lower jaw. This establishes a constant pressure when closed and provides the force necessary for positive thread reengagement. The tape is clamped between the upper jaw with a flat rubber gasket and the bottom jaw with an O-ring. A backing screen is provided on the lower (movable) jaw to protect the tape during filtration. The top jaw is connected through a pinch valve to the pump. The clamp motor is actuated for 7-second closure periods, permitting the inclusion of an extra period to be in the program for secure closure. A 2 inch wide tape, Gelman "Acropor" of 0.45 u pore size, is used.

b. Drier

Drying of the filter tape after the filtration cycle is accomplished by a heater which blows hot air across the freshly filtered section. Heat is provided by a GE Type 1143, 12-volt, 36 watt lamp and the hot air is circulated with a Micro-mo motor driven fan which is mounted over the bulb.



Title: Filter Clamp Mechanical Details	
Fig. 28	
CS INCORPORATED, Rockville, Maryland	
Date: Jan '71	Inst. / Scale
Cont. No: AT(30-1) 3993	Report Title: Phase II Report

The drier is programmed to operate for 2-minute cycles that must be repeated until sufficient drying time has elapsed. Tests of the ETM drier show that drying is accomplished in 8 minutes, (4 cycles). Complete drying is important since moisture will attenuate the emission of beta radiation from the tape. The drier and fan assembly are shown in the center of Figure 27.

c. Counter

The block-like object shown at the right end of the assembly in Figure 27 is the beta counter assembly. The counter consists of a Tracerlab Type 11002T thin-window Geiger-Mueller tube which has an active window of 1.75 inches in diameter. The window is positioned 0.25 inches away from the tape to keep the efficiency of the radiation assay system high without risking the possibility of contaminating the window. The geometric relationship of each filter as it is processed through the filtration system is maintained by the tape advancement mechanism described above. This locates each filter section exactly under the window as it is processed.

The electrical signal from the detector is coupled to an amplifier and high-voltage located in the electronic subsystem, returned through a common coaxial cable.

9. Waste Tank

The proper handling of radioactive waste from

the processing of the filters in the APPI was a serious problem in the development of the instrument. Although these wastes are diluted and do not pose a serious radioactive hazard, the uncontrolled disposal is not favored by most cognizant authorities except, perhaps, in the open seas. Since it is impractical to store the waste water in the open hull, a separate waste tank was provided within the hull. This tank, shown in Figure 29, is fabricated of mild steel with a capacity of 6000 ml, sufficient for more than 20 operational cycles. The interior plumbing of the tank is arranged so that the exhaust port removes only air from the very top of the tank and the inlet port discharges fluid near the bottom. This plumbing arrangement allows the easy removal of wastes from the tank during servicing simply by pressurizing the exhaust port and siphoning the fluid out of the tank. In normal operation, the exhaust port is connected to the vacuum pump through the CO_2 scrubber system.

10. Vacuum Pump

Filtration of turbid samples posed a serious problem until a means of lowering the pressure at the outlet from the filtration system was developed. A search for a vacuum pump which would operate on the limited 12 volts DC power failed to uncover a suitable unit, so the pump

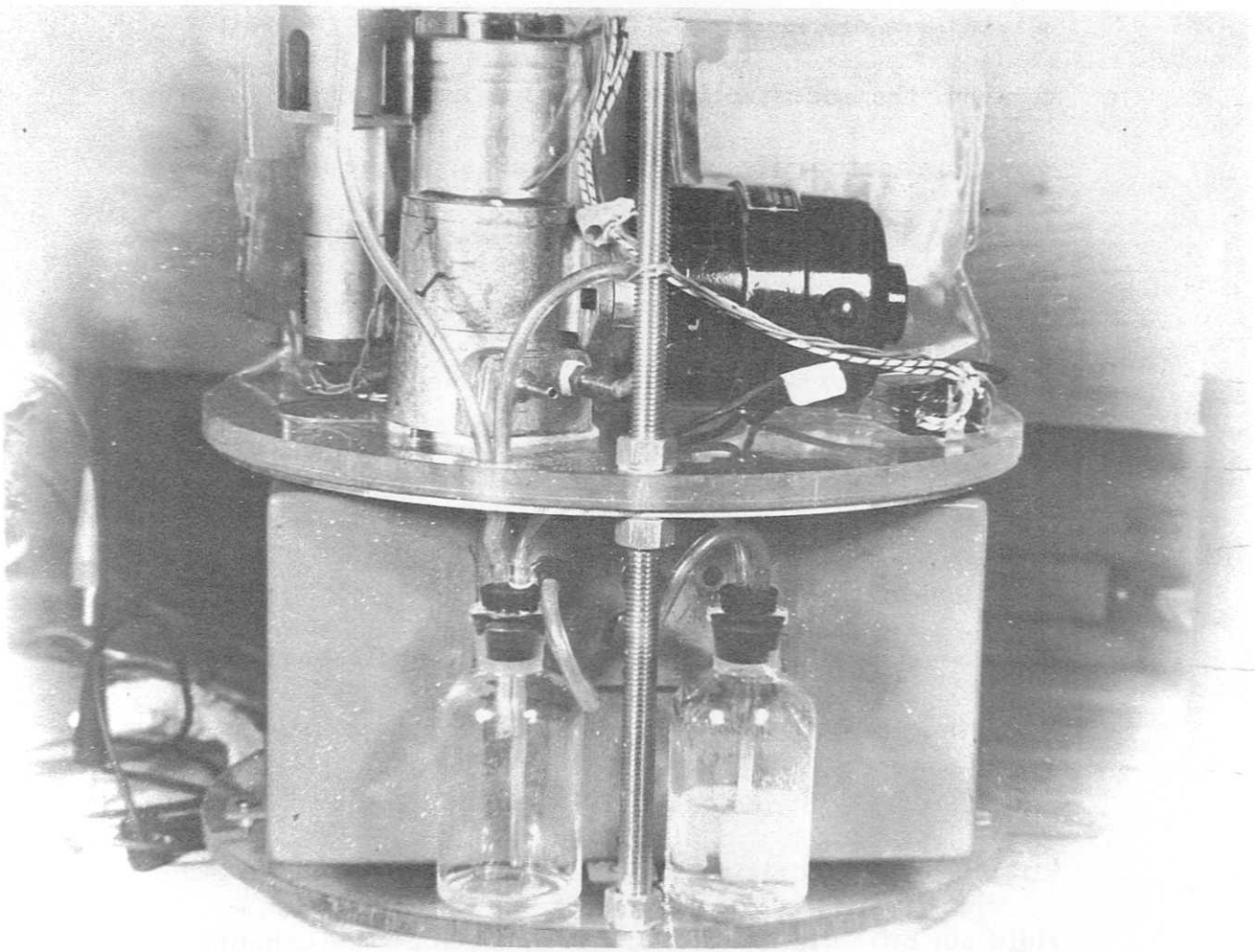


Figure 29

Waste Handling System

shown in Figure 29 was fabricated from available components. This pump can maintain a static vacuum of about -5 psig which is a sufficient pressure for the filtration application.

In addition to providing assistance in the filtration, the vacuum pump is also used to remove residual water from the filters while the filter clamp is being opened and for two minutes before the tape is advanced. This is to assure that excess liquid from the filters is completely removed.

11. CO_2 Scrubber

During the tests at the Occoquan Reservoir, an excessive background count developed, increasing with each cycle, until it finally reached a value where it was significant in comparison with the data. Upon returning the APPI to the laboratory, the hull was evacuated through a filter that was saturated with barium hydroxide which acted as a CO_2 getter. After the evacuation, the filter was dried and counted and found to be highly radioactive, indicating that the air in the hull contained an excessive quantity of $^{14}CO_2$. This $^{14}CO_2$ had been adsorbed on the APPI filters, and was included with the data counts as background. The hull was allowed to ventilate and, within an hour, the background count had returned to a normal state.

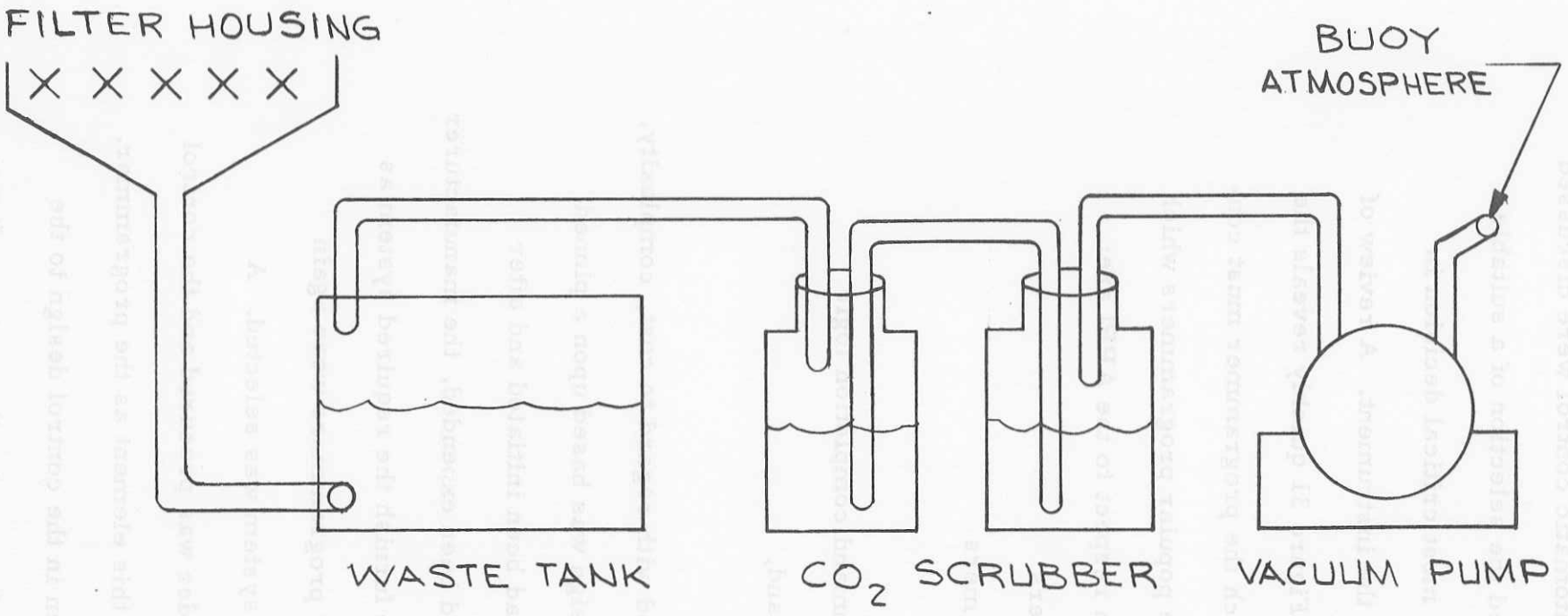
The CO_2 scrubber shown in Figures 29 and 30 was designed to adsorb the $^{14}CO_2$ as it is released by the waste tank, but before it can enter the hull cavity. This apparatus consists of two 100-ml stoppered bottles which contain LiOH in a saturated solution. The waste tank air is bubbled through these, thereby removing most of the CO_2 which will remain in the solution. The double bottles arrangement is required to prevent autosiphoning and prevent the LiOH from flowing back into the waste tank.

12. Electronic Cannister

Since the APPI is a feasibility model and the components being used are not designed for adverse environmental conditions, the electronic circuitry was packaged in a sealed cannister. The cannister is approximately the same diameter as the buoy hull within which it is housed and is fabricated of 1/8 inch cold rolled steel, with a welded bottom cover and a sealing flange on the top. The top cover is sealed with a cork gasket and fastened with socket head screws. The top cover also serves as the mounting surface to couple the unit electrically and mechanically to the APPI mechanism.

D. The Electrical Design

The electrical design of the APPI was predicated upon the series of functions which had been established by the biological program.



Title: Waste Processing Flow Diagram	
Fig. 30	
BCS INCORPORATED, Rockville, Maryland	
Date: Jan'71	Init. / Scale
Cont. No.: AT(30-1) 3993	Report Title: Phase II Report

The requirements for automatic control were discussed in Section IV-B which identified the selection of a suitable programming technique as the most critical decision in the successful development of the instrument. A review of the detailed block diagram in Figure 31 quickly reveals the complexity of the process which the programmer must control in the APPI. Some of the popular programmers which offer interesting features with respect to the APPI are:

a. Multi-cam timers

b. Pinned drum timers

c. Digital clocks

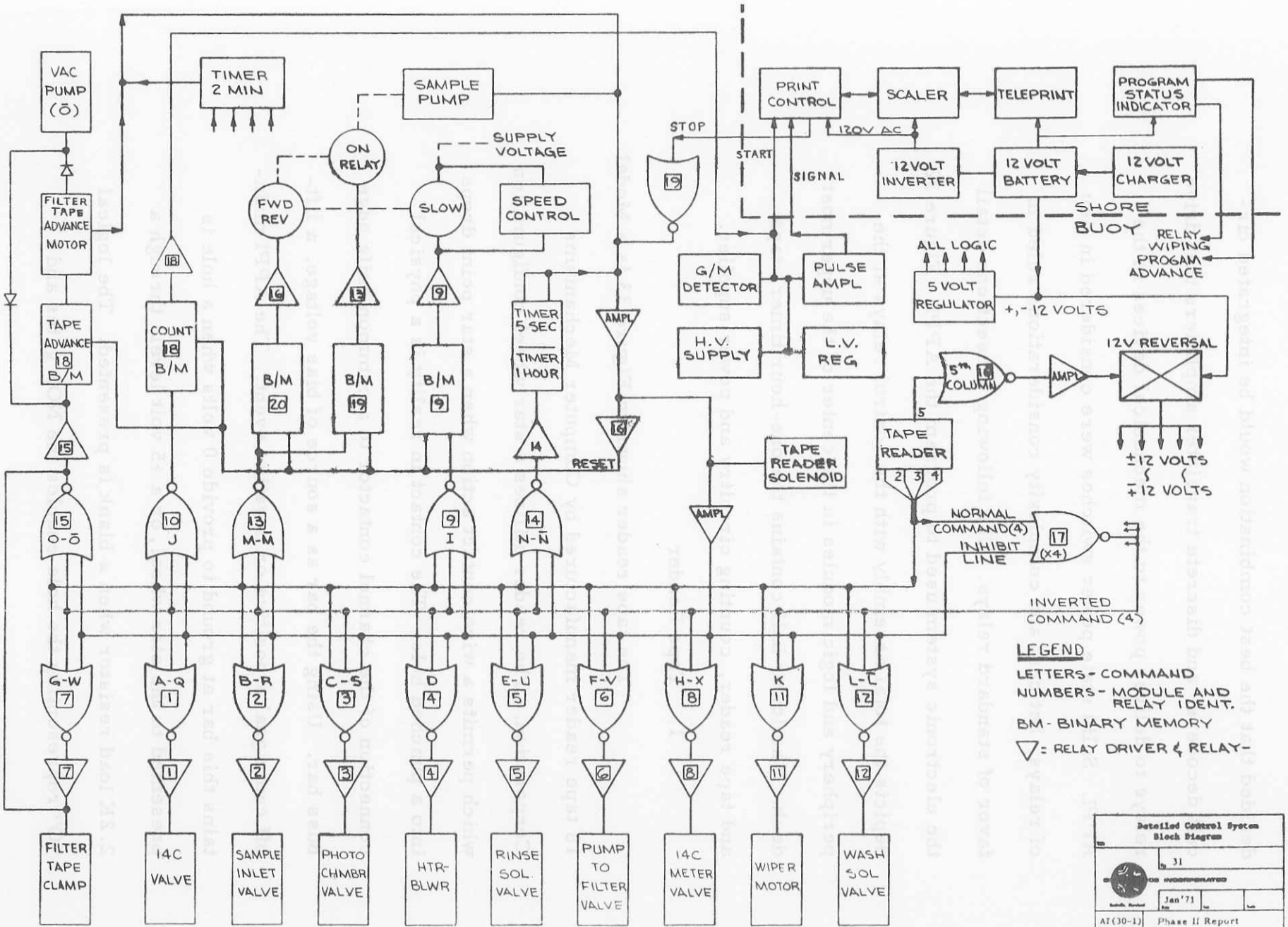
d. Command-command completion logic

e. Magnetic tape and,

f. Punched tape

Each of these were considered with regard to cost, complexity, and reliability. The first design was based upon a pinned drum timer. Procurement had been initiated and after considerable design effort had been expended, the manufacturer advised that he was unable to furnish the required system as originally quoted. The other programmers were again reviewed and a punched tape system was selected. A miniature 8 channel tape reader was procured and the control system was designed around this element as the programmer.

Consideration was given in the control design to the various circuit elements which comprise the system. It was


VAC PUMP (Ō)
TIMER 2 MIN
SAMPLE PUMP
PRINT CONTROL
SCALER
TELEPRINT
PROGRAM STATUS INDICATOR
STOP
START
SIGNAL
120V AC
12 VOLT INVERTER
12 VOLT BATTERY
12 VOLT CHARGER
SHORE
BUOY
RELAY WIPING PROGAM ADVANCE
ON RELAY
SUPPLY VOLTAGE
FWD REV
SLOW
SPEED CONTROL
FILTER TAPE ADVANCE MOTOR
TAPE ADVANCE 18 B/M
COUNT 18 B/M
B/M 20
B/M 19
B/M 9
TIMER 5 SEC
TIMER 1 HOUR
AMPL
RESET
G/M DETECTOR
PULSE AMPL
H.V. SUPPLY
L.V. REG
ALL LOGIC
5 VOLT REGULATOR
+,- 12 VOLTS
12V REVERSAL
5TH COLUMN
TAPE READER
TAPE READER SOLENOID
±12 VOLTS
∓12 VOLTS
NORMAL COMMAND(4)
INHIBIT LINE
17 (X4)
INVERTED COMMAND (4)
0-Ō
J
M-M̄
I
N-N̄
G-W
A-Q
B-R
C-S
D
E-U
F-V
H-X
K
L-L̄
FILTER TAPE CLAMP
14C VALVE
SAMPLE INLET VALVE
PHOTO CHMBR VALVE
HTR-BLWR
RINSE SOL. VALVE
PUMP TO FILTER VALVE
14C METER VALVE
WIPER MOTOR
WASH SOL VALVE
LEGEND
LETTERS - COMMAND
NUMBERS - MODULE AND RELAY IDENT.
BM - BINARY MEMORY
▽ = RELAY DRIVER & RELAY
Detailed Control System Block Diagram
Fig. 31
Jan '71
AT(30-1) 3993
Phase II Report

decided that the best combination would be integrated circuit decoders, and discrete transister amplifiers that drive relays to deliver power to the mechanical devices in the APPI. Solid state power switches were considered in lieu of relays, but cost and complexity considerations ruled in favor of standard relays. The following subsections detail the electronic system used to program the APPI. Figure 32 depicts the total assembly with the control relays at the periphery and logic modules in the center of the uppermost deck. The lower half contains the one-hour timer, tape and tape reader, counting circuitry and power supplies.

1. Tape Reader

The tape reader shown in Figure 33 is a Model 18 tape reader manufactured by Computer Mechanisms Corporation. The reader utilizes a star wheel configuration which permits a wire contact action when a star point drops into a punched hole. The contact in reality is a physical connection of the channel contactor to a common knife edge buss bar. Using the bar as a source of bias voltage, a lift-off can signal a positive or negative event. The APPI maintains this bar at ground to provide 0 volts when a hole is presented to the star wheel, or a +5 volt level, through a 2.2K load resistor when a blank is presented. The logical "O" representing the hole enables the NOR gates and

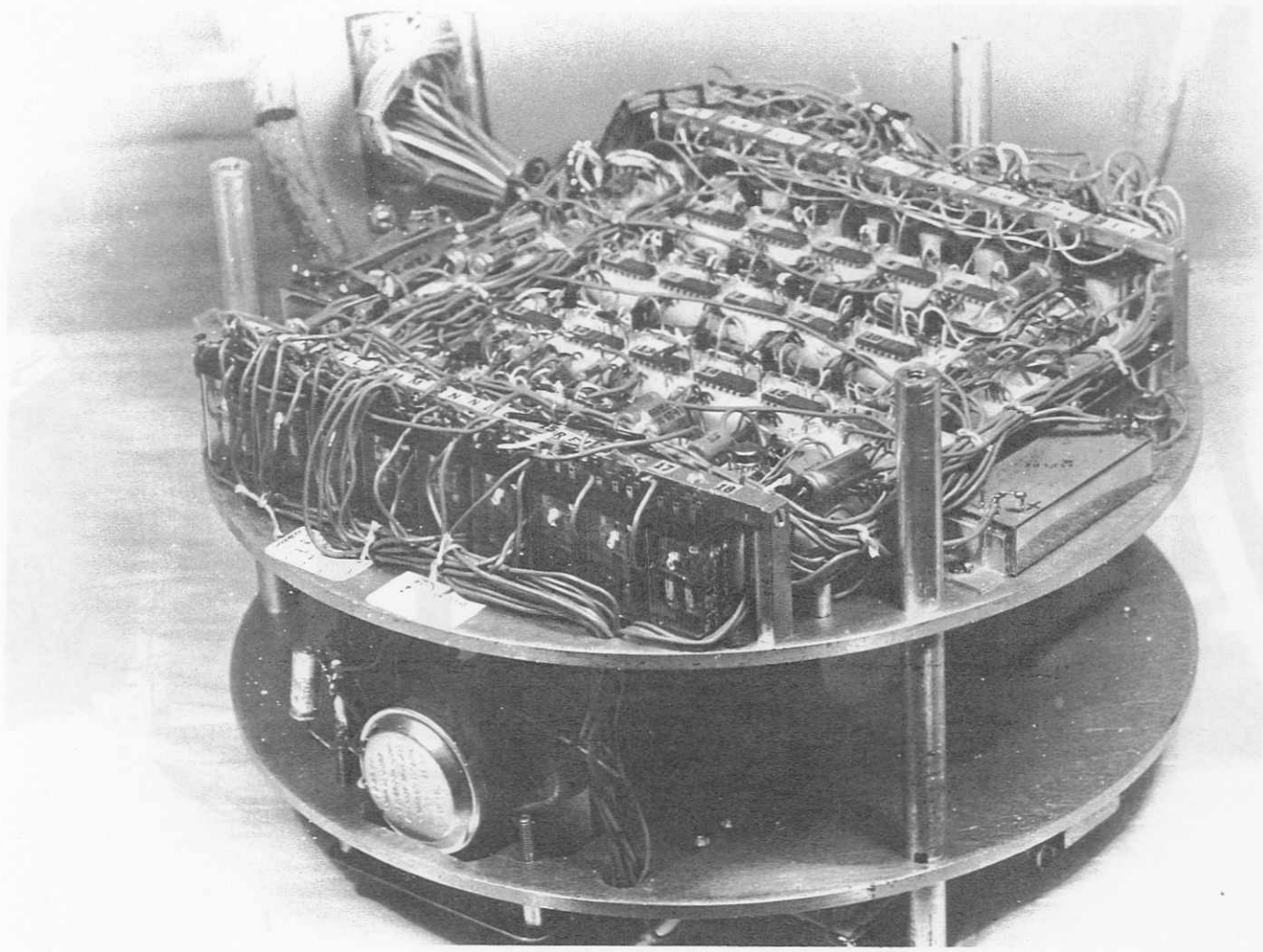


Figure 32

APPI Programming Electronics

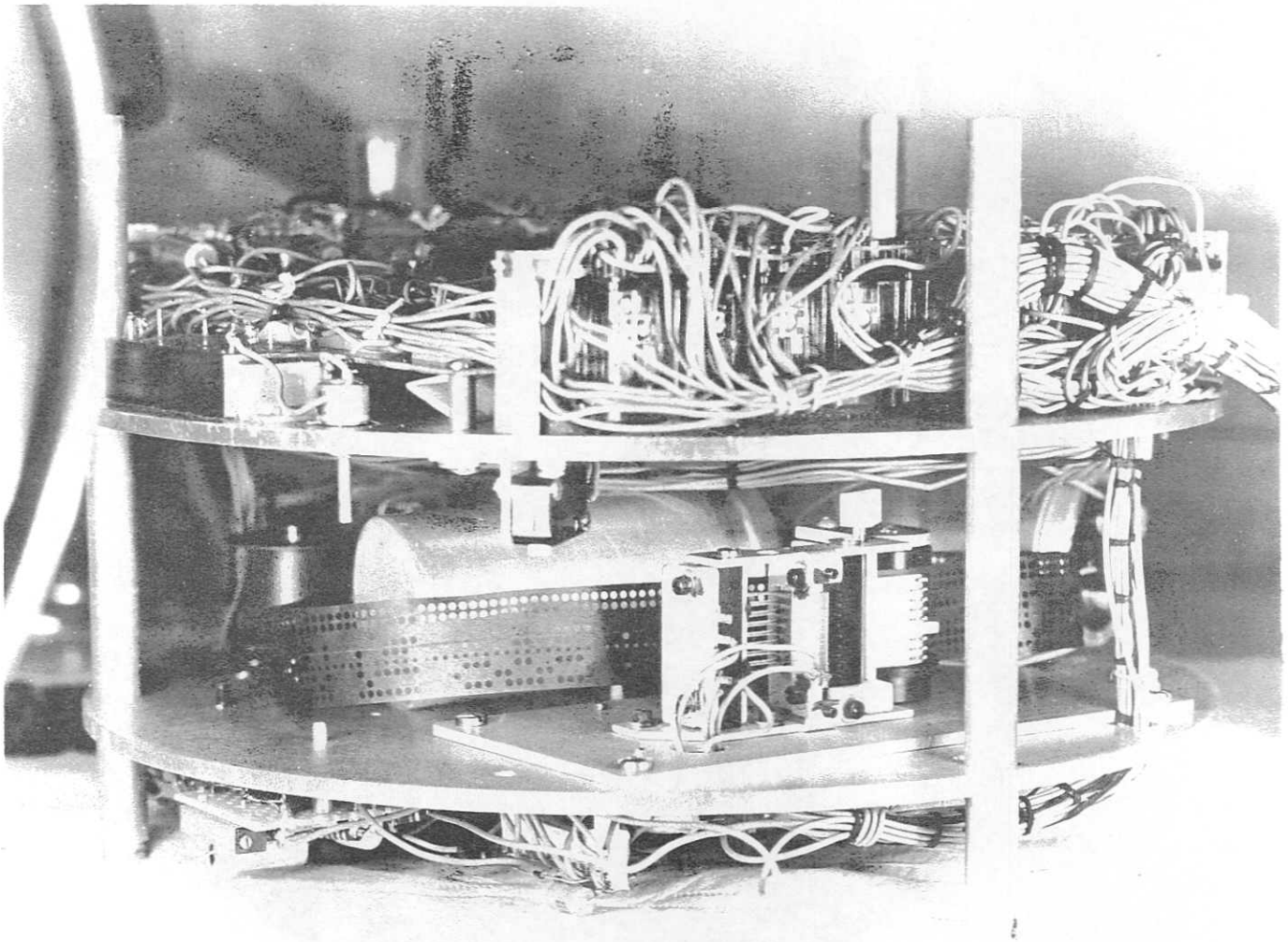


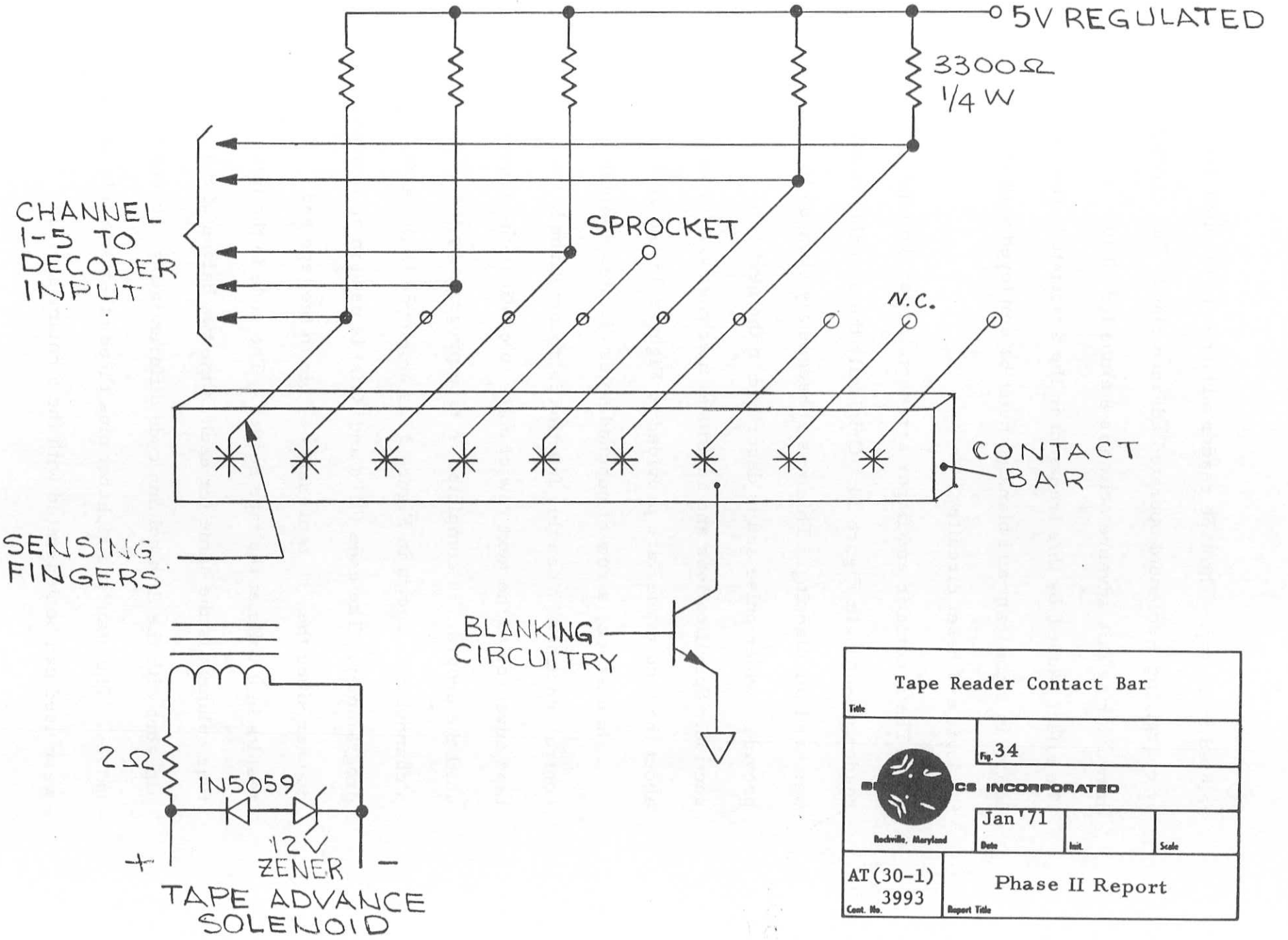
Figure 33

Program Tape Reader

related circuitry. The tape reader advances to the next row by energizing a solenoid operated advance claw. The current drawn during this advance action is 8 amps for 50 ms. The effect caused by this transient in the associated circuitry is significant and blanking must be employed with all the logic and reset circuitry.

The schematic and finger arrangement for the tape reader is shown in Figure 34. Originally the sprocket contact was used for blanking. This was subsequently changed to provide a wider pulse and is described in the next subsection. Specifications and additional mechanical information about the tape reader are provided in Figures 35 and 36.

In essence, a row of punched holes dictates a single control function. When this function is accomplished, the tape advances to the next row of holes to continue the sequence until the program is complete. A tape program by the USASCII 8 channel code shown in Figure 37 is employed in the APPI tape programmer. The code ("1"s and "O"s) is used in an inverted manner since the "1" represents a hole in the tape and results in 0 volts at the tape reader. The code in the first four columns is the same for each of the two halves of the alphabet with the fifth column code differentiating between the halves. The standard alphabet plus three other symbols which were used can be generated with the 5 columns. The



Tape Reader Contact Bar (Title)			
	Fig. 34		
CS INCORPORATED, Rockville, Maryland			
	Jan '71 (Date)	Init.	Scale
AT(30-1) 3993 (Cont. No.)	Phase II Report (Report Title)		

Solenoid Drive
Approximately 14 watts of power is required to operate the drive coil at rated voltages. Standard readers are available for 12, 24 and 48 volt D.C. operation. Readers for operation on other voltages are available upon special request.

Operate Time
The time required to operate the armature is approximately 15 milliseconds at rated power input. Coil can be overdriven to increase speed of operation.

Release Time
Release time is a function of the suppression circuit used. When a diode-zener series combination (with zener voltage ½ of drive voltage) is placed across the drive coil, release time is approximately 15 milliseconds. For simple diode suppression, release time is approximately 25 milliseconds.

Reading Rate
0 to 30 characters per second, asynchronously, when operated at rated power with appropriate suppression circuit. Speed is a function of solenoid operate and release times.

Reading Direction
Tape transport is unidirectional, from right to left.

Tape
Reader reads all standard perforated 5, 6, 7, or 8 channel tapes. All grades of paper or plastic tapes can be read. Readers capable of reading typesetter "advanced feed" tape (used in the printing industry) are available upon special request.

Starwheel Sensing Contacts
8 starwheels, sensing 8 tape channels, actuate 8 corresponding contacts. Each sensing contact consists of 2 silver contact wires and 1 stainless steel pressure wire. Contact wires close to a common plate. Contacts can switch .100 amperes, resistive load, for a life of 100 million cycles, and can carry steady state currents of 3 amperes.

Tape Loading Contact
Contact open during tape loading, closed when starwheels are in reading position.

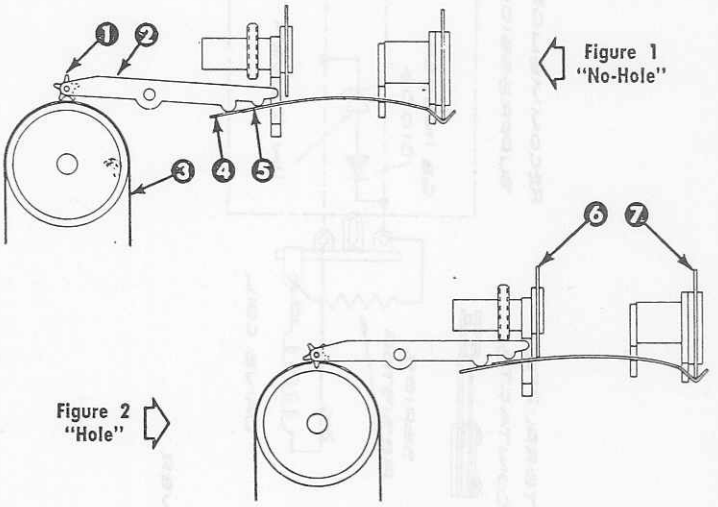
Interrupter Contacts
Normally closed at rest, contacts open when armature is pulled in, re-close during latter part of feed stroke. Contact material is tungsten. Contacts can switch 1 ampere, resistive load. Life exceeds 100 million cycles.

Mechanical Life
Exceeds 100 million cycles under normal environmental conditions.

Size
Panel size is ⅛" thick by 3½" high by 7" wide. Front of panel clearance is 2¼". Rear of panel clearance is 1½".

Weight
1½ lbs.

STARWHEEL SENSING



Starwheels are used to sense the holes in the tape. The sensing action is illustrated in Figures 1 and 2.

In Figure 1, the starwheel (1) is held against the tape (3) by the sensing arm lever (2) under the urging of the wire contacts (5) and the pressure wire (4). As the tape is advanced, the starwheel slides along the surface of the tape on two of its five points.

When a hole in the tape is encountered, the starwheel rolls into the hole and the resulting motion allows the wire contacts to close against the common plate (6) as shown in Figure 2. Electrical connection is thus established between the common plate and terminal pin (7).

A unique feature of starwheel sensing is that in the case of consecutive holes in the tape, the starwheel actuated contact remains closed and can be used as a holding circuit.

Starwheel sensing is easy on the tape. Paper tape program loops can be passed through the CMC Model 18 TAPE READER thousands of times.

TAPE DRIVE

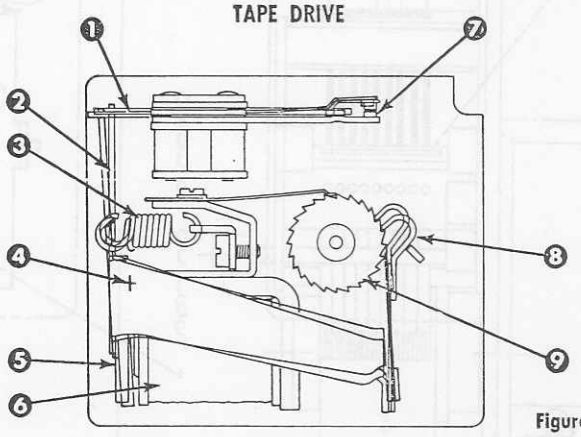


Figure 3

The method of feeding the tape is illustrated in Figure 3. When drive coil (6) is energized, armature (5) is attracted and moves about pivot point (4) to the position shown in colored lines, causing pawl (8) to engage the next tooth on ratchet (9). When the drive coil is de-energized, spring (3) returns the armature and steps the ratchet. The ratchet is fixed to the tape sprocket shaft.

An extension (2) of the armature operates a slider (1) which opens the interrupter contacts (7) when the drive coil is energized. By connecting the interrupter contacts in series with the drive coil, the device can be caused to self-step continuously. The interrupter switch also provides an interlock signal to connected equipment.

An important feature of this type of tape drive is that the tape is advanced by the spring-urged return stroke of the armature. The circuitry and control logic is considerably simplified. For example, if a command to step the tape is initiated by a character being sensed, that command is not wiped out by the energization of the drive coil.

Figure 35

Computer Mechanisms Corporation Model No. 18 Tape Reader

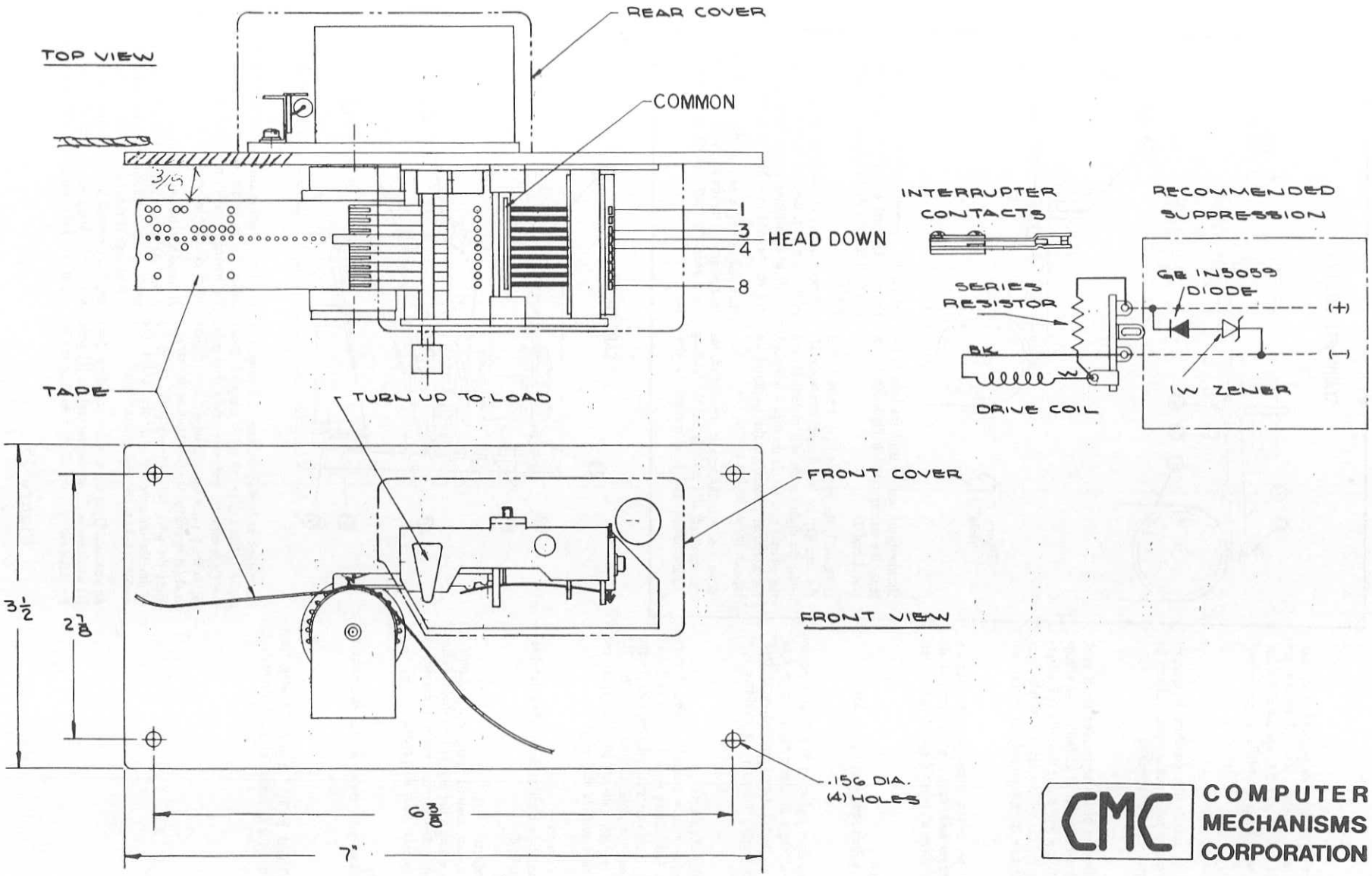


Figure 36

Computer Mechanisms Corporation Model No. 18 Tape Reader

BIT NUMBERS →								$0\,0\,0$	$0\,0\,1$	$0\,1\,0$	$0\,1\,1$	$1\,0\,0$	$1\,0\,1$	$1\,1\,0$	$1\,1\,1$
b_7	b_6	b_5	b_4	b_3	b_2	b_1	COLUMN → / ROW ↓	0	1	2	3	4	5	6	7
			0	0	0	0	0	NUL	DLE	SP	0	@	P	`	p
			0	0	0	1	1	SOH	DC1	!	1	A	Q	a	q
			0	0	1	0	2	STX	DC2	"	2	B	R	b	r
			0	0	1	1	3	ETX	DC3	#	3	C	S	c	s
			0	1	0	0	4	EOT	DC4	$	4	D	T	d	t
			0	1	0	1	5	ENQ	NAK	%	5	E	U	e	u
			0	1	1	0	6	ACK	SYN	&	6	F	V	f	v
			0	1	1	1	7	BEL	ETB	′	7	G	W	g	w
			1	0	0	0	8	BS	CAN	(	8	H	X	h	x
			1	0	0	1	9	HT	EM	)	9	I	Y	i	y
			1	0	1	0	10	LF	SUB	*	:	J	Z	j	z
			1	0	1	1	11	VT	ESC	+	;	K	[	k	{
			1	1	0	0	12	FF	FS	,	<	L	\	l	|
			1	1	0	1	13	CR	GS	−	=	M	]	m	}
			1	1	1	0	14	SO	RS	.	>	N	^	n	~
			1	1	1	1	15	SI	US	/	?	O	_	o	DEL

NUL	Null, or all zeros	DC1	Device control 1
SOH	Start of heading	DC2	Device control 2
STX	Start of text	DC3	Device control 3
ETX	End of text	DC4	Device control 4
EOT	End of transmission	NAK	Negative acknowledge
ENQ	Enquiry	SYN	Synchronous idle
ACK	Acknowledge	ETB	End of transmission block
BEL	Bell, or alarm	CAN	Cancel
BS	Backspace	EM	End of medium
HT	Horizontal tabulation	SUB	Substitute
LF	Line feed	ESC	Escape
VT	Vertical tabulation	FS	File separator
FF	Form feed	GS	Group separator
CR	Carriage return	RS	Record separator
SO	Shift out	US	Unit separator
SI	Shift in	SP	Space
DLE	Data link escape	DEL	Delete

Title: U. S. ASCII Code

Fig. 37

CS INCORPORATED

Rockville, Maryland

Date: Jan'71 | Init. | Scale

Cont. No.: AT(30-1)-3993

Report Title: Phase II Report

mechanical functions to be performed in the APPI required a reverse polarity mode in 9 operations. This suggested that the fifth channel be used as a reversing mode for each of the commands. This constitutes the basic rules which were used for the design of the programming subsystem.

Most of the commands are operated for a preset time interval which is controlled by single or repeated action by a 7-second, 2-minute or one-hour timer. The balance of the commands generates a tape advance command at their completion which disables the command, blanks the control until a new command is preset.

2. Decoder (Blanking)

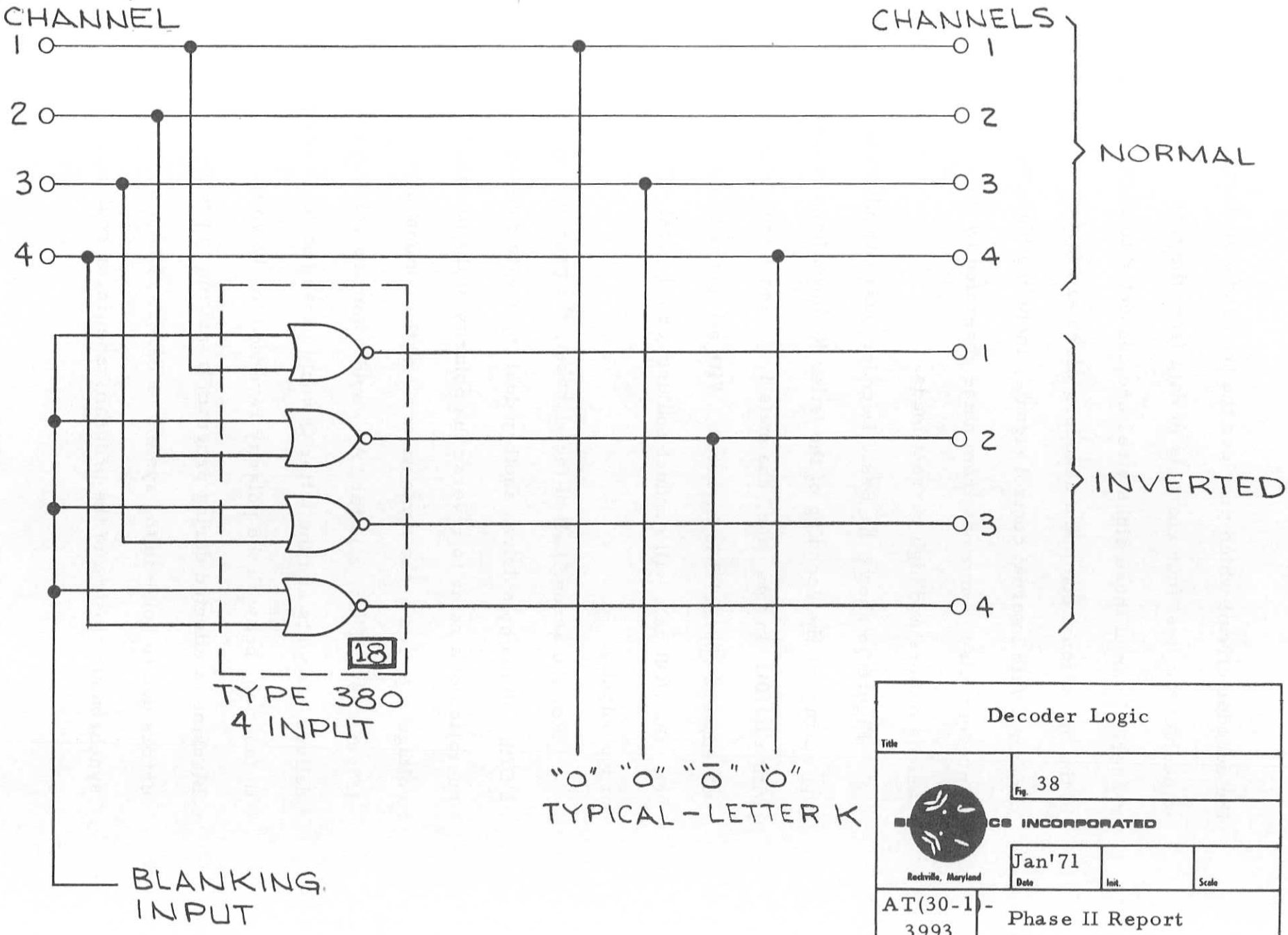
In all of the APPI decoding circuitry, Signetics Type 314A and 380A integrated circuit logic modules are used. Both of these integrated circuits are NOR gates with the 314A being a single 7-input gate and the 380A containing four 2-input gates. The NOR logic is used in the "AND" mode and therefore requires that all inputs be "O" for a "1" output. Any other combination will result in an "O" output.

Since each operational code is a combination of "O"s and "1"s, the data must be inverted so that the desired code can be processed with all "O"s. This is accomplished by

the decoder circuit which provides the true and complement of each of the first four channels of data from the tape reader. Four of these eight wires are connected to each of the operational command decoders which are further combined with internal control signals, including blanking to prevent false commands from noise generated by the various motors and high current loads.

Figure 38 shows the Basic Decoder Buss Circuit with the wiring for the decoding of the letter K shown (tape reader with a 0010). In this case, channels 1, 2, and 4 are not inverted, and channel 3 is inverted. With all inputs being "O", the NOR gate will conduct and deliver a logical "1" at its output.

For the second half of the alphabet, the presence of a fifth column hole drives another dual input gate causing operation of a relay to reverse the polarity of the primary voltage supplied to the contacts of all of the command relays (Figure 39). Thus, a letter "A" would command relay A to deliver +12 volts and the letter Q would cause the same relay to function, but with the polarity reversed to -12 volts. Blanking is effected during program transition to prevent errors due to noise in the system or errors caused by non-synchronous closure of the program tape finger contacts.



Title	Decoder Logic
Fig.	38
	CS INCORPORATED, Rockville, Maryland
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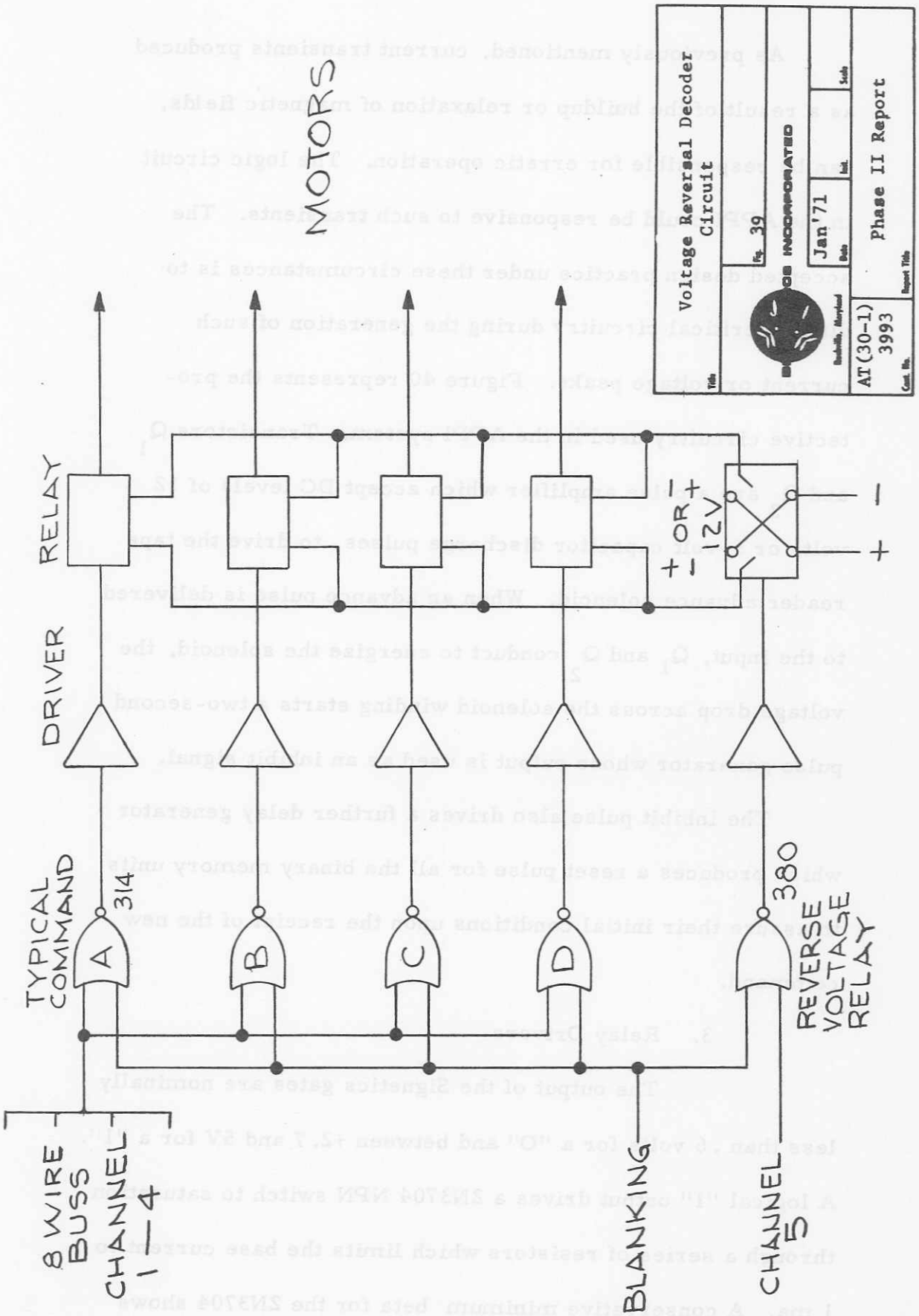
Scale	
Cont. No.	AT(30-1)-3993
Report Title	Phase II Report

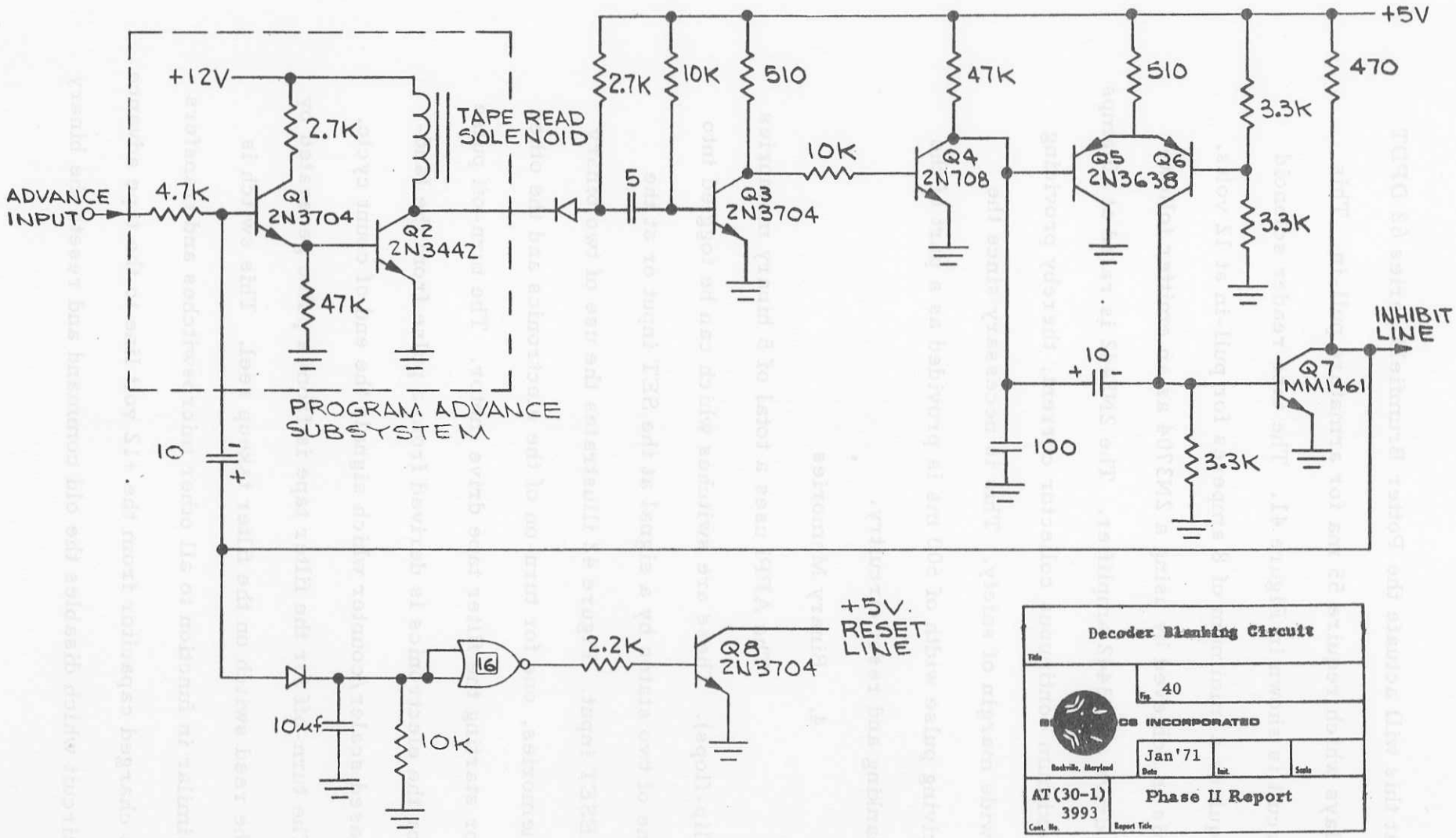

MOTORS
RELAY
DRIVER
TYPICAL COMMAND
A
B
C
D
314
380
8 WIRE BUSS CHANNEL 1—4
BLANKING
CHANNEL 5
REVERSE VOLTAGE RELAY
+ OR − 12V
− +
Voltage Reversal Decoder Circuit
Fig. 39
INCORPORATED
Jan'71
Phase II Report
AT(30-1) 3993

As previously mentioned, current transients produced as a result of the buildup or relaxation of magnetic fields, can be responsible for erratic operation. The logic circuit in the APPI could be responsive to such transients. The accepted design practice under these circumstances is to disable critical circuitry during the generation of such current or voltage peaks. Figure 40 represents the protective circuitry used in the APPI system. Transistors Q_1 and Q_2 are a pulse amplifier which accept DC levels of 12 volts or 5-volt capacitor discharge pulses, to drive the tape reader advance solenoid. When an advance pulse is delivered to the input, Q_1 and Q_2 conduct to energize the solenoid, the voltage drop across the solenoid winding starts a two-second pulse generator whose output is used as an inhibit signal.

The inhibit pulse also drives a further delay generator which produces a reset pulse for all the binary memory units to assure their initial conditions upon the receipt of the new command.

3. Relay Drivers

The output of the Signetics gates are nominally less than .6 volts for a "O" and between +2.7 and 5V for a "1". A logical "1" output drives a 2N3704 NPN switch to saturation through a series of resistors which limits the base current to 1 ma. A conservative minimum beta for the 2N3704 shows

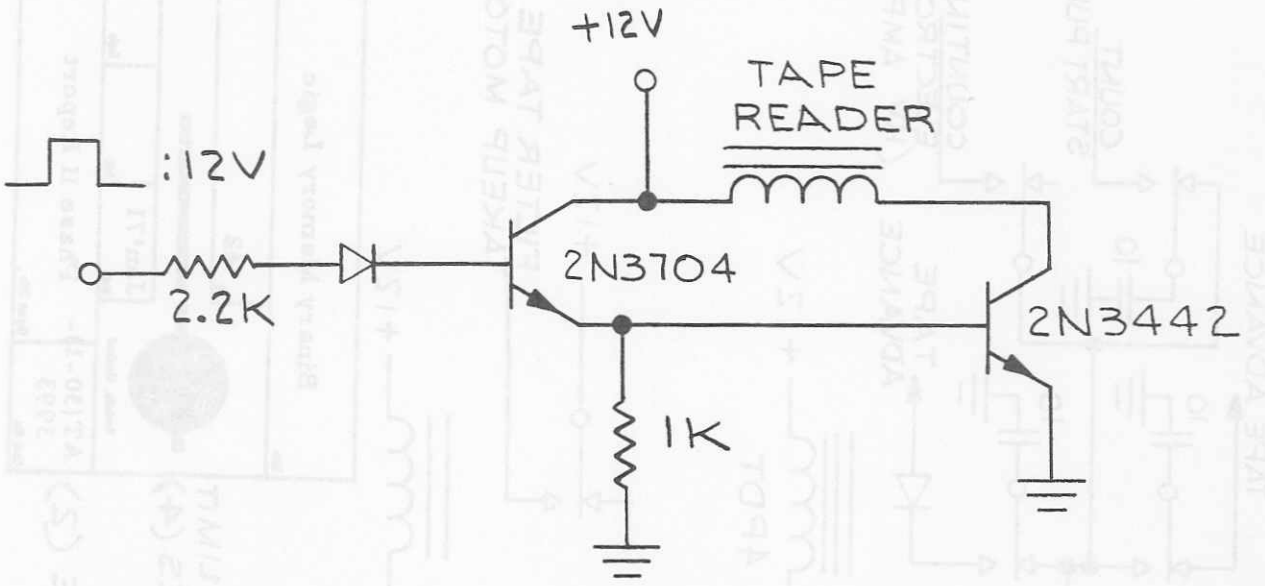


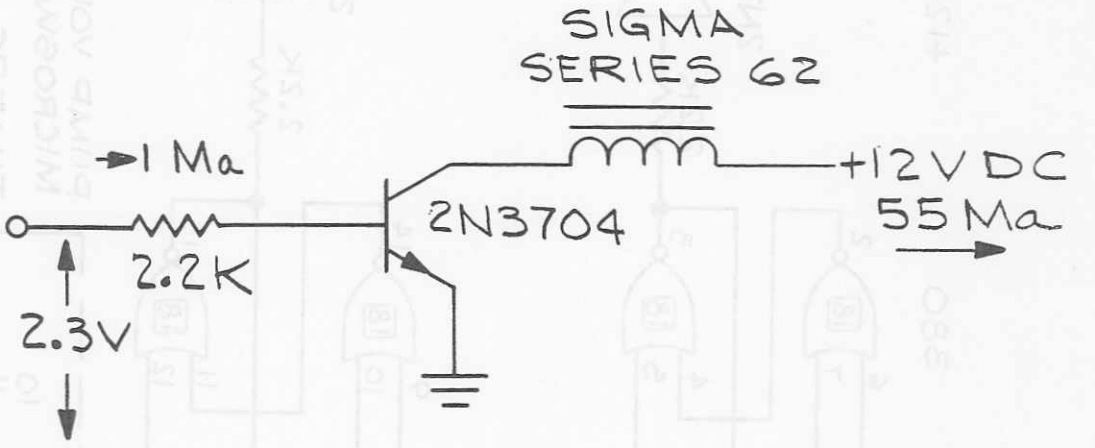
Title	Decoder Blanking Circuit	
	Fig. 40	
CS INCORPORATED Rockville, Maryland	Jan'71 (Date)	(Init.) (Scale)
AT(30-1) 3993 (Cont. No.)	Phase II Report (Report Title)	

that this will actuate the Potter Brumfield series 62 DPDT relays which require 55 ma for armature pull-in. This circuit is shown in Figure 41. The tape reader solenoid requires a minimum of 8 amperes for pull-in at 12 volts. This is achieved by using a 2N3704 as an emitter follower to drive a 2N3442 amplifier. The 2N3442 is rated at 10 amps maximum continuous collector current, thereby providing a wide margin of safety. This is necessary since the driving pulse width of 500 ms is provided as a part of the blanking and reset circuitry.

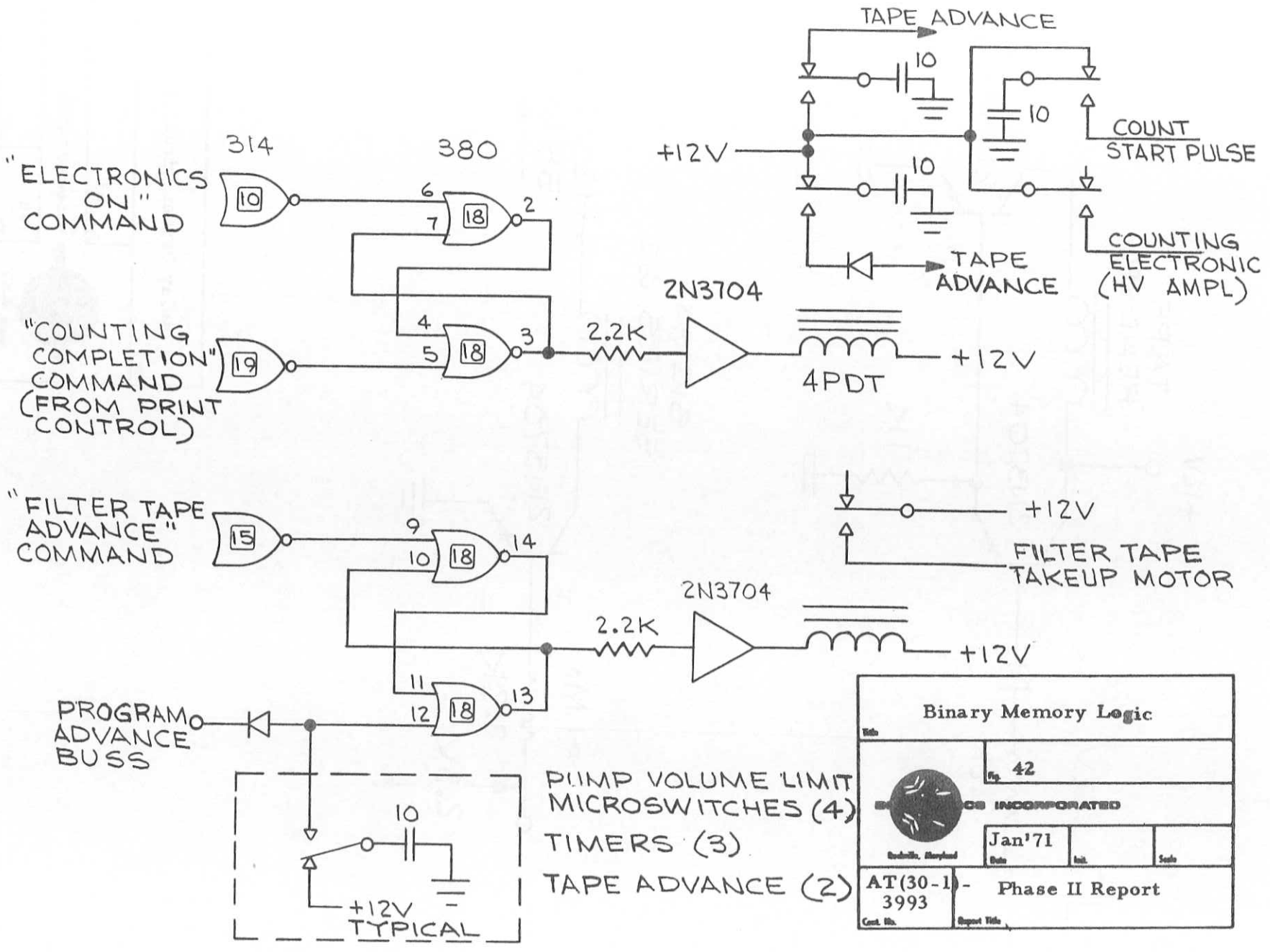
4. Binary Memories

The APPI uses a total of 5 binary memories (flip-flops). These are switches which can be toggled into one of two states by a signal at the SET input or at the RESET input. Figure 42 illustrates the use of two binary memories, one for turn-on of the electronics and the other for starting the filter tape drive motor. The turn-off pulse for the electronics is derived from a pulse from the land-based scaler/counter which signals the end of count cycle. The turn-off for the filter tape is from a pulse generated by the reed switch on the filter takeup reel. This switch is similar in function to all other microswitches and transfers a charged capacitor from the +12 volt line to the tape advance circuit which disables the old command and resets the binary





Relay Driver Circuits (Title)	
	Fig. 41
CS INCORPORATED (Rockville, Maryland)	
	Jan'71 (Date) / Init. / Scale
AT(30-1) 3993 (Cont. No.)	Phase II Report (Report Title)

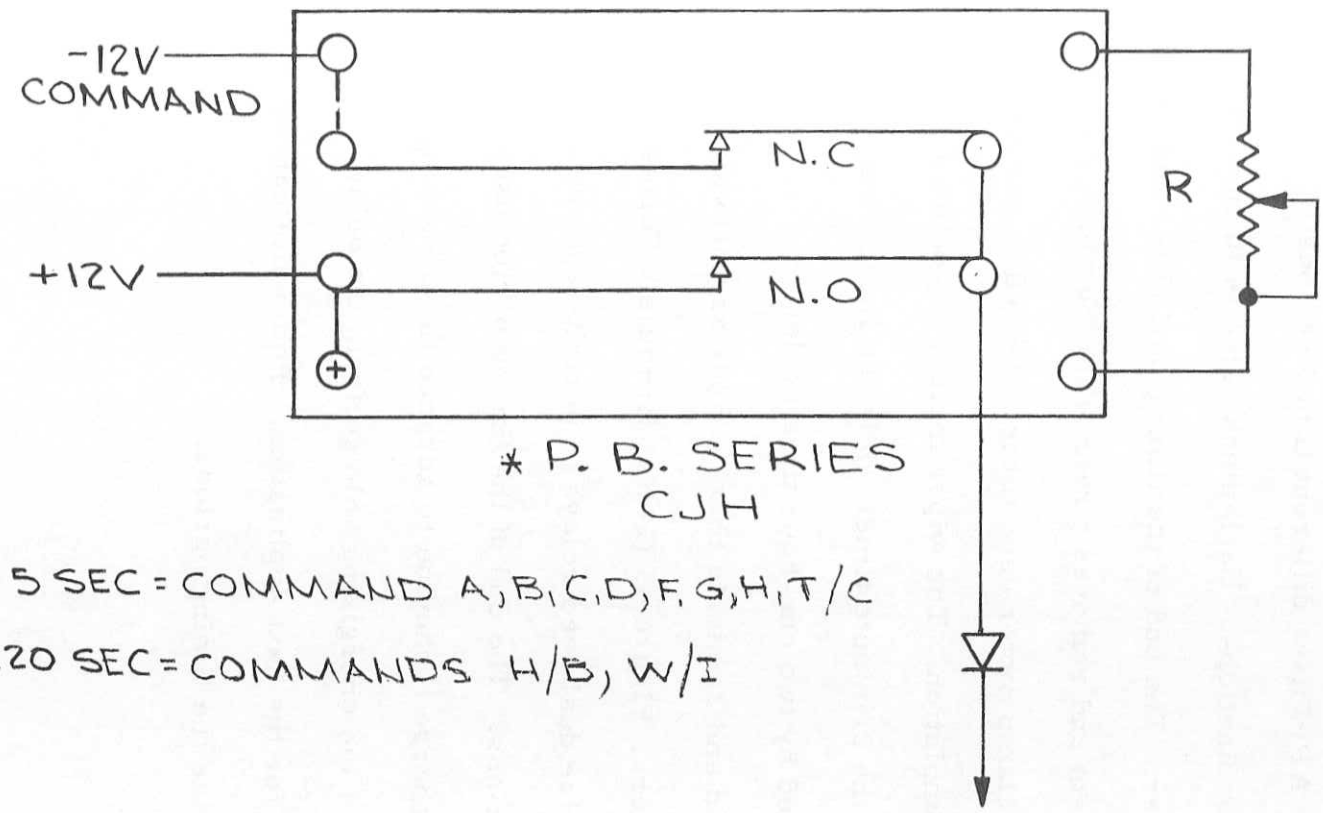

TAPE ADVANCE
10
10
10
COUNT START PULSE
+12V
COUNTING ELECTRONIC (HV AMPL)
TAPE ADVANCE
314
380
"ELECTRONICS ON" COMMAND
10
6
7
18
2
2N3704
4
5
18
3
2.2K
"COUNTING COMPLETION" COMMAND (FROM PRINT CONTROL)
19
4PDT
+12V
"FILTER TAPE ADVANCE" COMMAND
15
9
10
18
14
+12V
FILTER TAPE TAKEUP MOTOR
2N3704
2.2K
11
12
18
13
+12V
PROGRAM ADVANCE BUSS
PUMP VOLUME LIMIT MICROSWITCHES (4)
TIMERS (3)
TAPE ADVANCE (2)
10
+12V
TYPICAL
Binary Memory Logic
Fig. 42
INCORPORATED
Jan'71
AT(30-1)-3993
Phase II Report

memories. The binary memory circuit uses two gates from a Signetics 380A package. The output from the binary memory is used to drive relay drivers as described previously. Figure 42 also illustrates the capacitor discharge circuitry employed throughout the APPI.

5. Timers (7-second, 2-minute, one-hour)

The APPI uses different timed periods to accomplish various functions. Each pinch valve is turned on by a 7-second timer. The end of the timing period is used to advance the program and removes power from the pinch valve motor. The tape clamp motor uses three repeated 7-second cycles to allow completion. The wiper motor, vacuum pump and dryer all use the 2-minute timer while the incubation period is controlled by two one-hour time cycles.

Seven second and 2-minute time periods are provided by solid state timers, Figure 43 (Potter Brumfield Series CJH). These utilize dual reed relays to permit separate NO and NC operations. The end of the timing period uses the capacitor discharge technique to advance the program. This also removes the energizing voltage from the delay unit, resetting it for the next application. Both units can be adjusted to optimize the timing periods.



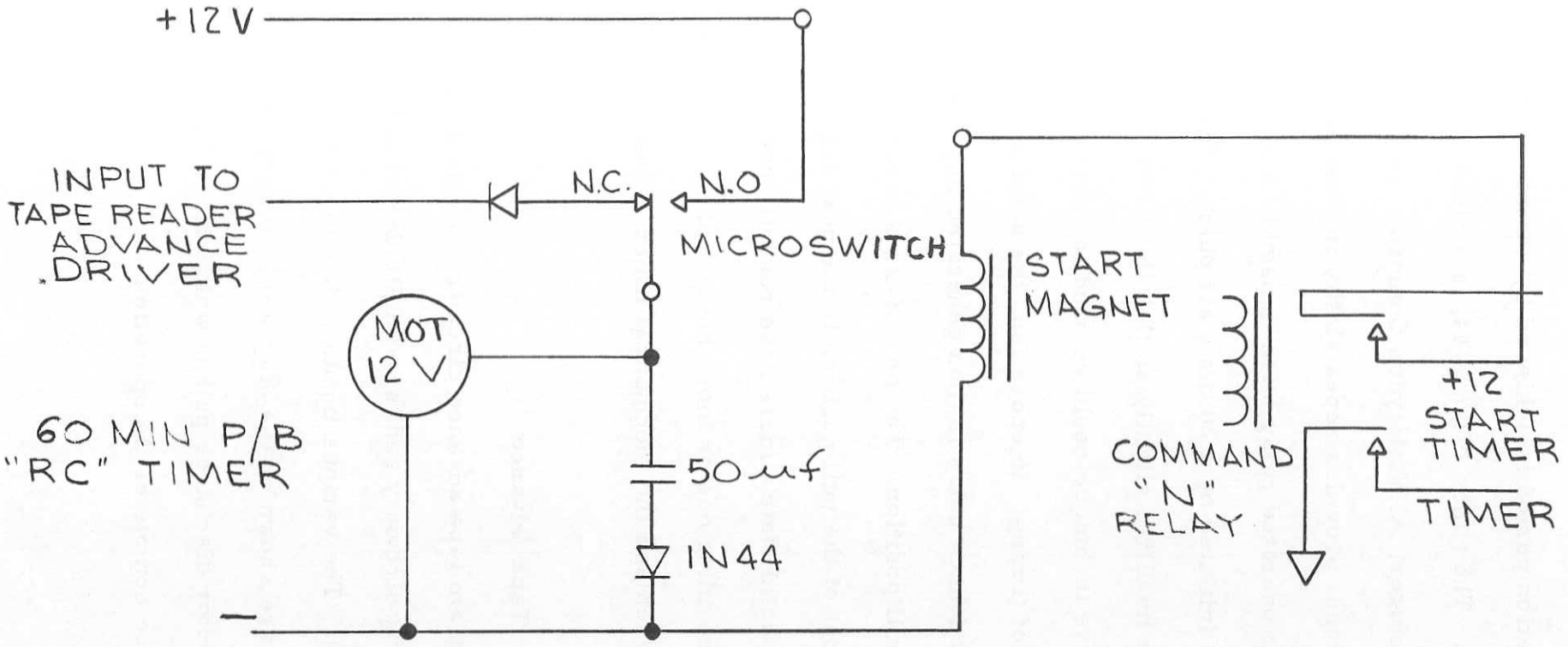
* POTTER BRUMFIELD

Title: Solid State Timer Circuitry	
Fig. 43	
BCS INCORPORATED, Rockville, Maryland	
Date: Jan'71	Init. / Scale
Cont. No.: AT(30-1) 3993	Report Title: Phase II Report

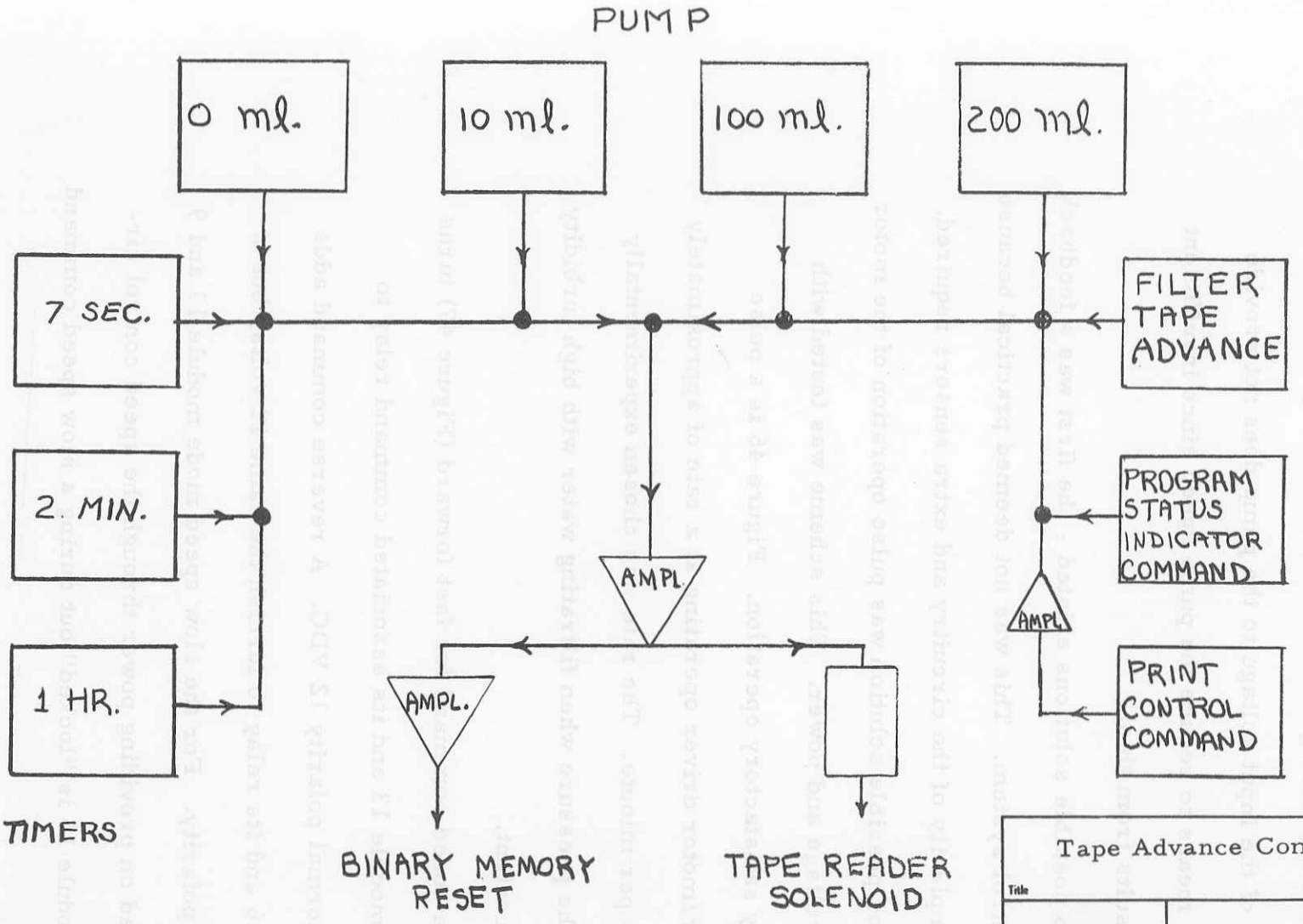
The 2-hour incubation period is achieved by commanding a one-hour timer twice. The timer (Figure 44) is a clock timer modified by the manufacturer, A.W. Haydon Company, to operate on 12 DC. The unit provides repeatability in time to better than 1%. A pulse-operated relay latch is used to lock motor voltage on and is initiated by a 20 ms start pulse. The microswitch wiring was modified slightly so that the APPI capacitor discharge pulse technique could be used to advance the program at the end of timing. In operation, the start pulse is commanded, and an advance pulse is then generated to place the program tape in a null position. The end-of-time pulse advances the program out of the null position to the next active command. Completely solid state timers have recently been announced capable of providing one or more hours. It is assumed that these can replace the mechanical timer in the future.

6. Program Tape Advance

The program tape advance circuitry is combined with the decoder blanking circuitry and is partially described under this section (D-2). The various inputs which generate the advance commands are shown on the diagram in Figure 45. Most of these are capacitor discharge pulses which are generated as each source completes its operation.



Title: One-Hour Timer Circuit				
	Fig. 44			
BIONICS INCORPORATED, Rockville, Maryland				
	Jan'71 Date	Init.	Scale	
AT(30-1) 3993 Cont. No.	Phase II Report Report Title			



Tape Advance Command Inputs

Fig. 45

CS INCORPORATED

Rockville, Maryland

Jan'71 (Date) | (Init.) | (Scale)

AT(30-1)-3993 (Cont. No.)

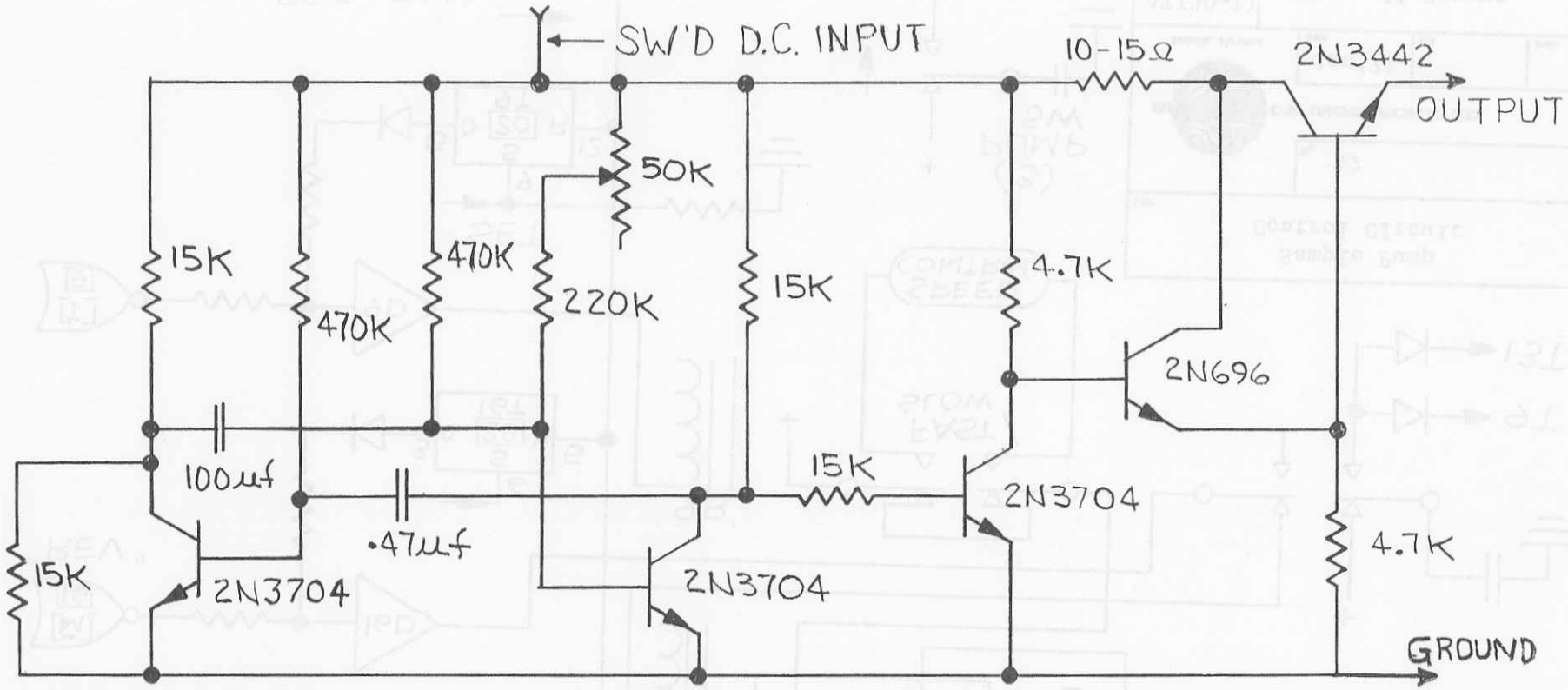
Phase II Report (Report Title)

7. Pump Circuitry

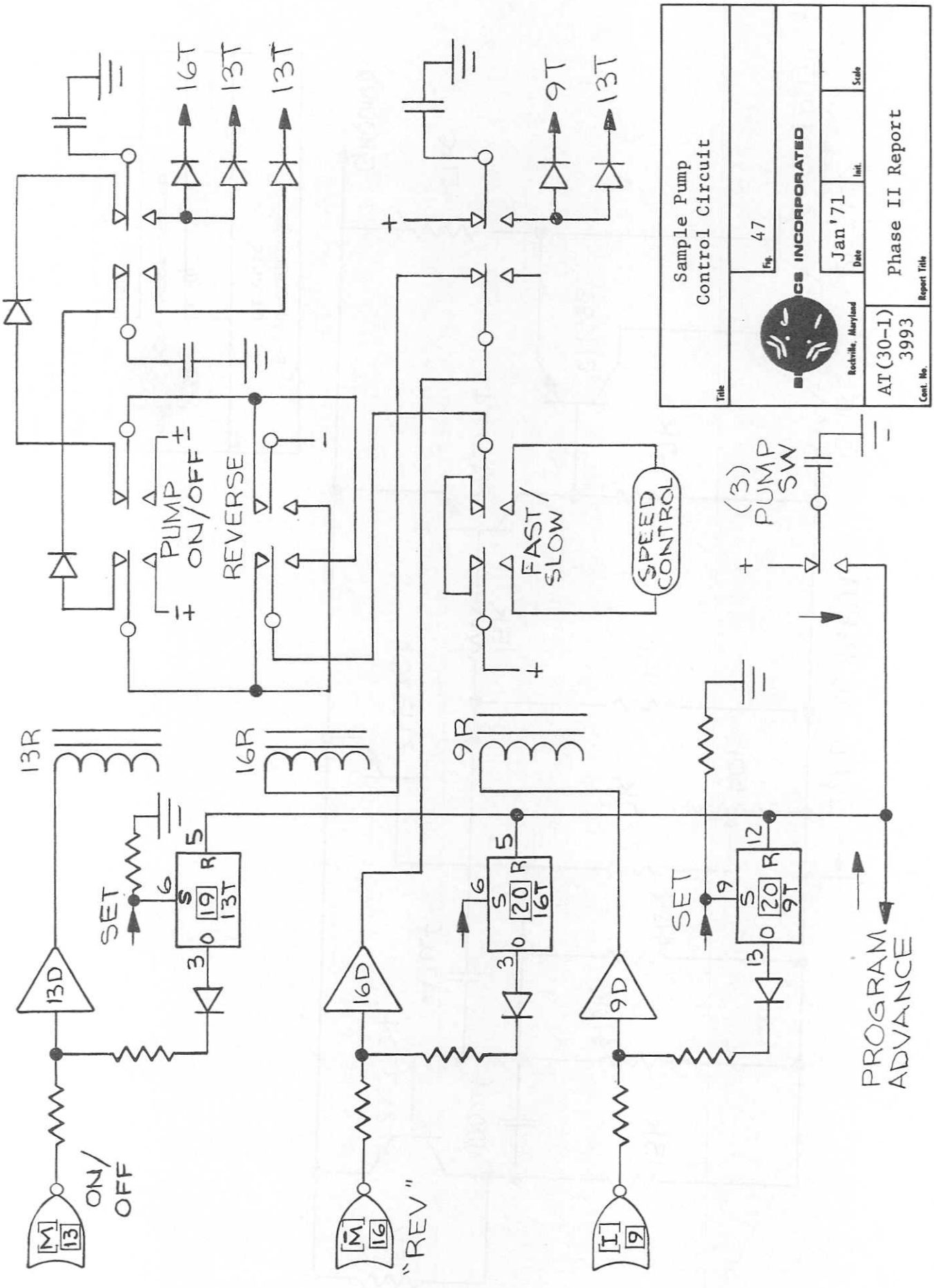
The sample pump in the APPI must be capable of operating at normal and reduced speed to prevent excessive pressure buildup during filtration of turbid samples. Reduction of the input voltage to the pump does not provide a suitable means to reduce the pump speed since insufficient torque results from this.

Two possible solutions existed - the first was a feedback speed control system. This was not deemed practical because of the complexity of the circuitry and extra sensors required. The second possible solution was pulse operation of the motor at rated voltage and power. This scheme was tested with extremely satisfactory operation. Figure 46 is a pulse generator/motor driver operating at a rate of approximately 55 pulses per minute. The rate was chosen experimentally to limit the pressure when filtrating water with high turbidity or algal content.

The coded command for fast forward (Figure 47) turns on logic module 13 and its associated command relay to furnish normal polarity 12 VDC. A reverse command adds module 16 and its relay to furnish the same 12 volts, but of opposite polarity. For the slow speed mode module 13 and 9 are turned on providing power through the speed control circuit. Module 16 is "locked" out during a slow speed command



Title: Speed Control Circuit	
	Fig. 46
CS INCORPORATED, Rockville, Maryland	
Jan'71 (Date) / Init. / Scale	
Cont. No.: AT(30-1) 3993	Report Title: Phase II Report


Sample Pump
Control Circuit
Title
Fig. 47
CS INCORPORATED
Rockville, Maryland
Jan'71
Date
Init.
Scale
AT(30-1)
3993
Cont. No.
Phase II Report
Report Title
16T
13T
13T
9T
13T
PUMP
ON/OFF
REVERSE
FAST/
SLOW
SPEED
CONTROL
(3)
PUMP
SW
13R
16R
9R
SET
SET
13D
16D
9D
ON/
OFF
"REV"
PROGRAM
ADVANCE

sequence to prevent any possibility of reverse operation. These three modules are binary memory elements and are automatically reset with each program advance. The pump has four microswitches, positioned for the 0 ml, 10 ml, 100 ml and 200 ml volumes. These microswitches are illustrated in Figure 48, and provide the program tape advance pulse.

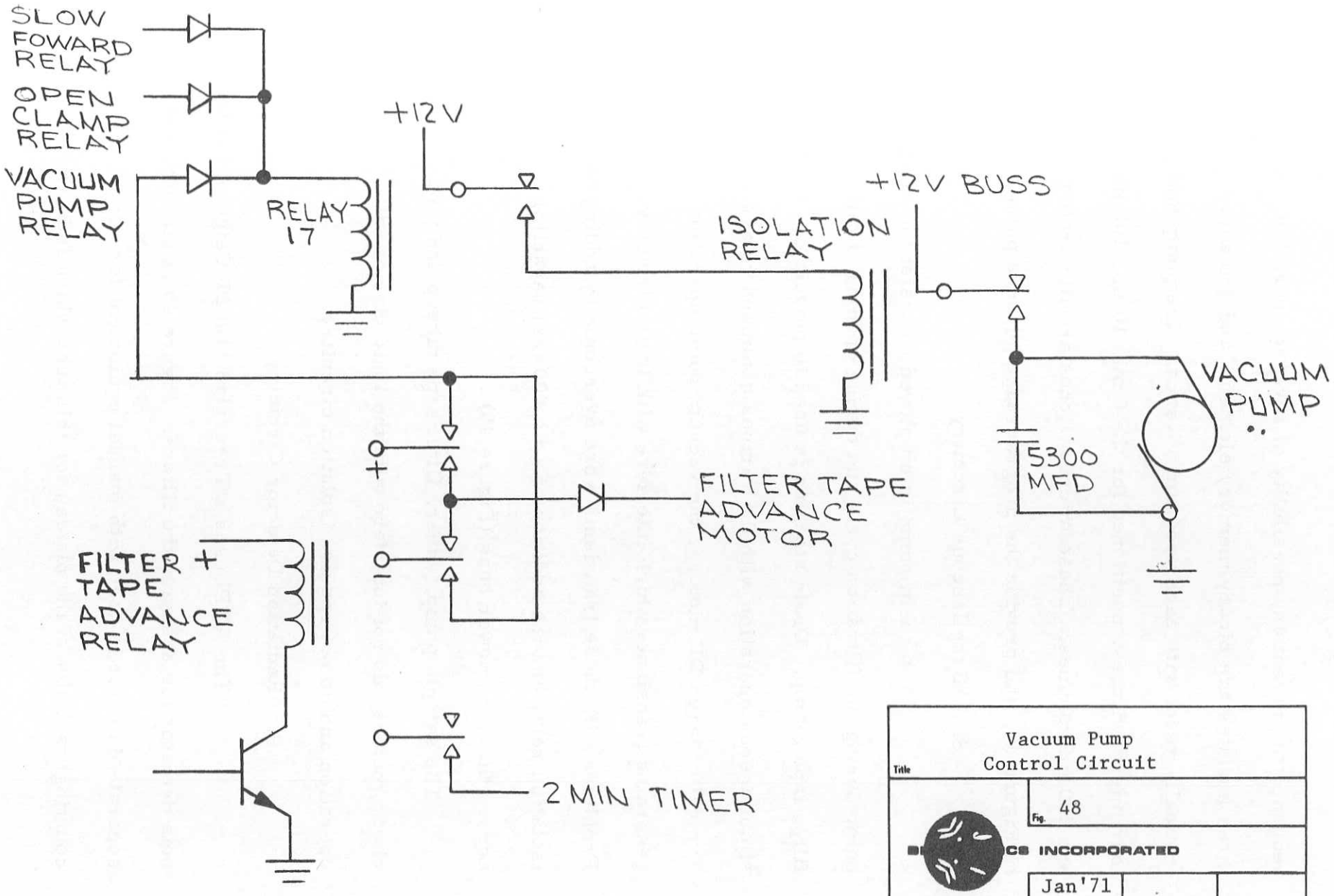
8. Miscellaneous Circuitry

A vacuum pump is employed to assist the sample pump during the filtration cycle and during opening of the filter tape clamp. Diode steering is used to permit simultaneous operation with the various command relays. Typical of large DC motors, the vacuum pump operation generates transients which interfere with logic circuitry. Problems with these transients were overcome by adding an isolation relay and the addition of a 53,000 mfd capacitor across the main power buss (Figure 48) .

The sample pump, wiper, filter tape takeup and tape clamp motors, do not interfere with the logic circuit with operation and do not require isolation circuitry.

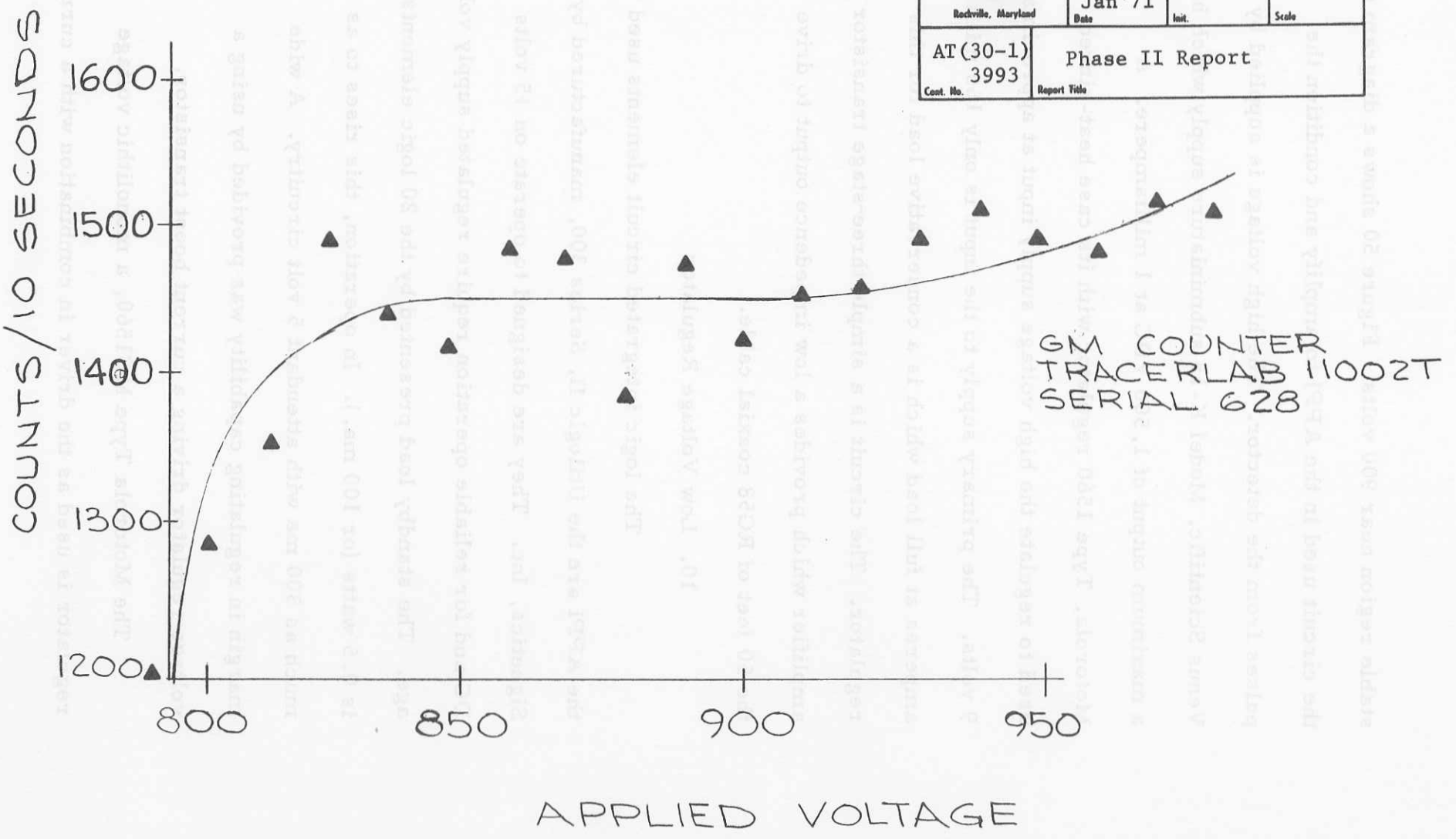
9. Radiation Detector Circuitry

The APPI uses a Tracerlab 11002T Geiger-Mueller beta detector for assaying the filters. Figure 49 (a similar, but separate detector is used with manual equipment for comparative counting) is a plot of the plateau for this tube which indicates a



Title	Vacuum Pump Control Circuit
Fig.	48
	BIOTECS INCORPORATED, Rockville, Maryland
Date	Jan'71
Init.	
Scale	
Cont. No.	AT(30-1) 3993
Report Title	Phase II Report

Title	Beta Detector Response
Fig.	49
	CS INCORPORATED, Rockville, Maryland
Date	Jan'71
Init.	
Scale	
Cont. No.	AT(30-1) 3993
Report Title	Phase II Report

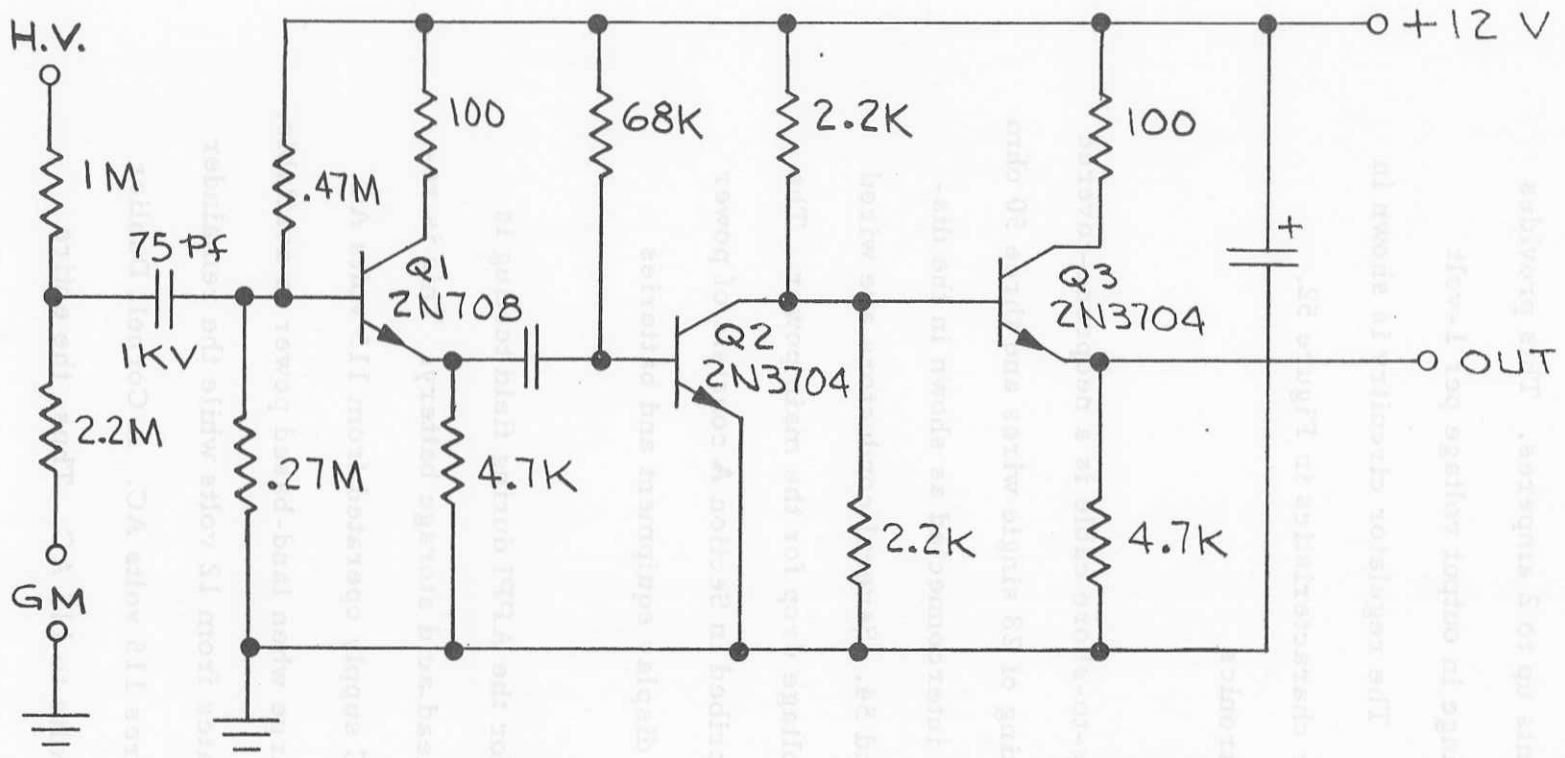


stable region near 900 volts. Figure 50 shows a diagram of the circuit used in the APPI to amplify and condition the pulses from the detector. The high voltage is supplied by a Venus Scientific, Model K-15 subminiature supply which has a maximum output of 1,500 VDC at 1 milliampere. A Motorola, Type 1560 regulator with its case heat-sinked, is used to regulate the high voltage supply input at approximately 9 volts. The primary supply to the input is only 165 milliamperes at full load which is a conservative load for this regulator. The circuit is a simple three-stage transistor amplifier which provides a low impedence output to drive the 50 feet of RG58 coaxial cable.

10. Low Voltage Regulator

The logic integrated circuit elements used in the APPI are the Utilogic II, Series 300, manufactured by Signetics, Inc. They are designed to operate on +5 volts DC and for reliable operation require regulated supply voltage. The standby load presented by the 20 logic elements is 0.5 watts (or 100 ma.). In operation, this rises to as much as 300 ma with attendant 5 volt circuitry. A wide margin in regulating capability was provided by using a voltage regulator driving a current boost transistor.

The Motorola Type MC1560, a monolithic voltage regulator is used as the driver in combination with a current



Title: Pulse Amplifier	
	Fig. 50
Rockville, Maryland	Date: Jan'71 / Init. / Scale
Cont. No.: AT(30-1) 3993	Report Title: Phase II Report

boost transistor 2N3442 used as a conventional regulator to furnish 5 volts at currents up to 2 amperes. This provides a maximum of .03% change in output voltage per 1-volt change in input voltage. The regulator circuitry is shown in Figure 51 and regulator characteristics in Figure 52.

E. Support Electronics

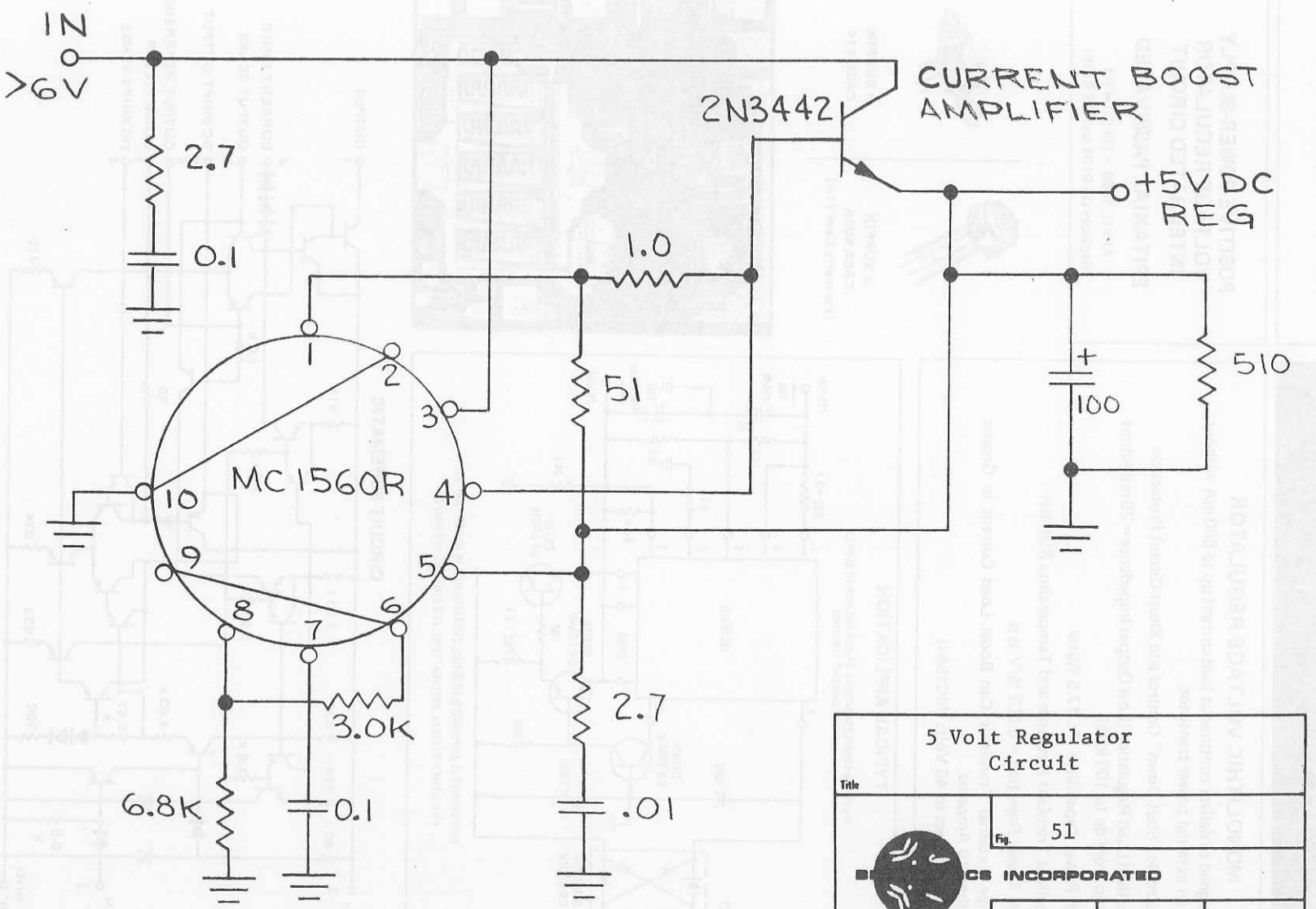
1. General

The buoy-to-shore cable is a neoprene-covered television cable consisting of 28 single wires and three 50 ohm coaxial cables which is interconnected as shown in the diagrams in Figures 53 and 54. Several conductors are wired in parallel to reduce voltage drop for the main power. The support equipment described in Section A consists of power supplies, counting and display equipment and batteries (Figure 55).

Primary power for the APPI during field testing is provided by a 12-volt lead-acid storage battery. This is recharged by a 4 amp DC supply operated from 115 volts AC to maintain proper charge when land-based power is available. The Mite printer operates from 12 volts while the remainder of the equipment requires 115 volts AC. A Cornell Dublier inventor converts 12 volts to 115 AC. Thus, the entire support equipment is portable.

2. Pulse Counting

The most economical and practical method of



Title: 5 Volt Regulator Circuit	
Fig. 51	
Rockville, Maryland	
Date: Jan'71	Init.	Scale
Cont. No.: AT(30-1) 3993	Report Title: Phase II Report

MOTOROLA Semiconductors

BOX 20912 • PHOENIX, ARIZONA 85036

MC1560, MC1561
MC1460, MC1461

MONOLITHIC VOLTAGE REGULATOR

. . . designed to deliver continuous load current up to 500 mA without use of an external power transistor.

- Electronic "Shut-Down" Control and Short-Circuit Protection
- Excellent Load Regulation (Low Output Impedance = 20 milliohms typ from dc to 100 kHz)
- High Power Capability: To 17.5 Watts
- Excellent Transient Response and Temperature Stability
- High Ripple Rejection = 0.002 %/V typ
- Single External Transistor Can Boost Load Current to Greater than 10 Amperes
- Input Voltages to 40 Volts (MC1561)

POSITIVE-POWER-SUPPLY VOLTAGE REGULATOR INTEGRATED CIRCUIT

EPITAXIAL PASSIVATED

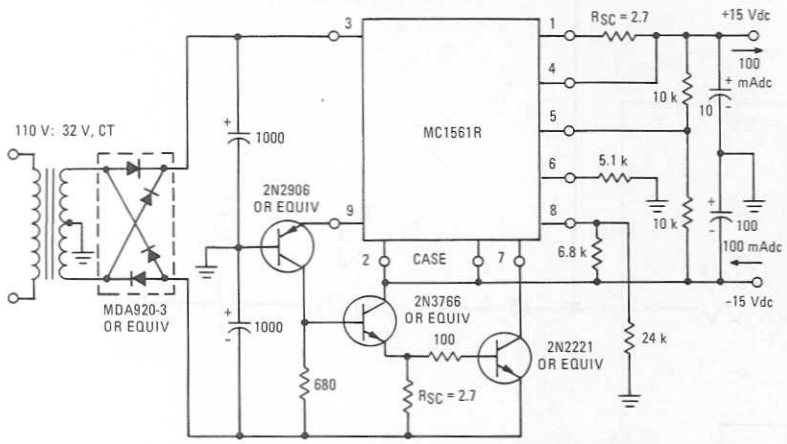
APRIL 1969 – DS 9104 R1
(Replaces DS 9104 and DS 9116)

G SUFFIX
CASE 602A
(Formerly Case 71A)

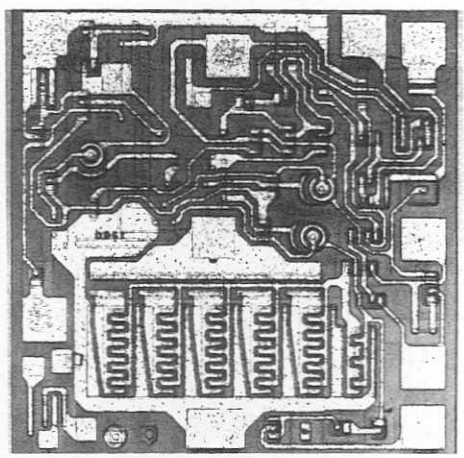
R SUFFIX
CASE 614

TYPICAL APPLICATION

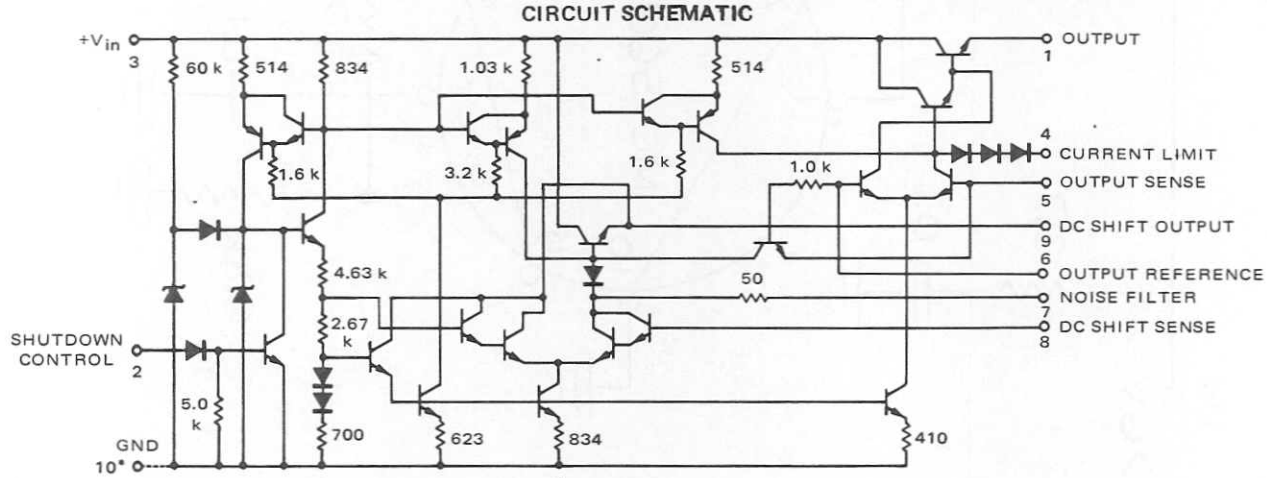
A ±15 Vdc COMPLEMENTARY TRACKING POWER SUPPLY WITH CURRENT LIMITING



RESISTANCE IS EXPRESSED IN OHMS, CAPACITANCE IN MICROFARADS.
FOR OTHER TYPICAL APPLICATIONS, SEE FIGURES 16 THRU 29



CIRCUIT SCHEMATIC



*"G" package – pin 10 is ground, "R" package – case is ground.

Figure 52

The regulator characteristics for the Motorola MC 1560

Buoy Interconnections
Plug P-1

Positive Terminals	Function	Negative Terminals	Command
1	^{14}C Valve	21	H
2	^{14}C Meter Valve	22	A
3	Sample Inlet Valve	23	B
4	Light Chamber Valve	24	C
5	Wash Solution Valve	25	L
6	Clean Solution Valve	26	E
7	Pump to Fill Valve	27	F
8	Pump Motor	28	M
9	Wiper Motor	29	K
10	Tape Advance Motor Locked On - Reset Off	30	O
11	Count Locked on Electronics And Start Count	31	J
12	Tape Clamp 15 Second On-Off	32	G
13	Heater/Blower Timed Turn-On	33	D
14	Program Advance +5 - +12 (Input)	34	
15	Turn-Off Electronics No Program Advance	35	
16	Filter Completion Pulse (Advance Program)	36 +12 VDC	
17	Reset Pump to Off	37 GM Signal Input	
18	Ground	38	
19	Vacuum Pump +12	39	

Figure 53

Buoy Interconnections
Plug P-2

Buoy Connector Pin No.	Color	P-2 Pin No.	Function	Relay Source
A	Black	1	Sample Valve	B
B	Brown	20	^{14}C Meter Valve	H
C	Red	2	^{14}C Valve	A
D	Yellow	21	Photosynthesis Chamber	C Group A
E	Green	3	Filter Valve	F
F	Blue	22	Tape Clamp	G
G	White	4	Tape Advance	O
H	Black	23	Wash Valve	L
J	Brown	5	Rinse Valve	E
K	Red	24	Wiper Motor	K
L	Yellow	6	Fast Pump, Empty	M Group B
M	Green	25	Slow Pump, Empty	I
N	Blue	7	Fast Pump, Fill	Reverse
P	White	26	Driver	D
R	Black Coax	8	Start Count	J Cap. Contact
S	Brown/ Blue	27	Ground	Ground
T	Green/ Orange	9	+12	Bus
U	White Coax	28	Stop Count	Pin 15 P1 Conn.
V	Red Coax	10	Signal	Pin 19 P1 Conn.

Figure 54

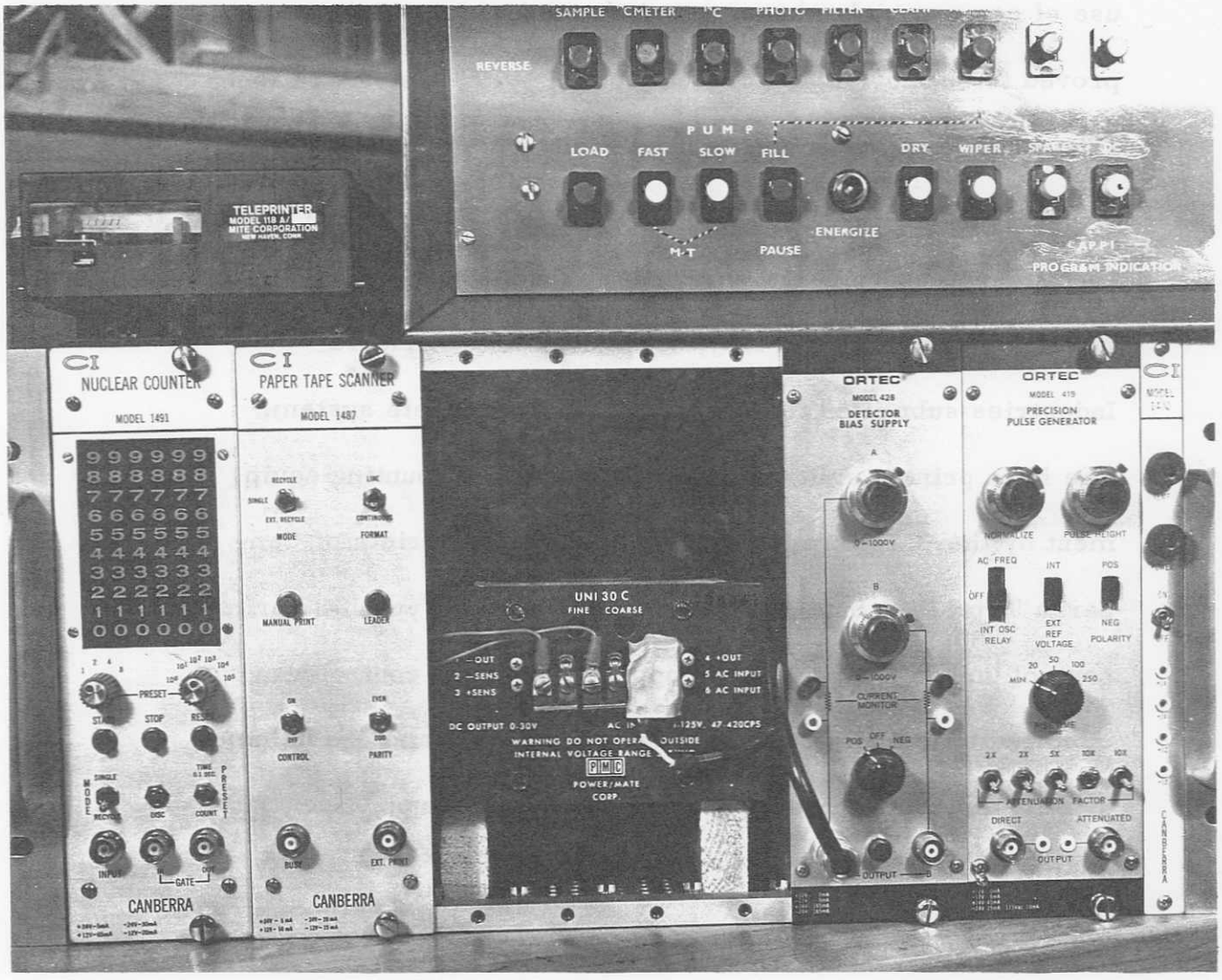


Figure 55

Shore Based Support Equipment

counting the radiation assay signal from the APPI is by the use of commercial, shore operated, equipment which proved to be more than adequate for the APPI demonstration. (The same equipment can be miniaturized to be included in the buoy during advanced program development). When the equipment requirements were established, quotations were requested from several major suppliers and Canberra Industries submitted the lowest bid for a complete system. The Mite printer unit was integrated with the counting equipment by the Canberra factory. The system consists of Canberra Type 1491 Scaler in combination with a Type 1487 print control unit and a bin power supply. The print control was modified to include a set of 8 solenoid drivers and one hammer driver to permit direct operation of the Mite paper tape printer.

The Canberra system provides the capability of counting for periods from 1 to 10,000 seconds. Counting periods for APPI were standardized at 100 seconds. Duplicate background counts and duplicate counts of each light sample on the filter tape are programmed.

3. Data Storage

A number of systems were considered to provide permanent record of the APPI data. The format from the counting equipment is digital, so various tape printers,

punch tape, cards, and magnetic tape were considered for data storage. A Mite Corporation Model 118A tape printer was chosen as the most compatible with the standard equipment. Specifications for this unit are shown in Figure 56. The unit is a subminiature printer approximately 1/20 cubic foot in volume and capable of printing 100 words per minute on a 5/16 inch tape.

4. Program Monitor

The APPI was designed to be a self-contained instrument, controlled by its own program. Recognizing the fact that there could be programming problems, a program monitor was designed to indicate the initiation of various functions. The monitor consists of a series of pilot lights wired in simplex fashion to indicate 24 active functions. In addition, the primary power, signal output for counting, start count, reset and a manual program advance are wired through the monitor. The cable to the APPI utilized 16 separate wires and three coaxial cables. The program status indicator shown in Figure 57 operates as follows. Pilot lights are arranged in combinations of 2, each in series with a common diode. With this arrangement, the upper lamp is energized by grounding the connecting wire and the lower lamp by connecting +12 VDC to the wire. With no signal, the pilots are separated at half voltage to indicate

The MITE Corporation Model 118-A Subminiaturized Tape Printer is designed for use in applications where a highly portable, very small size printer is required, such as mobile, airborne and interior communications systems.

All connections are made through a printed-circuit or "Amphenol" type connector at the rear of the equipment.

The equipment may be furnished with any 16, 32 or 64 character alphabet; standard Baudot communications, standard weather, ASCII Dense Sub-Set, Fieldata, or special characters and symbols as required.

TECHNICAL DATA

CHARACTER DATA:

Size:	.098" high x .062 wide
Number per inch:	9.3
Center-to-Center Spacing:	.107"
Number per 3" roll of tape:	Approximately 22,000

RECORDING MEDIUM:

Type:	Self-contained, impact sensitive carbonless high speed printer paper
Size:	5/16" wide 3" roll
Length:	Approximately 170 feet
Thickness:	.003"
Weight:	1 ounce per roll

OPERATIONAL DATA:

Speed: Infinitely variable up to 10 characters per second (100wpm)

SIGNAL INPUT DATA:

Type: 4, 5, and 6-wire intelligence plus 1-wire print command

Levels:

BIT	COIL	26± 4 VDC		47 ± 4 VDC	
		Resistance (OHMS)	Current (MA)	Resistance (OHMS)	Current (MA)
b_1	L1	75	350	200	235
b_2	L2	125	210	310	152
b_3	L3	58	450	210	224
b_4	L4	75	350	200	235
b_5	L5	125	210	310	152
b_6	L6	58	450	210	224
Print Comm	L7	18	1500	72	653

Voltage:	26 ± 4 or 47 ± 4 Volts DC
Standby Power:	None
Average Power:	32 Watts
Maximum Power:	85 Watts
Duty Cycle per Character: 100 ms:	Intelligence – Time 0 thru time 80 Print Command – Time 50 thru time 75 Reset – Time 80 thru time 100

PHYSICAL CHARACTERISTICS APPROXIMATELY:

Size:	4-13/16" wide by 10-11/16" long by 1-7/8" high (Not including feet or mating connector)
Weight:	3 lbs., 12 oz.

SPECIAL FEATURES:

1. Last character printed always visible.
2. No tools required for paper tape replacement. Low Paper Tape Alarm Kit available.
3. Variety of mounting bases and flange adapters available.
4. 3" recording tape roll self-contained. Provisions can be made for mounting larger roll externally.

Figure 56

The specifications for the MITE Model No. 118A Teleprinter

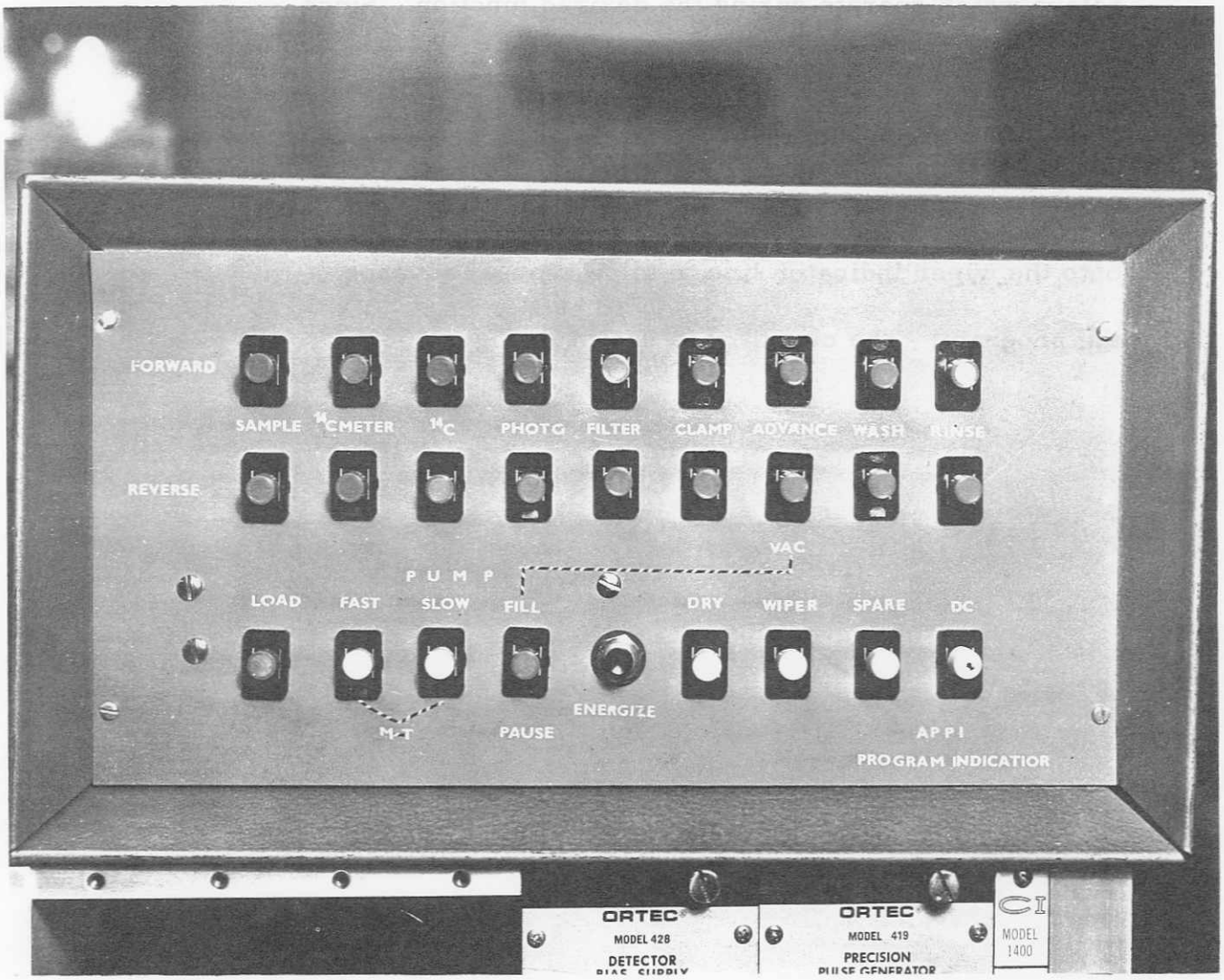
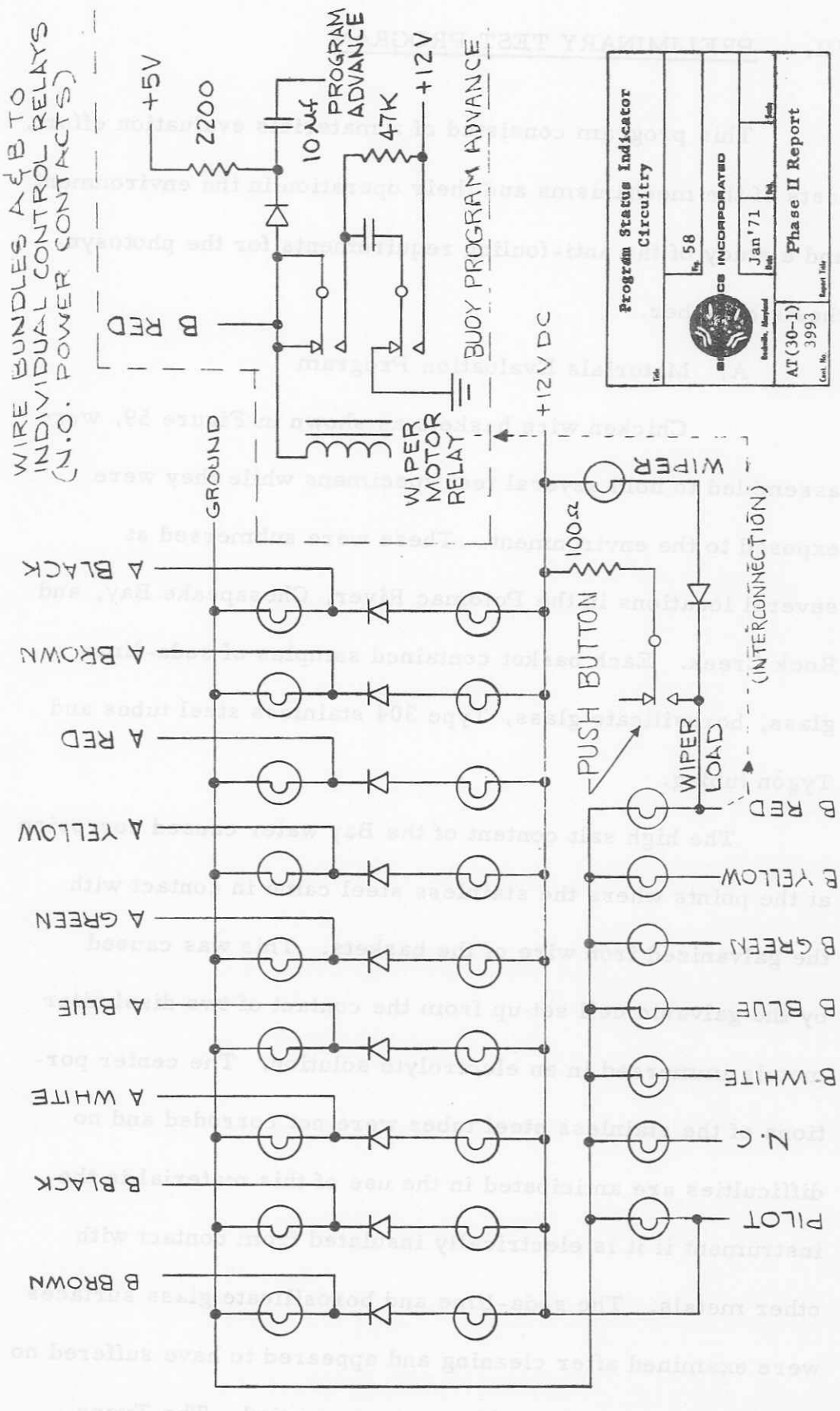


Figure 57

Program Status Indicator Unit

defective lamps. The signals are obtained from the command relays which operate during the desired function. Since both negative and positive lines are switched and/or reversed in the control circuitry, the proper signals are available. A special isolation circuit is used to couple a 12-volt pulse onto the wiper indicator line to allow manual advance of the program. The circuitry is shown in Figure 58.


WIRE BUNDLES A&B TO INDIVIDUAL CONTROL RELAYS (N.O. POWER CONTACTS)
+5V
2200
10 μf
PROGRAM ADVANCE
47K
+12V
B RED
BUOY PROGRAM ADVANCE
WIPER MOTOR RELAY
+12V DC
GROUND
WIPER
100Ω
PUSH BUTTON
WIPER LOAD
(INTERCONNECTION)
A BLACK
A BROWN
A RED
A YELLOW
A GREEN
A BLUE
A WHITE
B BLACK
B BROWN
B RED
B YELLOW
B GREEN
B BLUE
B WHITE
N.C
PILOT
Program Status Indicator Circuitry
Fig. 58
CS INCORPORATED
Jan'71
AT(30-1) 3993
Phase II Report

VII. PRELIMINARY TEST PROGRAM

This program consisted of a materials evaluation effort, tests of the mechanisms and their operation in the environment and a study of the anti-fouling requirements for the photosynthesis chamber.

A. Materials Evaluation Program

Chicken wire baskets, as shown in Figure 59, were assembled to hold several test specimens while they were exposed to the environment. These were submersed at several locations in the Potomac River, Chesapeake Bay, and Rock Creek. Each basket contained samples of soda-lime glass, borosilicate glass, Type 304 stainless steel tubes and Tygon tubing.

The high salt content of the Bay water caused corrosion at the points where the stainless steel came in contact with the galvanized iron wire of the baskets. This was caused by the galvanic cell set up from the contact of two dissimilar metals immersed in an electrolyte solution. The center portions of the stainless steel tubes were not corroded and no difficulties are anticipated in the use of this material in the instrument if it is electrically insulated from contact with other metals. The soda-lime and borosilicate glass surfaces were examined after cleaning and appeared to have suffered no permanent effects from this exposure period. The Tygon

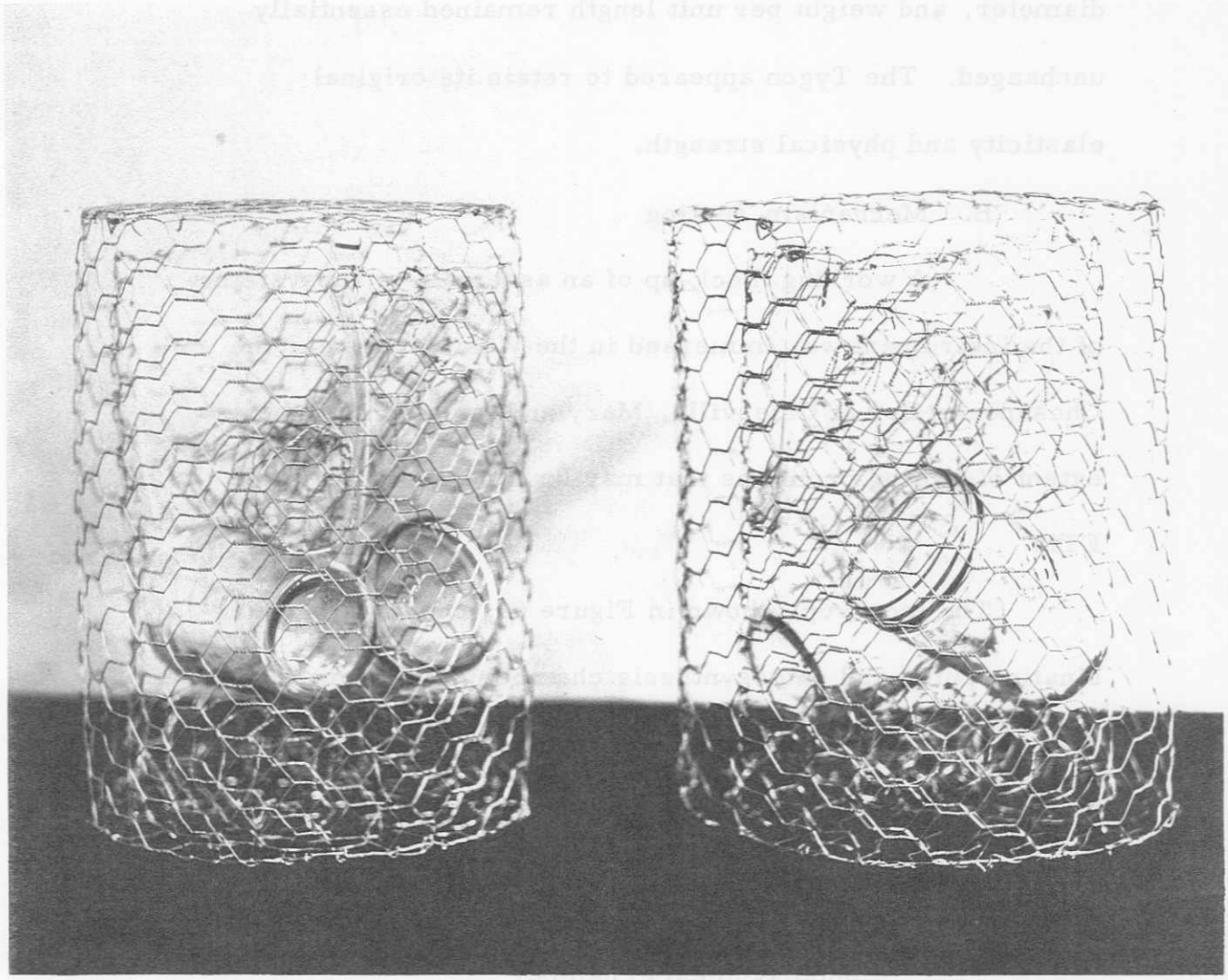


Figure 59

Materials Used for Anti-Fouling Tests

tubing had discolored slightly; however, its wall thickness, diameter, and weight per unit length remained essentially unchanged. The Tygon appeared to retain its original elasticity and physical strength.

B. Mechanism Testing

A working mock-up of an assembly of subsystems of the instrument was immersed in the West River near the Chesapeake Bay at Galesville, Maryland to determine the extent of fouling problems that may be experienced with the ETM.

The mock-up, shown in Figure 60, consists of a combination pump and photosynthesis chamber, a cleaning solution reservoir, and the valves and timer needed to regulate the mock-up's operation. The operating cycle consisted of the following repetitive stages:

1. Sample incubation for 2 hours in the pump/photosynthesis chamber
2. Cleaning of pump/photosynthesis chamber with a solution for 1 hour
3. Three wash cycles of pump/photosynthesis chamber rinsing using natural water

The unit was installed approximately four feet below the water surface within several feet of a marina sewage outfall in a eutrophic section of the West River. It was operated continuously for three and one-half months with the exception

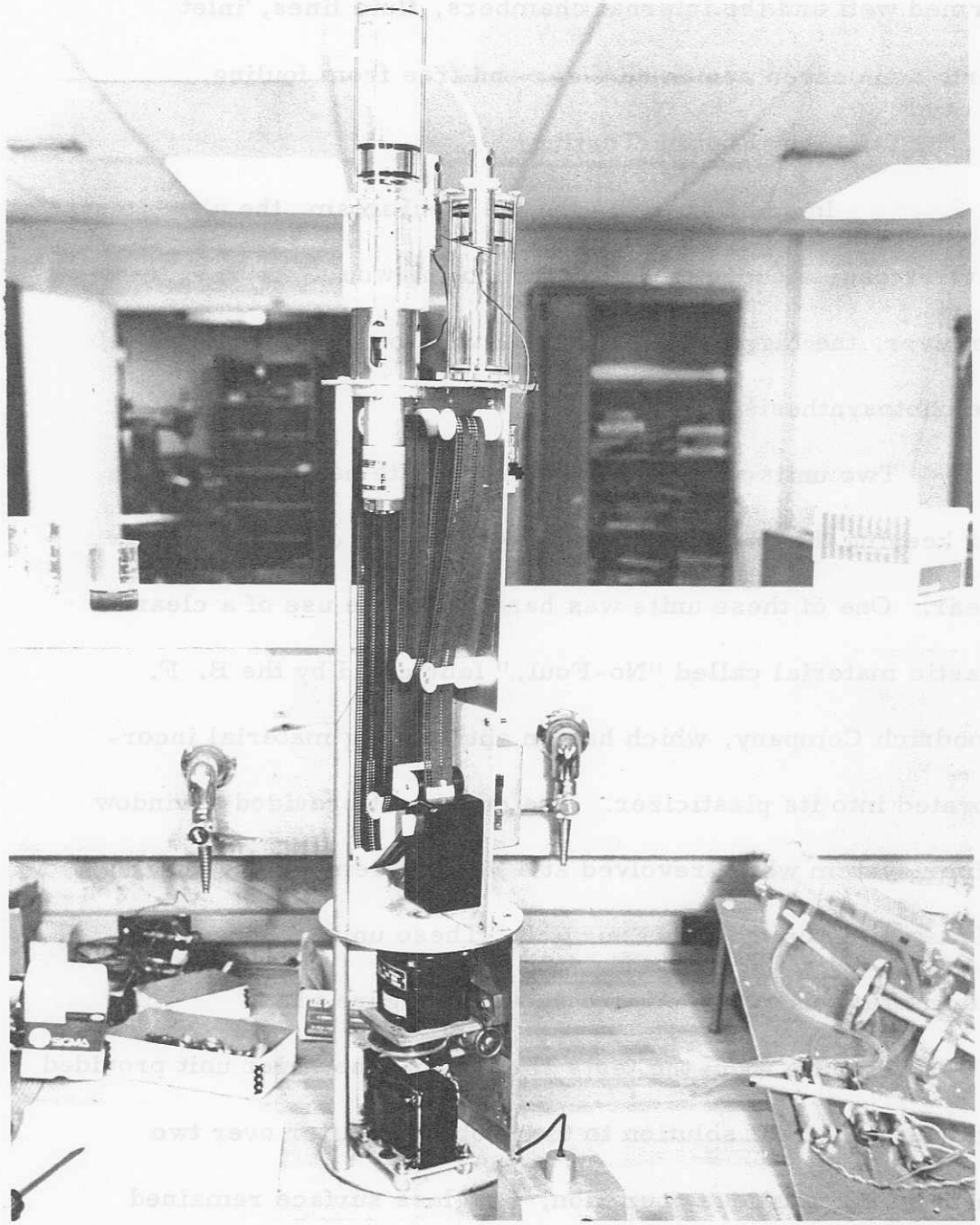


Figure 60

APPI Subsystem Mock-Up

of a few days that were required for maintenance. The unit performed well and the internal chambers, fluid lines, inlet port, and screen remained clear and free from fouling.

C. Anti-Fouling Testing

In addition to testing the mechanism, the above test gave strong evidence that internal fouling would not be a problem. However, the biggest fouling threat was to the transparency of the photosynthesis chamber.

Two units were designed to evaluate potential methods for keeping the surface of the photosynthesis chamber window clear. One of these units was based upon the use of a clear plastic material called "No-Foul," fabricated by the B. F. Goodrich Company, which has an anti-fouling material incorporated into its plasticizer. The other unit provided a window wiper system which revolved at 4 RPM to remove any buildup of materials on the glass surface. These units, along with the instrument mock-up, are shown in Figure 61.

Results from the tests showed that the wiper unit provided the best practical solution to the problem. After over two months of continuous operation, the glass surface remained completely free of any undesirable material buildup and without physical degradation due to scratches, etc., as shown in Figure 62. The rubber wiper blades showed no signs of degradation. The only minor problem was the filamentous

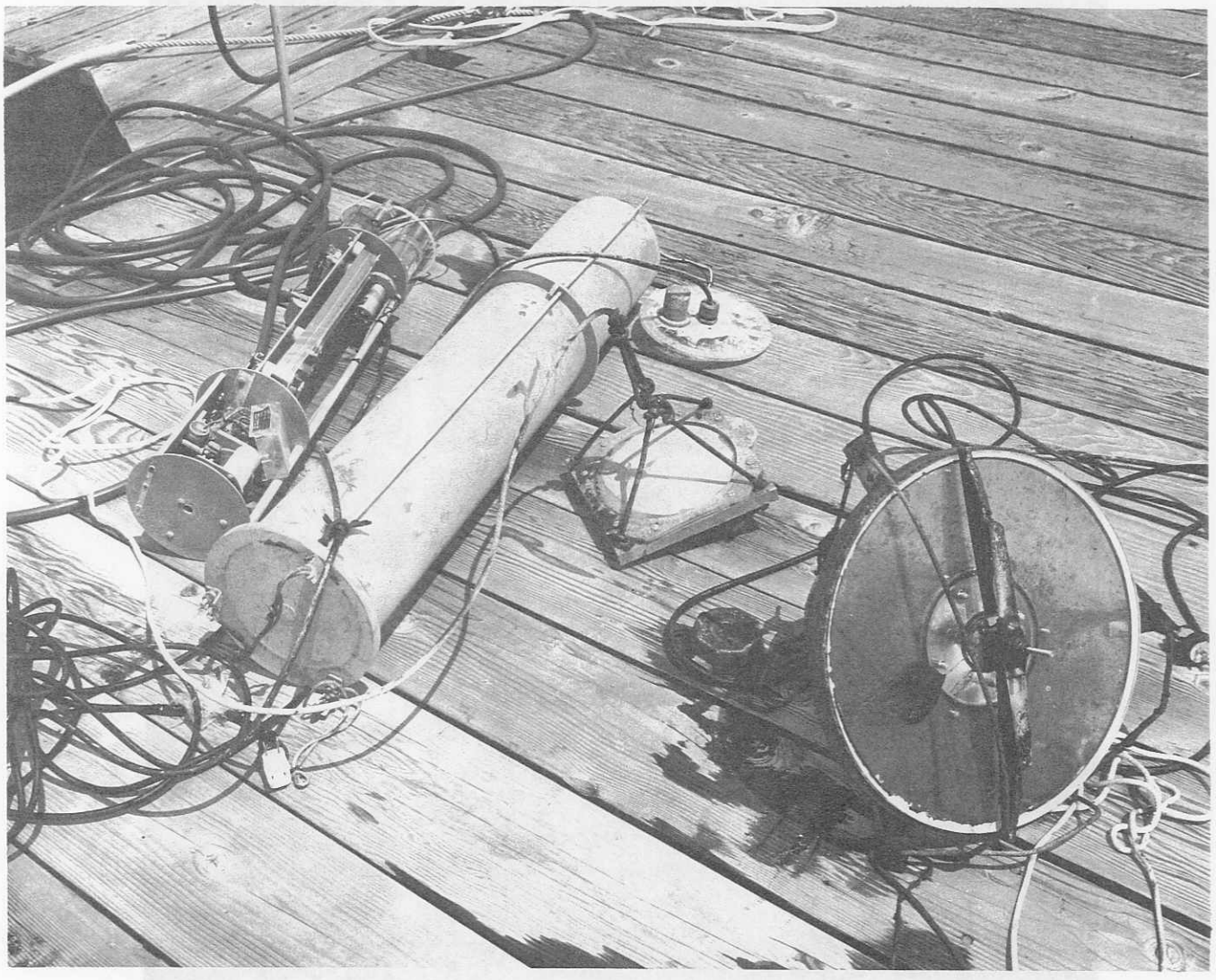


Figure 61

Preliminary Test Apparatus

At the left: APPI mechanism mock-up next to its clear plastic case.
Center: Goodyear "No-Foul" material. Right wiper system.

Figure 62

Wiper Anti-Fouling Test Unit

Upper surface is completely free from fouling or sedimentation buildup.

algae which became attached to the wiper arm, but this did not have a noticeable effect upon the unit's performance. In operation on the ETM, the wiper arms are positioned away from the sample photosynthesis chamber so that such algae would have no practical screening effect on the light entering the chamber.

The "No-Foul" material was successful in preventing biological fouling, but became coated with sedimentation. A thin white surface deposit was observed which was removed easily with light wiping after the two-month immersion. Although the "No-Foul" maintained its original transparency, it was felt that this sedimentation buildup was unacceptable and the material was dropped from consideration.

VIII. FIELD TESTING PROGRAM

A. In-House Evaluation of the APPI

Upon the completion of the assembly tasks described previously, the APPI was tested in the laboratory to evaluate the efficiency of its mechanical operation and the accuracy of the performance of the primary productivity assay. The initial phase of this effort was directed toward the testing of the mechanical and electronic subsystems and the programmer control which are shown in Figure 63, as they were integrated into the overall system. The integration testing was followed by another series of tests to determine the instrument's characteristics while handling reagents. On 5 August 1970, the APPI was ready for the first primary productivity assays.

Triplicate manual assays were conducted by the procedure described in Appendix II in order to check the results obtained from the APPI. During the first 12 laboratory tests, the light bottles were incubated while lying on their sides at the same distance from the light as the APPI photosnythesis chamber. Because this procedure caused some of the sample bottles to become heated, exceeding the temperature of the sample within the APPI, an alternate procedure was initiated whereby the bottles were incubated

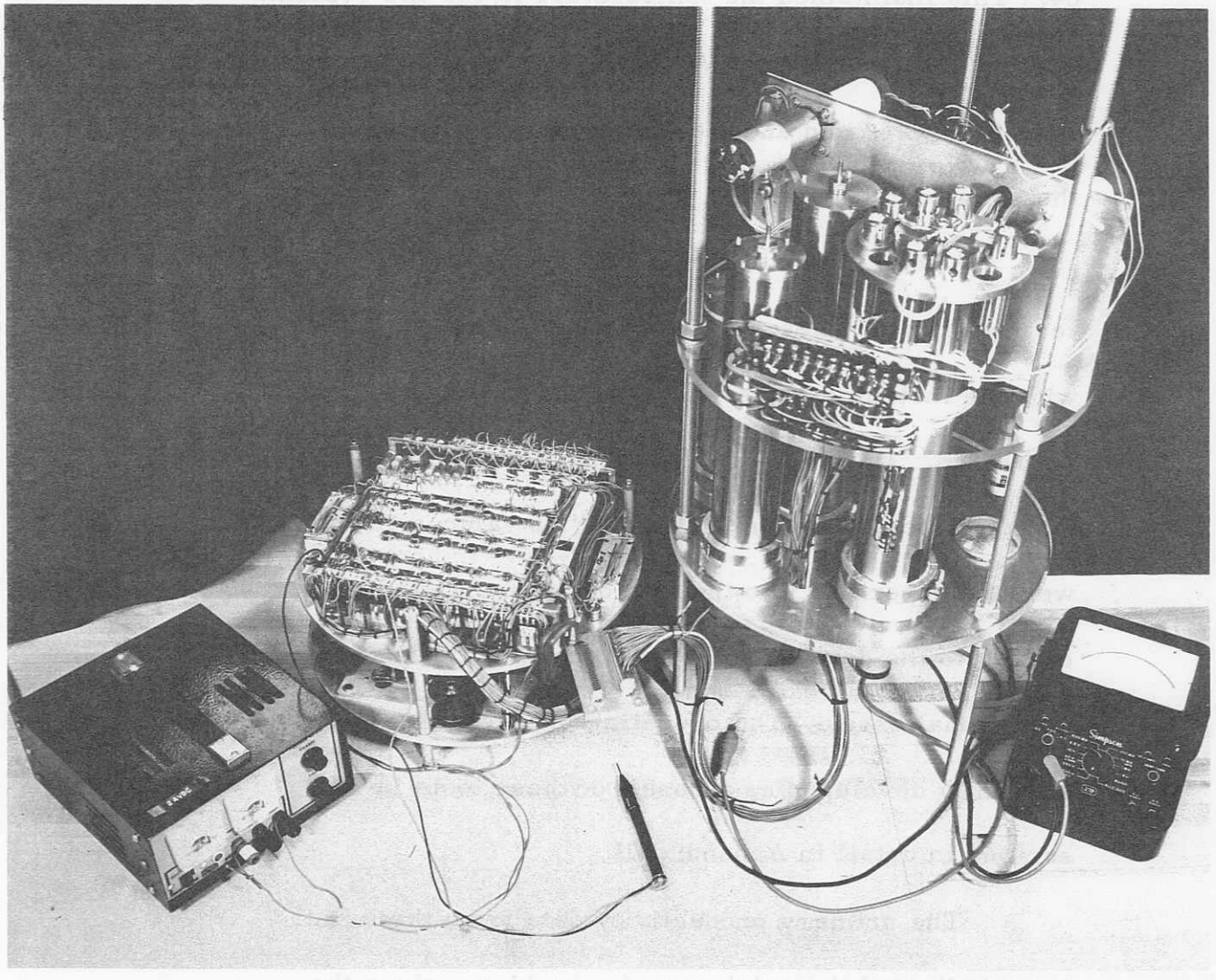


Figure 63
Integration Testing of the APPI

The electromechanical subsystem was tested with the programmer as they were integrated into the final configuration

within a water bath, alongside the APPI, as shown in Figure 64. This maintained the temperature in the two systems within 2°C. In all of the APPI tests, the automatic programmer was used and the instrument was operated in the open, outside the hull.

A total of 23 tests were conducted in the in-house testing phase. These helped to identify several problems with the instrument operation which were subsequently corrected. During these tests, the program tape which controls the assay was "debugged" as various refinements were incorporated into the test cycle in an attempt to correlate the data from the instrument with those from the manual assay. The operating program that was subsequently developed as a result of these tests is described in detail in Appendix III.

The primary productivity data from the initial tests indicated the existence of a problem, since the manual assay results were consistently greater than those from the APPI. Examination of each of the operational steps in the instrument and manual systems failed to reveal any component operational failure. Among these tests were the following:

1. Comparison of the incubation chambers of the instrument and manual technique by performing a parallel sample

Figure 64

Laboratory Testing of the APPI

Manual assay light bottles were incubated in water bath shown in foreground. Illumination was provided by fluorescent and incandescent lighting

inoculation and incubation, then removing the sample from the instrument and filtering and counting it along with the manual samples. Results from this test are shown in Table 11.

2. Comparison of the filtration and counting systems by incubating a large quantity sample in one bath, then performing simultaneous filtrations and counting with instrument and manual systems. The results shown in Table 12 were subsequently used to compute the counter efficiency difference between the instrument and manual systems.

3. This was an experiment to extend the tests described above in Table 11. In this experiment, variations in the optical properties of the manual light bottles were effected which reduced their illumination to a value similar to the APPI photosynthesis chamber. These tests, which are reported in Table 13, concur with the data in Table 11.

Table 11

Test to Determine Effect of Incubation Chamber *

Incubation Chamber	Assay
Manual Bottles - Average of 3	85,460
APPI Photosynthesis Chamber (1) -	63,171
Counting Ratio: APPI/Manual =	0.74

* Samples from Test No. 3 were incubated in artificial light, filtered and counted with manual apparatus.

Table 12

Comparison of APPI Counting vs. Manual *

Sample No.	APPI Assay	Manual Assay
1	28,534	23,809
2	25,952	22,108
3	27,083	23,064
4	24,612	23,330
5	25,667	22,732
6	26,882	20,573
7	24,950	22,582
Average	26,240	22,600

Counting Ratio:

$$\frac{\text{APPI}}{\text{Manual}} = 1.16$$

* Data taken in Test No. 10, performed 8 August 1970, using a common sample, individually filtered and counted.

Table 13

Test (No. 11) to Compare Incubation Chamber Effect on Assay

Incubation Chamber *	Assay	Ratio: (Test/Control)
Test Tubes in Air (Control)	72,909	-
Test Tubes under Lucite	63,587	.87
Test Tubes Immersed in DC 500 Silicone Oil	55,696	.76
APPI Photosynthesis Chamber	56,514	.77

* All samples incubated in diffused skylight, filtered and counted with manual apparatus.

4. Repetitive tests of the isotope metering apparatus were made. It was determined that the reproducibility of this technique was considerably better than 5%. These tests were combined with a radioisotopic assay of the strength of the $NaH^{14}CO_3$ solution that was stored within the instrument. Results from the assay failed to show any measurable changes as the result of storage.
5. The radioactivity counting apparatus was tested several times to determine the stability of the electronics system. Except for a brief period when one GM tube failed because of a degradation of the plateau, the 900 volt operating potential was always well within the stable region for the tubes being used.
6. In preparation for the field testing, several tests were conducted of the hull seal to determine its ability to withstand depths up to 20 feet. After correcting a problem with a warped

flange (caused in the welding of the hull), the hull was immersed in a tank of water, see Figure 65, and evacuated to a pressure of 0.25 atmospheres for 15 hours. (This is equivalent to a 24-foot immersion). No measurable leakage was observed.

Subsequent to the above tests, a series of operational tests was conducted to help optimize the steps in the automated assay program. Data from these tests were compared by computing a ratio of the APPI assay divided by the manual assay; Table 14 summarizes these ratios for tests 16 through 23. As these tests proceeded, the ratio seemed to improve (a ratio of 1.0 would be obtained if the two assays were equal), although the magnitude of the variation indicated that a serious problem still existed.

During these tests, detailed observations were made of the moving parts of the photosynthesis chamber. These observations detected a major loss of sample. Phytoplankton tend to collect on the walls of the photosynthesis chamber during incubation. As the sample was removed from the chamber by the sample pump, the piston in the chamber gently scrapes the walls, thereby concentrating the phytoplankton along the circumference of the piston. This concentration of labeled organisms remained in the few tenths

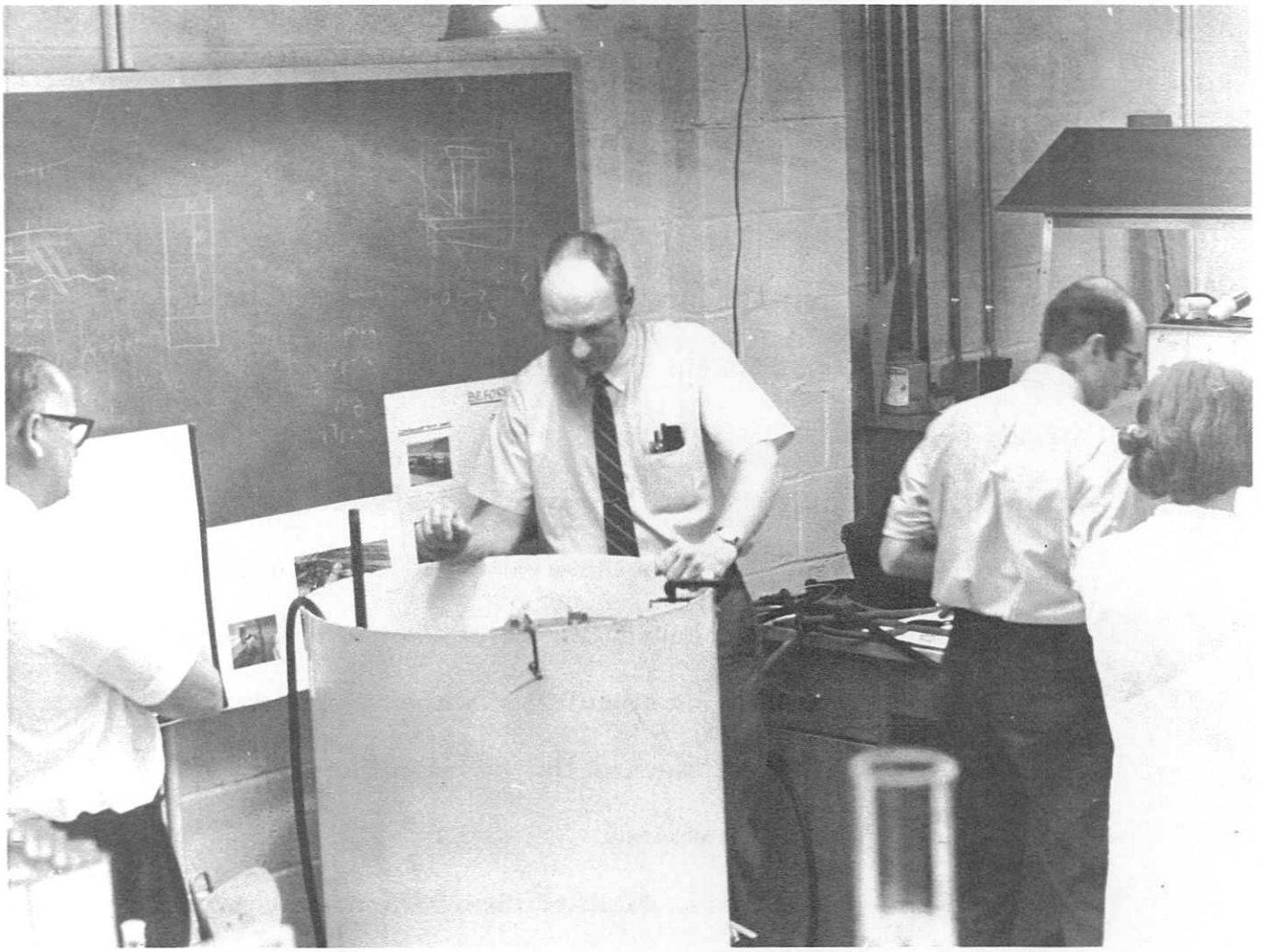


Figure 65
APPI Hull Immersion Tests

Hull was immersed in water and evacuated to 0.25 atmospheres to simulate a 24-foot immersion

Table 14

Summary of Operational Testing Data Obtained
While Optimizing the Assay Program

Test No.	Ratio of APPI/Manual		
	Light	Dark	L-D
16	.51	1.0	.41
17	.51	1.1	.86
18	.93	1.1	.59
19	.58	.83	.57
20	.63	.98	.59
21	.94	.40	.96
22	.78	.89	.78
23	.67	3.2	.62

of a milliliter trapped in the chamber and tubing and was a significant portion of the sample and varied for each assay cycle. The problem was solved by re-programming the controller so that the piston would mix the sample prior to withdrawing it. Only a trace of the phytoplankton remained in the chamber after this operation, and even this was recovered by routing the wash solution (0.01 N H_2SO_4) into the chamber before it was forced through the filter. Three such wash cycles were performed for each assay.

On 13 August 1970, the tape program and apparatus modifications were completed, and a series of calibration tests was started. Results from these 11 tests are analyzed in Table 15. These data show that yields from the manual assay are consistently greater than those from the APPI assay - findings which are in agreement with the data shown previously in Table 13. The coefficient of variation for these data, excluding those where trouble was experienced, was 13%. This provided a strong argument that there was an enhancement of the photosynthetic process within the manual bottles, which was in agreement with a simple analysis of the geometric optics for the two chambers. This effect is shown diagrammatically (for the laboratory

Table 15

Summary of Comparative Laboratory Tests of APPI

Test No/ Date	^{14}C Concen. A/M	Relative Counter Eff. A/M	Data	Background cts/100s	Light cts/100s	Dark cts/100s	L-D cts/100s	Ratio - APPI/Manual			Note
								Light	Dark	L-D	
#24	0.895	1.160	APPI	154	12,496	517	11,979	.78	1.33	.78	
Aug. 13			Manual	65	15,217	327	14,890				
#25	1.183	1.160	APPI	131	41,810	1,202	40,608	.71	.44	.72	
Aug. 14			Manual	75	43,142	1,860	41,282				
#26	1.183	1.160	APPI	164	57,082	1,423	55,659	.68	.69	.69	
Aug. 14			Manual	85	61,001	1,422	59,579				
#27	1.036	1.160	APPI	161	155,948	4,943	151,005	.86	1.55	.84	
Aug. 17			Manual	64	151,438	2,626	148,812				
#28	1.036	1.160	APPI	947	131,074	6,598	124,476	.65	.73	.65	
Aug. 17			Manual	86	166,445	6,512	159,933				
#29	0.893	1.160	APPI	129	24,393	-	24,373	.96	-	1.05	(1)
Aug. 18			Manual	59	24,557	2,211	22,346				
#30	0.893	1.224	APPI	171	23,181	1,784	21,397	.71	1.07	.69	
Aug. 18			Manual	60	29,831	1,439	28,382				
#31	0.893	1.224	APPI	164	3,235	1,605	1,630	1.46	4.92	.90	
Aug. 18			Manual	61	1,981	329	1,652				
#32			APPI								
Aug. 18			Manual								
#33	0.654	1.224	APPI	236	4.411	837	3.574	.46	3.26	.41	(2)
Aug. 19			Manual	57	11,197	287	10,910				

Table 15
(continued)
Summary of Comparative Laboratory Tests of APPI

Test No/ Date	^{14}C Concen. A/M	Relative Counter Eff. A/M	Data	Background cts/100s	Light cts/100s	Dark cts/100s	L-D cts/100s	Ratio - APPI/Manual			Note
								Light	Dark	L-D	
#34	1.04	1.224	APPI	496	23,531	3,842	19,689	.74	1.65	.69	
Aug. 20			Manual	92	39,134	2,631	35,503				
#35	0.658	1.224	APPI	78	14,989	1.725	13,264	.46	.23	.42	(1)
Aug. 21			Manual	71	40,016	929	39,087				
#36	1.03	1.224	APPI	571	17,847	2,476	15,371	.60	1.21	.57	
Aug. 21			Manual	56	22,751	1,479	21,272				
#37	1.03	1.224	APPI	69	37,479	3,070	34,410	.79	.84	.78	
Aug. 24			Manual	70	37,821	2,908	34,913				

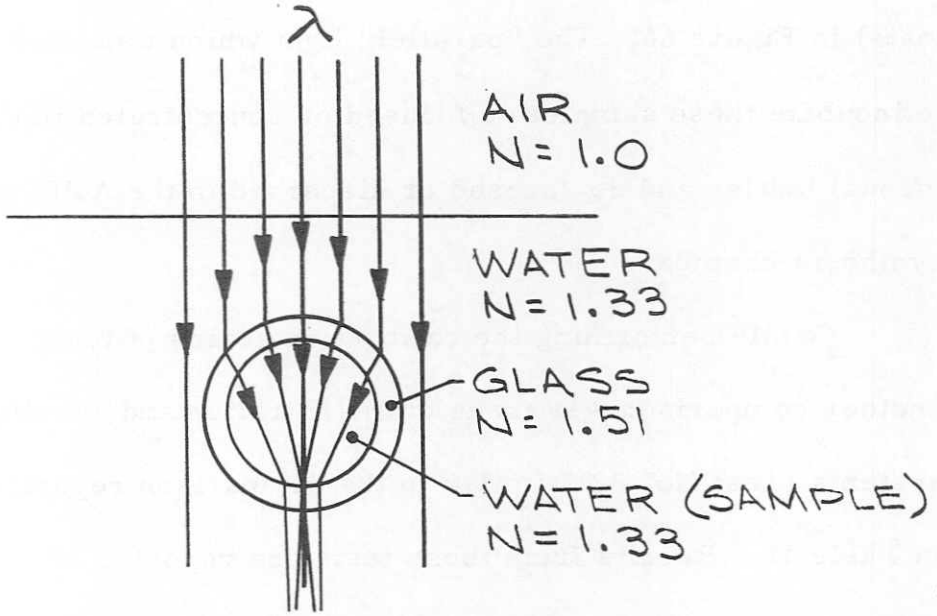
NOTE: (1) Filter tape positioning mechanism accidentally moved. (2) Residual rinse caused insufficient sample.

L-D Average (excluding 29, 33 & 35) = .72
Coefficient of Variation = 13%

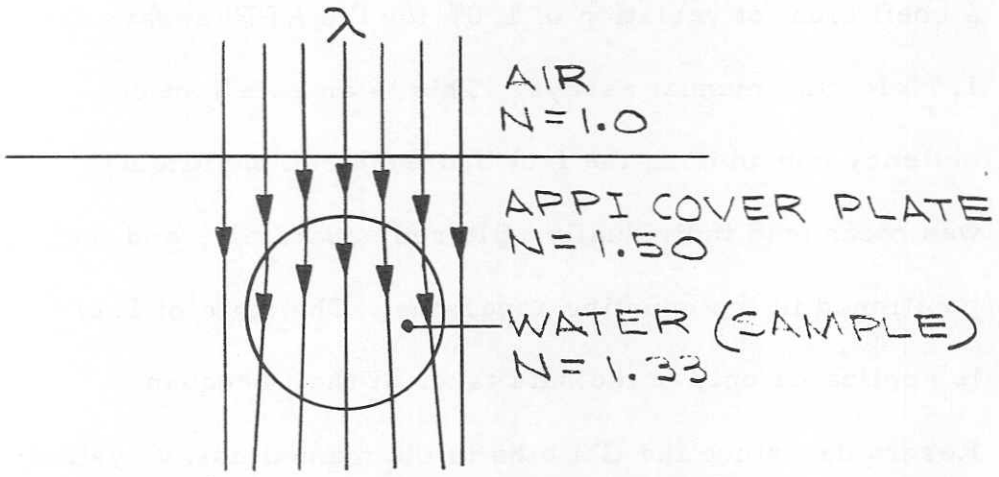
tests) in Figure 66. The "parallel" light which was used to incubate these samples is focused or concentrated in the manual bottles and de-focused or dispersed in the APPI photosynthesis chamber.

While performing the calibration series of tests, another comparison was made of the filtration and counting systems (Test No. 32) similar to the comparison reported in Table 12. Results from these tests are reported in Table 16 and show a ratio of 1.224 for the APPI compared to the Manual assay. Consistency of this data shows a coefficient of variation of 1.0% for the APPI assays and 1.7% for the manual assays. This is an excellent consistency considering the fact that each sample aliquot was measured individually, filtered separately, and then positioned in the counting apparatus. The ratio of 1.224 is applicable only to the data taken at the Occoquan Reservoir, since the GM tube in the manual assay system was damaged after those tests and the new tube had a reduced efficiency which increased the ratio to 1.254 subsequently.

Except for a human error which caused an improper operation of the filter tape metering device when the tape position indicator was moved accidently in Tests No. 29 and 35, the APPI operated without mechanical or electronic



OPTICAL PATH FOR MANUAL BOTTLES



OPTICAL PATH FOR APPI PHOTOSYNTHESIS CHAMBER

Title: Comparison of Manual and APPI Incubation Chamber Optics	
	Fig. 66
BIOMEDICAL SERVICES INCORPORATED, Rockville, Maryland	
	Date: Jan'71 / Init. / Scale
Cont. No.: AT(30-1)-3993	Report Title: Phase II Report

Table 16

Comparison of APPI Counting vs. Manual *

Sample No.	APPI Assay	Manual Assay
1	62,794	52,317
2	63,665	51,370
3	63,350	51,053
4	62,894	52,269
5	62,620	49,979
6	61,763	51,140
Average	62,848	51,355

Counting Ratio:

$$\frac{\text{APPI}}{\text{Manual}} = 1.224$$

* Data taken in Test No. 32, performed 18 August 1970, using a common sample, individually filtered and counted.

failure during the entire calibration series. The system appeared to be working perfectly and it was decided to proceed with the first cycle of the field testing phase of the program.

B. Environmental Testing and Evaluation

1. The Test Plan

The criteria for the test program are described in detail in the Test Plan (Appendix IV), which incorporates two cycles of testing. The first cycle was designed to be conducted at a local open water site and the second, at another site selected by the FWQA. Several potential local sites were explored to find one which was suitable for these tests. It was felt that a good test site must have the following features:

(a) Restricted access to ensure the security required to leave the APPI unguarded overnight.

(b) Minimum depth at test site of 20 feet.

(c) Low turbidity so that the assay would not be affected greatly by changes in depth during the test, and between the APPI photosynthesis chamber and the manual light bottles.

(d) Moderate algal growth so as to allow a reasonable primary productivity assay.

(e) Approval by the cognizant authority.

A variety of local sites was investigated,including the Dalecarlia Reservoir (Corps of Engineers, District of Columbia), Tridelphia Reservoir (Washington Suburban Sanitary Commission), and the Occoquan Reservoir (Fairfax County Water Authority) - FCWA - Virginia. The Occoquan Reservoir best met the above requirements, and the necessary approvals were obtained through the assistance of Mr. James Corbalis, Engineer/Director of the FCWA.

The Flat Creek tributary to Lake Lanier, Georgia, was recommended by the FWQA for its cooperative field test. Flat Creek carries the discharge from the Gainesville sewage treatment plant (and several animal processing plants), is normally quite turbid, experiences considerable algal blooms, and has a depth of about 10 feet.

During the initial conduct of testing at Flat Creek, it became obvious that this test site could be difficult because of the previously mentioned problems and another test site on Lake Lanier - Snug Harbor Marina - was chosen for several of the tests.

2. The Test Equipment

Figure 67 shows the APPI system which was

Figure 67

The APPI System

Showing the instrument and its photosynthesis chamber, hull and cable, and support electronics

used for the field testing. It consisted of the instrument with its hull and a 50-foot cable, the data recording system, program status indicator box, and the manual filter assay counter. The ASR-33 Teletypewriter was used in the preparation of program tapes for the control of the APPI programmer and was not required in the field for testing. The actual conduct of the testing required the use of the components shown in Figure 67, along with ropes and rigging equipment (used for the deployment of the APPI into the water body), reagent supplies, manual sampler, filtration apparatus, spare components, and several radioactive waste recepticles.

3. The Occoquan Test Cycle

The APPI system was tested in the Occoquan Reservoir (Virginia) on 25, 26, and 27 August 1970, working from the dam structure as shown in the photograph in Figure 68 and in the diagram in Figure 69. During the past few years, the FCWA has been troubled with excessive eutrophication which has caused algal blooms. During the tests, the instrument was submersed to a depth of 1.5 feet and held 20 feet from the dam face with a 150-foot tether rope from the opposite shore. This location was only about 25 feet from the dam head gate screen which resulted in rather dynamic changes in the water conditions from test-to-test.

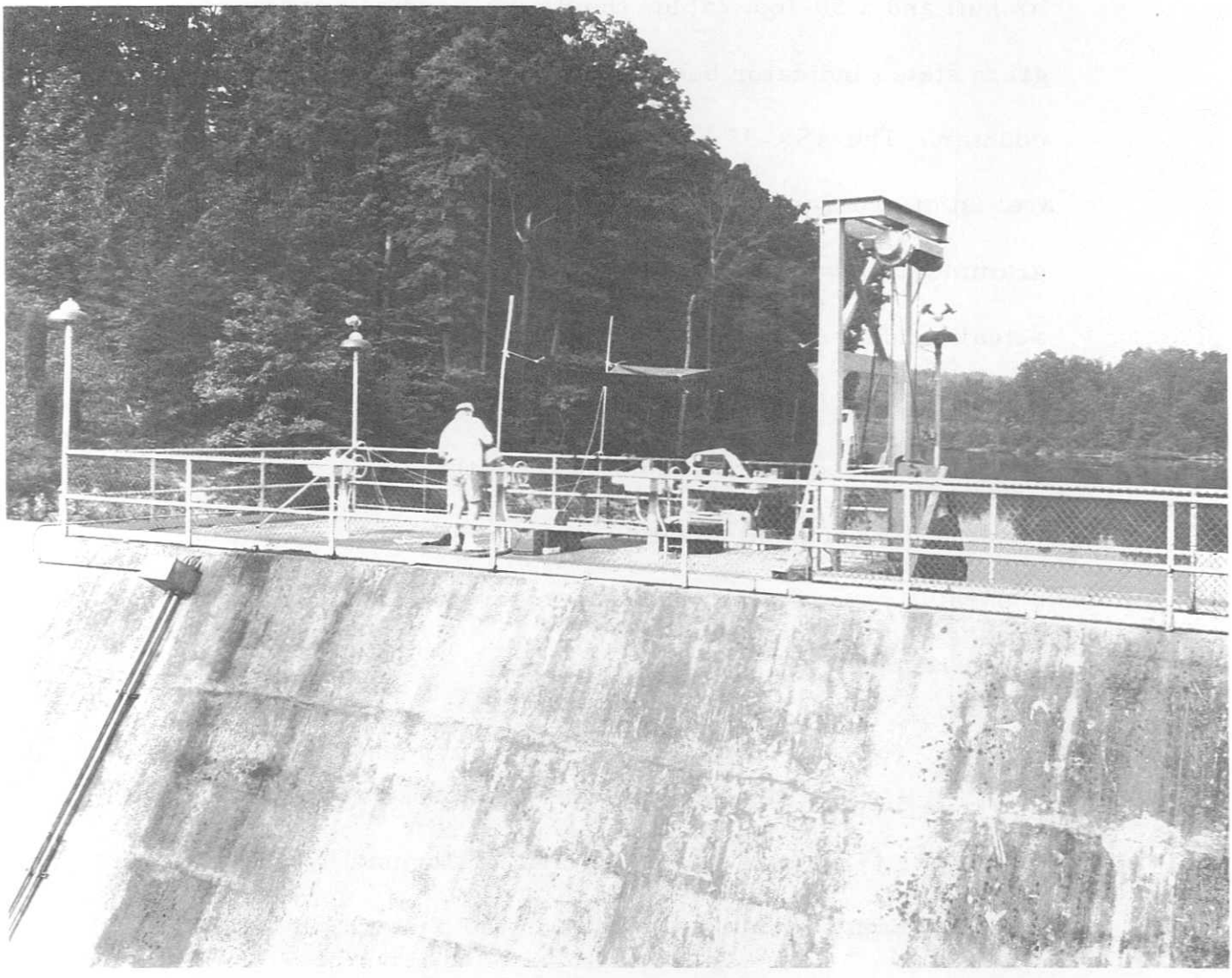
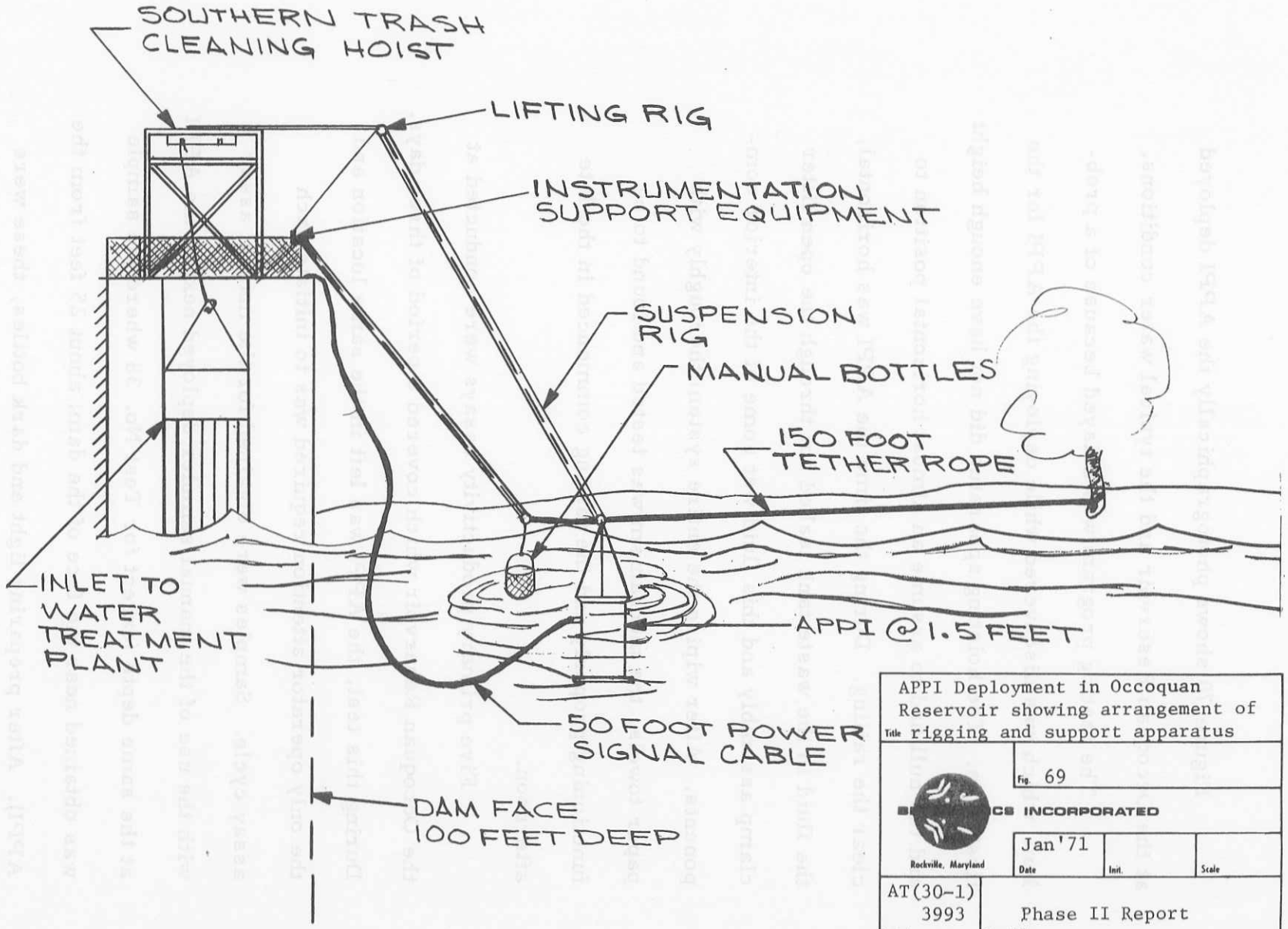


Figure 68
The Occoquan Reservoir Test Site

View of field testing of APPI from dam face.
Reservoir is shown in background



APPI Deployment in Occoquan Reservoir showing arrangement of rigging and support apparatus

Fig. 69

CS INCORPORATED

Rockville, Maryland

Jan'71 (Date)

AT(30-1) 3993 (Cont. No.)

Phase II Report (Report Title)

Figure 70 shows photographically the APPI deployed at the Occoquan Reservoir and the typical water conditions.

The testing program was delayed because of a problem which was discovered while deploying the APPI for the first time. The hoisting apparatus did not have enough height and the hull had to assume an almost horizontal position to clear the railing. During the time the APPI was horizontal, the fluid in the waste tank leaked out through the open filter clamp assembly and this fluid wet some of the interior components. After wiping the entire system thoroughly with paper towels, the mechanism was tested and found to be functioning properly and the testing commenced in the late afternoon.

Five primary productivity assays were conducted at the Occoquan Reservoir which covered a period of three days. During this test, the APPI was left in the same location and the only operator attention required was to initiate each assay cycle. Samples were obtained for the manual assay with the use of the manual sampler deployed next to the APPI at the same depth (except for Test No. 38 where the sample was obtained near the face of the dam; about 25 feet from the APPI). After preparing light and dark bottles, these were loaded into a wire basket and returned to the sampling point for incubation. Upon the initiation of the program to filter

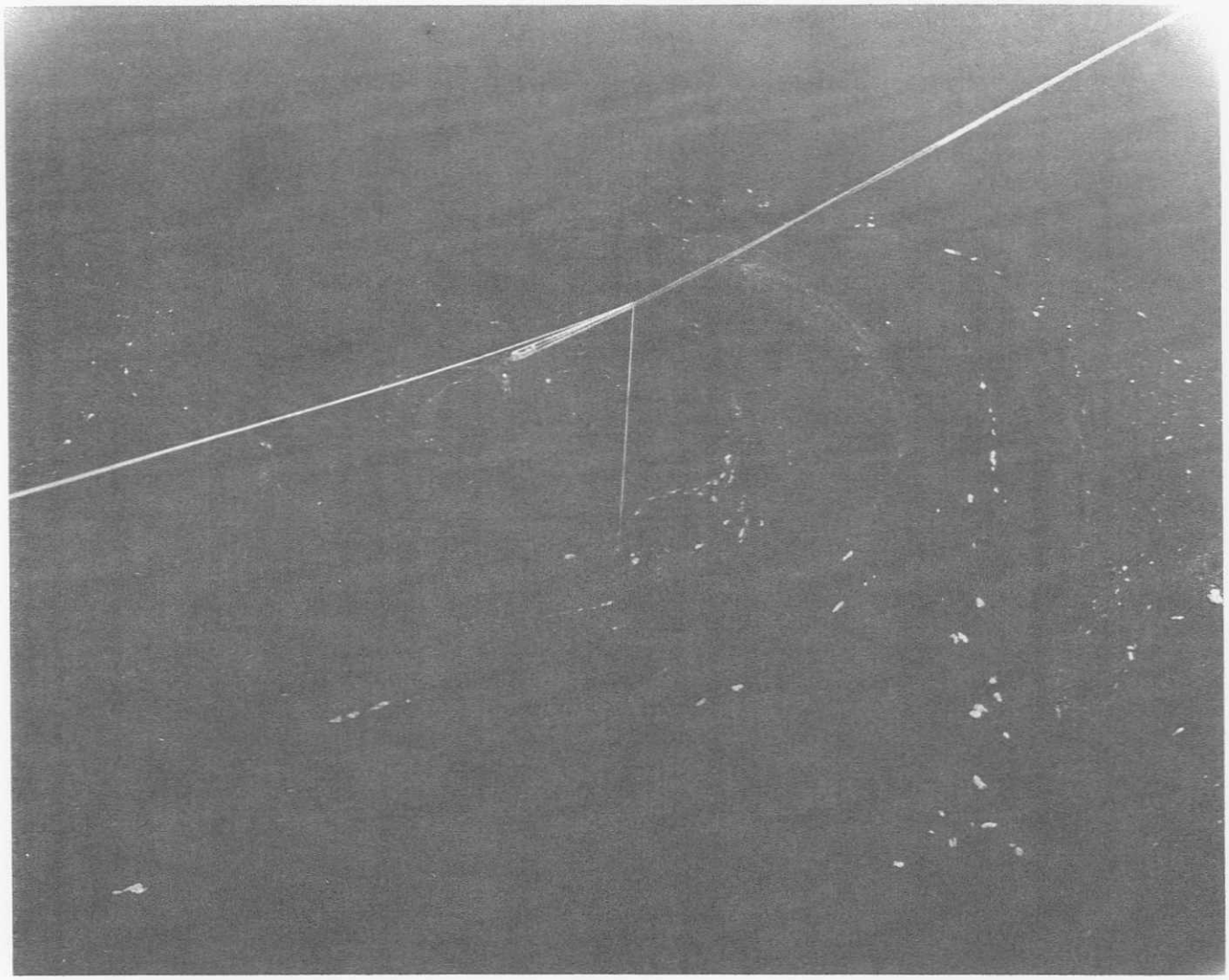


Figure 70
APPI in Occoquan Reservoir

Showing faint outline of hull and manual bottles
suspended in the turbid water

the APPI's light sample, the bottles were brought up and filtered, dried and counted.

The data which were obtained during this test cycle are shown in Table 17. Except for Test No. 38, where there was a significant difference in the locations (about 25 feet apart) where the samples were obtained, the agreement between the APPI and manual assays were excellent with a coefficient of variation of 20%, which predicted a favorable outcome for the second test cycle scheduled for Georgia.

In general, the APPI's operation was excellent although several minor operational problems were encountered during these tests which had little effect on the primary productivity assay data. These problems, which were subsequently corrected before further testing, included:

(a) The liquid spill from the waste described above imposes an added problem. This liquid contains a small quantity of radioisotope and can induce a contamination problem as well as a corrosion and electrical shorting problem. The problem was corrected by always maintaining the instrument's normal attitude during deployment and by routinely removing all liquids from the instrument during transportation.

Table 17

Summary of Data From Occoquan Tests

Test No.	A/M	Date Time of Day	Background cts/100s	Light cts/100s	Dark cts/100s	L-D cts/100s	Ratio: APPI/Man.[1] Light	Dark	L-D	Note
#38	APPI	8/25/70	76	24,800	1,925	22,875	2.3	5.2	2.2	(2)
	Manual	17:25	60	8,820	353	8,467				
#39	APPI	8/26/70	1,120	18,935	2,427	16,508	.91	1.8	.88	
	Manual	09:00	61	16,053	645	15,408				
#40	APPI	8/26/70	2,654	52,530	3,115	49,415	.94	.30	.93	
	Manual	12:35	55	43,599	1,302	43,297				
#41	APPI	8/27/70	3,485	31,200	4,095	27,105	.85	.88	.85	
	Manual	08:25	90	26,578	657	25,921				
#42	APPI	8/27/70	3,973	149,600	4,732	144,868	1.27	.19	1.3	
	Manual	11:55	80	93,744	3,239	90,455				

NOTE: 1 Relative Counter Efficiency (A/M) = 1.224

2 APPI and Manual samples were taken separately 20 feet apart

L-D Average (excluding #38) = .99
Coefficient of Variation = 21%

(b) The time required for the instrument to perform a filtration increased from 4 minutes to over 10 minutes. Examination of the sample pump indicated that the drive mechanism was being overloaded and the power requirements exceeded the output of the slow-speed drive circuit. The pump was completely overhauled after the testing at the Occoquan Reservoir and the driving screw, rider nut, and piston O-rings were replaced. (Bench tests in the laboratory indicated that this problem was corrected. However, the problem again surfaced immediately while setting up the instrument at Lake Lanier, Georgia).

(c) The radiation background which is monitored during each assay cycle was observed to increase from a normal level (less than 100 counts/100 seconds) to over 3,000 counts/100 seconds. The cause of this problem was found to be the free $^{14}CO_2$ which is released by acidification of the waste fluid containing $NaH^{14}CO_3$ during each

assay cycle. This gas is pumped into the hull from the waste tank by the vacuum booster pump. The solution to the problem was found to be the addition of a CO_2 scrubber to the exhaust of the vacuum pump. This device reduced the background buildup by a factor of 10.

4. Lake Lanier, Georgia Test Cycle

After the completion of the instrument modifications detailed above, it was decided that the APPI was ready for the tests at Lake Lanier, Georgia. The instrument and its array of rigging and support equipment were loaded into a station wagon and trailer and driven to Georgia on 3 September 1970.

Friday morning (4 September 1970), Mr. Donald Brockway of FWQA reviewed the test site and aquatic conditions of the Lake. The equipment was loaded onto a 34-foot houseboat that was rented from the Bald Ridge Marina at Cumming, Georgia. The remainder of the day was spent assemblying the support equipment and the APPI was prepared for the first tests.

During the preparatory checkout, it was discovered that the pump drive mechanism, just refurbished before leaving Rockville, was binding to such an extent that it was not

possible to perform an assay. It was necessary to perform emergency repairs to the pump. While performing the repairs, the test site at Flat Creek was visited and Test No. 43 was conducted with the manual technique in order to establish a suitable sampling depth for the joint FWQA-Biospherics tests.

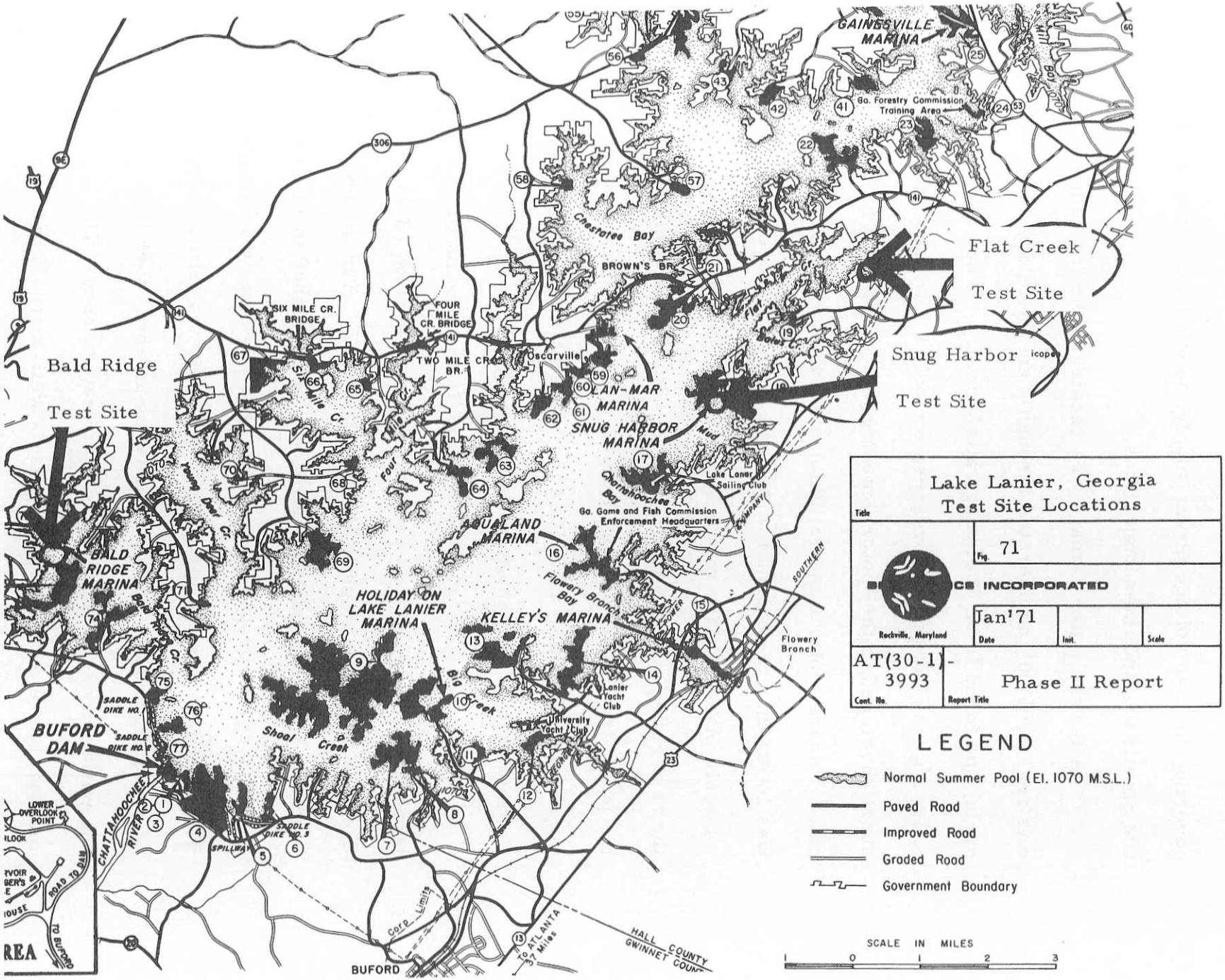
On 6 September 1970, the overhaul of the instrument had been completed and it was decided to commence testing in Flat Creek the next morning. Test No. 44 began as planned, but immediately after initiating the assay program, bubbles appeared near the vicinity of the APPI. It was impossible to be certain that the bubbles were arising from the APPI, since the entire Flat Creek was also bubbling from subsurface gas evolution.

An attempt was made to bring the instrument back on-board to inspect it, but the hoist rigging parted and it took 15 minutes to repair. In the meantime, the bubbling continued. Upon removing the APPI from the water and removing the instrument from the hull, it was found that the sealing flange had become eroded since it was last used at the Occoquan Reservoir and had allowed about 2 gallons of water to cover the interior mechanism (mainly the electronics cannister and sample pump motor). Further inspection of the electronics cannister revealed about $1\frac{1}{2}$ inches of water

covering the high voltage power supply, tape advance mechanism, GM amplifier, and interconnections.

After the water damage was repaired, the instrument was reassembled and operated through a complete dry-run cycle; and found to be operating satisfactorily. When the following test had been in progress for only a few minutes, a short developed in the motor, causing the commutator to burn-out. A spare motor was available, and even though its torque was lower than the original motor and the pump's operation was only marginal, the motor operated satisfactorily for the next eight tests (through Test No. 52). On the ninth test, the pump drive screw stripped which required another major overhaul of the pump as well as the services of a Gainesville, Georgia, mechanic shop to fabricate a new piece.

The test sites which were used in the conduct of this test cycle were described previously in this section (B-1) and are detailed in the maps in Figures 71 and 72. The method to deploy the APPI into the water body is described in Figure 73. It had been proposed originally that this test cycle should be performed with the instrument in operation continuously, at a single location, without manual assistance other than the initiation of the start of each automated assay. The original test site at Flat Creek was a well-traveled area for carp fishermen and the local lake people strongly



Lake Lanier, Georgia
Test Site Locations

Fig. 71

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Rockville, Maryland

Jan'71 (Date) | Init. | Scale

AT(30-1)-3993 (Cont. No.)

Phase II Report (Report Title)

LEGEND

- Normal Summer Pool (El. 1070 M.S.L.)
- Paved Road
- Improved Road
- Graded Road
- Government Boundary

SCALE IN MILES
1 0 1 2 3

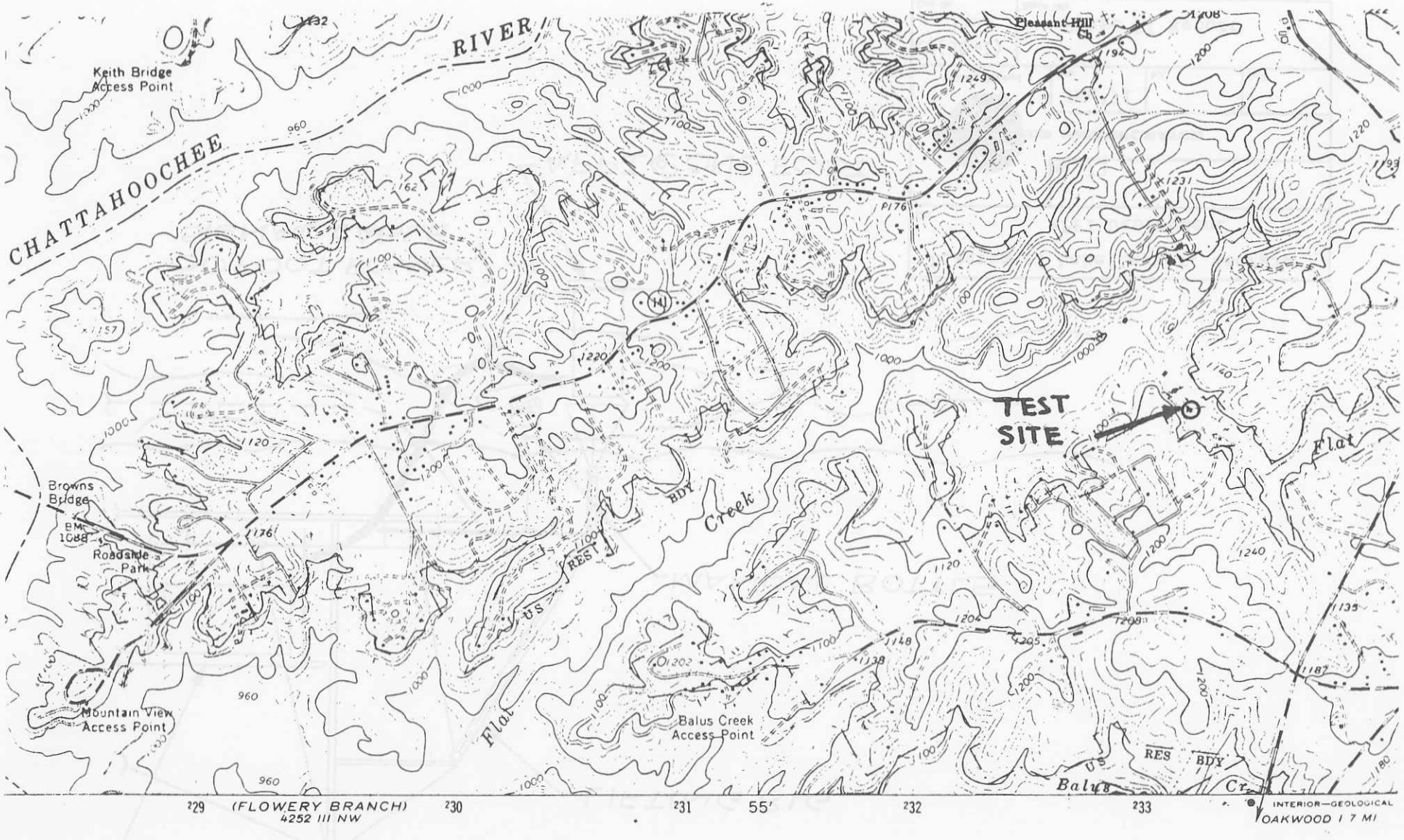

Figure 72

U. S. Geological Survey Map Showing Flat Creek Test Site

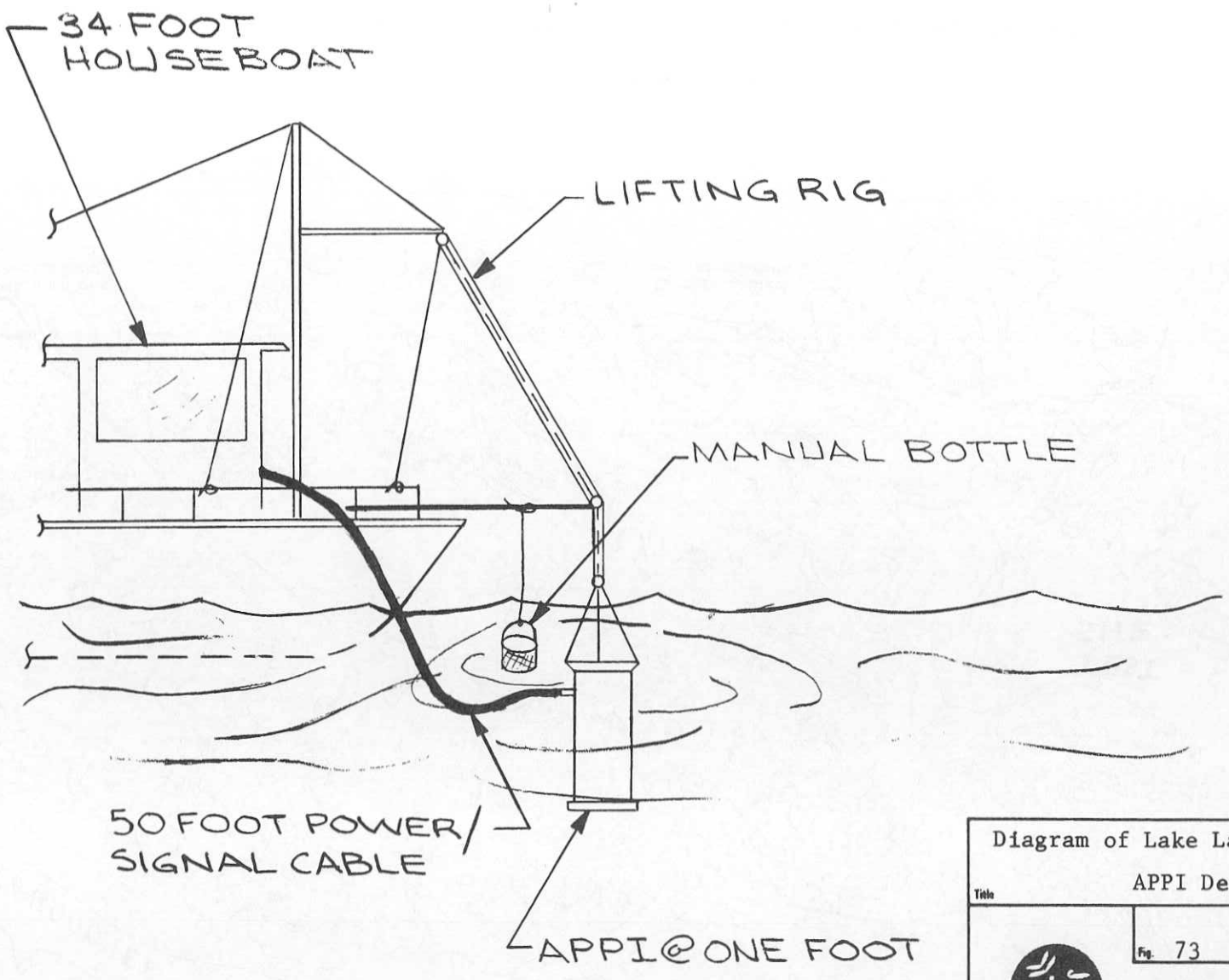

Diagram of Lake Lanier, Georgia
APPI Deployment

Fig. 73

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suggested that the plans be changed to have the instrument kept onboard. Mr. Oscar Bizzell, the AEC Program Manager who participated in these tests, agreed with this alternate plan.

The tests at Lake Lanier were planned so that there was a total of 10 assay cycles to be conducted at a rate of two per day. Personnel from the FWQA (Miss Patricia Kerr and a field group) participated on two days by making parallel primary productivity assays as well as other pertinent measurements which included inorganic carbon. The test cycles were performed in the same manner as the Occoquan Reservoir tests. The APPI was deployed into the water each morning and manual samples were taken at a point next to the inlet and after preparation into the light and dark bottles, they were resubmersed at the same point for incubation. At the end of the day's operation, the APPI was hauled out of the water and stowed on deck. Except for the difficulties with the sample pump noted previously, the APPI performed well during these tests.

A summary of the data taken during the testing at Lake Lanier, Georgia, is shown in Table 18. These results show a large disagreement between the two assays (the APPI and manual) which must be explained. Unfortunately, it was

Table 18

Summary of Data From Lake Lanier, Georgia Tests

Test No. A/M	Date Time of Day	Background cts/100s	Light cts/100s	Dark cts/100s	L-D cts/100s	Ratio: APPI/Man.[1] Light	Dark	L-D	Note
#43 Flat Creek 1 ft.	9/6/70	70	241,525	3,850	237,675				
(Manual only) 3 ft.	14:45	70	245,572	5,050	240,522				
5 ft.		70	35,239	6,133	29,106				
#44 Hull leaked stopped test	9/7/70 07:00								
#45 Flat Creek -APPI	9/8/70	116	42,880	1,531	41,349	2.2	1.5	2.3	
Manual	07:00	71	15,590	843	14,547				
#46 Flat Creek -APPI	9/8/70	164	173,050	2,800	170,250	2.2	.85	2.3	
Manual	12:05	65	62,603	2,551	60,052				
#47 Snug Harbor -APPI	9/9/70	240	12,810	1,930	10,880	5.9	6.2	5.8	(2)
Manual	11:15	52	1,762	270	1,492				
#48 Snug Harbor -APPI	9/9/70	249	9,100	1,772	7,328	3.3	5.3	3.1	
Manual	15:00	66	2,207	296	1,911				
#49 Flat Creek -APPI	9/10/70	-	205,800	3,134	202,666	2.0	1.7	2.0	
Manual	08:32	65	83,203	1,527	81,676				
FWQA (Average)		-	338,000	126,000	212,000	2.6	.11	4.12	
(High)		-	671,000	277,000	394,000	1.3	.05	2.2	
(Low)		-	191,000	40,000	151,000	4.6	.33	5.8	
#50 Flat Creek -APPI	9/10/70	412	529,957	7,182	522,775	2.0	.3	2.2	
Manual	14:30	129	210,281	16,571	193,710				
FWQA (Average)		-	945,000	368,000	577,000	2.4	.08	3.9	
(High)		-	1,005,000	254,000	751,000	2.3	.11	3.0	
(Low)		-	723,000	496,000	227,000	3.2	.06	9.9	

NOTE: 1 Relative Counter Efficiency (A/M) = 1.254
2 Data adjusted for sampling error.

Table 18
(continued)

Summary of Data From Lake Lanier, Georgia Tests

Test No. A/M	Date Time of Day	Background cts/100s	Light cts/100s	Dark cts/100s	L-D cts/100s	Ratio APPI/Man. Light	Dark	L-D	Note
#51 Snug Harbor -APPI	9/10/70 22:00	568	3,400	3,760	-360				
		Test of instrument in dark - no manual test.							
#52 Snug Harbor -APPI	9/11/70	363	8,185	2,364	5,821	6.5	.68	6.5	
Manual	08:10	98	1,052	333	719				
#53 Snug Harbor -APPI	9/11/70	-	2,960	1,150	1,810	2.8	4.0	2.4	(3)
Manual	16:52	-	832	229	603				
#54 Bald Ridge -APPI	9/12/70	174	3,160	2,175	1,085	4.3	8.7	2.4	(4)
Manual	08:50	-	552	184	368				
FWQA (Average)		-	6,620	994	5,622	3.9	17.3	1.7	
(High)		-	13,000	2,800	10,200	2.0	6.1	.91	
(Low)			4,900	643	4,257	5.2	26.8	2.2	

NOTE: 3 Filter tape adjustment error during re-assembly after repair of mechanical problems.
4 Data adjusted for sampling error.

not possible during the tests to determine whether the instrument's operation was failing in some manner which had not been discovered in the previous testing. The problem here was that in most all cases in which component failures are conceivable, the resultant effect on the data would be lower assay data in the APPI, rather than greater values as experienced.

An analysis of the specific problems encountered during these tests again failed to solve the quandry. Also, after re-analyzing the problems which had been encountered in the entire program, it is difficult to attribute any to the explanation of the higher APPI assay.

C. Data Analysis

The data obtained at the Occoquan Reservoir and Lake Lanier were used to calculate the primary productivity values shown in Table 19. The wide disagreement between the manual and APPI results at Lake Lanier (Test Nos. 43 through 54) differs markedly from the Occoquan Reservoir results, (Test Nos. 38 through 42) where there was good agreement.

D. Post-Testing Diagnosis

Subsequent to the field testing, the APPI instrument and its operations, the technique used in the performance of the manual assay, and possible biological reasons for the discrepancy in the test results were investigated.

Table 19

Summary of Primary Productivity Data from * Occoquan Reservoir (38-43) and Lake Lanier (45-54) Tests

Test No.	pH	Inorganic C mg/M^3	Primary Productivity ($\frac{mgC}{M^3 . Hr.}$) APPI	Manual
38	7.0	13,600	106	48
39	6.6	17,000	96	110
40	6.8	13,400	226	224
41	6.8	12,800	118	113
42	7.0	10,200	505	388
43	8.2	6,000	-	599 607 74
45	6.6	5,420	77	33
46	6.8	6,080	354	154
47	7.1	4,200	16	2.6
48	7.1	3,640	9.1	2.9
49	7.4	5,400	374	186 95 (FWQA - Average) 176 (FWQA - High) 68 (FWQA - Low)
50	9.6	3,960	708	323 189 (FWQA - Average) 246 (FWQA - High) 74 (FWQA - Low)
52	8.2	3,600	7.2	1.1
53	7.0	3,830	2.4	1.0
54	7.0	5,220	2.0	.8 1.2 (FWQA - Average) 2.2 (FWQA - High) 0.9 (FWQA - Low)

* These data were computed from primary productivity equation shown in Section VI using L-D data from Tables 17 & 18. R for APPI = 1.55×10^6, for Manual = 1.26×10^6, for FWQA (49 & 50) = 6.40×10^6, and FWQA (54) = 1.29×10^7.

1. Instrument Checkout

The various components of the instrument were subjected to a step-by-step examination. The first of these tests was to recount the radioactivity on the manual assay filters to verify the original data. Results from this test did not show a significant difference from the counts obtained at Lanier.

The next test was the recheck of the APPI filter tape to verify the original count. This test was performed by stretching the tape on the shelf of the manual assay counter in the same position in which the manual filters are counted. The data from this test were compared to the original count to compute a ratio of rerun/original. These are summarized in Table 20, which shows that the average of the light assay ratios was 0.78 and dark assay ratios was 0.77. In previous tests, when the manual counter's efficiency was experimentally compared to the APPI counter, the ratio was found to be 0.80. This test had shown conclusive evidence that the radioactivity counting of the APPI had not contributed to the error in the Lanier test results.

In another analysis, the data taken during the Lanier tests were analyzed to verify the instrument's self-checking feature. In the operational program, each assay commences with a radiation check of the previous light bottle filter by

Table 20

Recheck of APPI Filter Tape from Lake Lanier Test

Tests were made of radioactivity on APPI filter tape by positioning the sample discs for each test in the manual filter assay apparatus. Previous ratio of Manual/APPI counter was found to be .80.

Test No.	Light Assay		Ratio	Dark Assay		Ratio
	Orig. Data (1)	Recheck (2)	(2)/(1)	Orig. Data (1)	Recheck (2)	(2)/(1)
45	42,880	36,650	.85	1,531	1,220	.80
46	173,050	115,340	.67	2,800	2,490	.89
47	9,606	7,135	.74	2,414	1,610	.67
48	9,100	6,960	.77	1,772	1,420	.80
49	205,800	174,550	.85	3,134	2,410	.77
50	529,957	324,320	.61	7,182	6,220	.87
51	3,400	2,894	.85	3,760	2,680	.79
52	8,185	5,956	.85	2.364	1,671	.71
54	3,788	2,900	.75	1,739	1,150	.66
Average of ratios:			.78			.77

of the previous light assay data, the proper functioning of the radiation detector and its associated electronics, and a third data point in the data record. The counter verification data are summarized in Table 21, which shows that the difference between the first two assays and the re-check on the third was small. The high levels of radio-activity on the filters may possibly have been caused by incompletely removed inorganic ^{14}C. If this was the case, a more rigorous acid treatment of the filters would further reduce their activity. The filters from Test Nos. 46 and 47 were counted and then placed over fuming concentrated HCl acid for 30 minutes. The filters were again counted and the data compared. Table 22 is a summary of these data, and shows that there is no measurable difference in the filters before or after treatment. Also, the differences between this test and the recheck tests are not significant.

A retest of the operation of the GM counters was also performed to assure that the detectors were being operated in a stable region. Figure 74 shows a plot of the plateau for the APPI's detector and indicates that the selection of 900 volts for an operating voltage was correct. The plateau was plotted with the use of two different sources,

Table 21

APPI Counter Verification Test

Test No.	Date/Time of Day	Counts/100 Sec	% Difference
38	Aug. 25 19:30	24,800 (1)	
Rerun	Aug. 26 11:30	24,493	-1.8%
39	Aug. 26 11:45	18,935 (1)	
Rerun	Aug. 26 15:15	18,814	-0.8%
40	Aug. 26 15:45	52,530 (1)	
Rerun	Aug. 26 10:45	51,541	-1.8%
46	Sept. 8 14:45	173,050 (1)	
Rerun	Sept. 9 13:45	170,461	-1.5%
47	Sept. 9 14:15	9,605 (1)	
Rerun	Sept. 9 17:45	9,213	-4.3%
49	Sept.10 11:15	205,800 (1)	
Rerun	Sept.10 17:00	206,139	+0.15%
50	Sept.10 17:30	529,957 (1)	
Rerun	Sept.11 00:45	510,198	-3.9%
Rerun	Sept.22 10:00	479,572 (2)	-10.2%
51	Sept.11 01:15	3,400 (1)	
Rerun	Sept.11 10:45	3,415	+0.44%
52	Sept.11 11:15	8,185 (1)	
Rerun	Sept.11 19:30	8,042	-1.8%
53	Sept.11 19:50	1,714 (1)	
Rerun	Sept.12 11:45	1,638	-4.7%

Note: (1) indicates original data

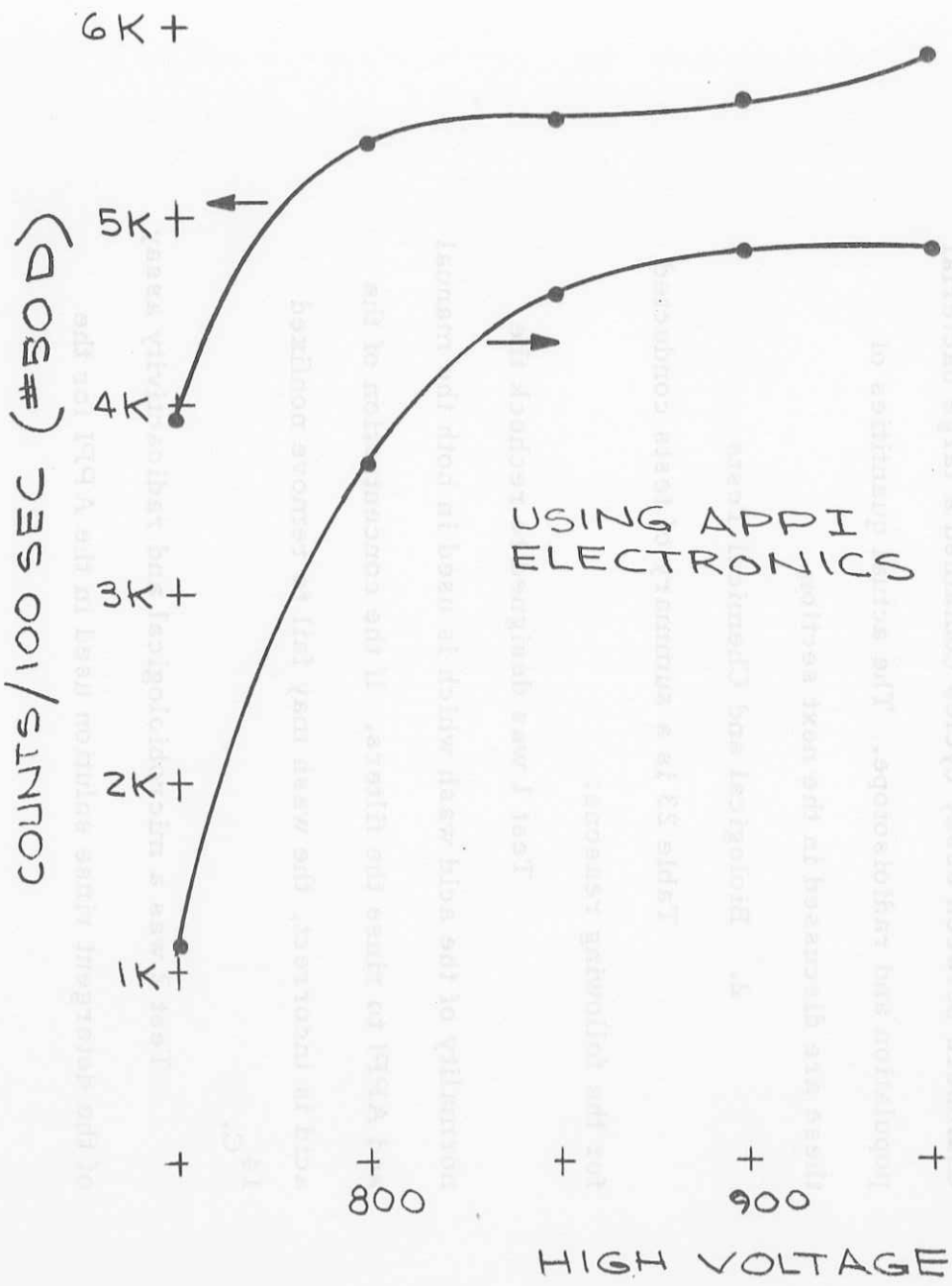
(2) repositioned in APPI detector after 12 days

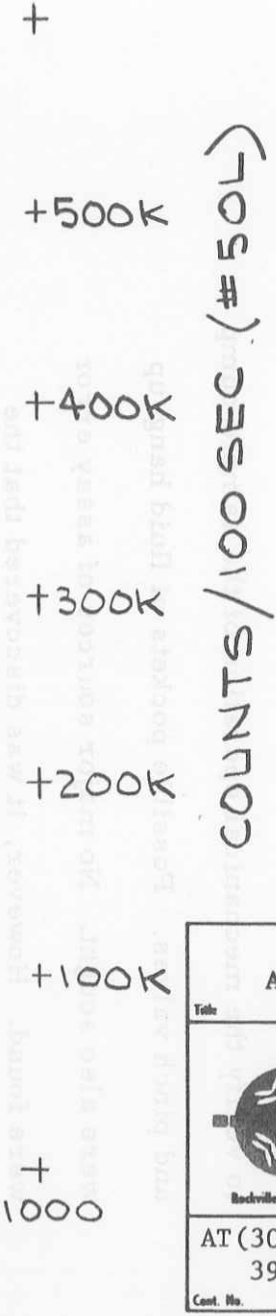
Table 22

Results from Acid Fuming of Filters

Test No.	Recheck 9/15	Acid Fuming 11/30 Before	Acid Fuming 11/30 After
46 Dark	2,490	1,958	1,932
46 Light	115,340	123,135	123,346
47 Dark	1,610	1,506	1,520
47 Light	7,135	5,176	5,279

Background: 51





Title	APPI Detector Tests
	Fig. 74
Rockville, Maryland	CS INCORPORATED
Date: Jan'71	Init. / Scale
Cont. No.: AT(30-1) 3993	Report Title: Phase II Report

one in the 400,000 counts/100 second region and the other in the 5,000 counts/100 second region. The plateau with the higher source is shortened because of dead-time losses created by the differences in quenching in the detector.

Several other tests and analyses were conducted to verify the mechanical operation of the sample pump and pinch valves. Possible pockets of fluid hang-up were also sought. No major sources of assay error were found. However, it was discovered that the detergent rinse which is stored in the light and dark chambers between assay cycles contained a large bacterial population and radioisotope. The actual quantities of these are discussed in the next section.

2. Biological and Chemical Tests

Table 23 is a summary of tests conducted for the following reasons:

Test 1 was designed to recheck the normality of the acid wash which is used in both the manual and APPI to rinse the filters. If the concentration of the acid is incorrect, the wash may fail to remove nonfixed ^{14}C.

Test 2 was a microbiological and radioactivity assay of the detergent rinse solution used in the APPI for the

Table 23

Summary of Preliminary Diagnostic Laboratory Tests Performed After Georgia Tests

Test 1: Examination of filter wash solution to check normality (0.01N H_2SO_4).

a. Manual reagent: 0.010N H_2SO_4
b. ETM reagent: 0.012N H_2SO_4

Test 2: Assay of detergent rinse from ETM reservoir. (This fluid had been used to rinse the incubation chambers between assay cycles).

a. Total plate count: 50,000 cells/ml
b. Assay for algae by incubating for one-week in light (4-500 ft. candles), with air (plus 5% CO_2) bubbled. No algae isolated in this test
c. Assay of solids: 22 cpm/ml filtered liquid
d. Filtered liquid assay: 0.02 uCi/ml (Sample is inoculated to 0.06 uCi/ml)

Test 3: Determination of "R" for primary productivity calculation:

$$R\ (ETM) = 2.58 \times 10^5 \frac{\text{counts}}{100\ \text{sec} \times \text{uCi}}$$

$$R\ (Manual) = 2.1 \times 10^5 \frac{\text{counts}}{100\ \text{sec} \times \text{uCi}}$$

Table 23
(continued)

Test 4: Comparative test of the effect of scraping walls of manual light and dark bottles to simulate AMML piston action.

Sample	Light Bottle		Dark Bottle	
	No Scraping	With Scraping	No Scraping	With Scraping
1. West River Water	7,731	6,844	200	219
2. Detergent from ETM	804	794	-	-
3. Snug Harbor, Ga., Water	11,450	11,256	143	146
4. Flat Creek, Ga., Water	53,097	49,297	1,164	1,184

(Data in counts/100 sec)

Test 5: Determination of the effect of detergent solution (0.5% "Woolite") contamination upon primary productivity of Flat Creek, Ga., water. 125 ppm PO_4

Detergent Solution Added (%)	Light (Counts/100 sec)	Dark
0.0005	13,010	498
0.005	11,475	420
0.05	10,760	312
0.5	10,315	364
5.0	9,795	296

Lanier tests. It was found there was a significant bacterial population in the detergent which must have been removed from the walls of the incubation chambers as they were rinsed. However, only a few algae were isolated in this test after about four weeks of incubation. The tests also indicated a sizeable quantity of isotope in the detergent which was analyzed.

Test 3 was the experiment which was used to determine the counter efficiency which was needed for the calculation of primary productivity. The experiment was performed by the technique described by Strickland (5).

Test 4 was used to test a hypothesis that suggested that the water samples in the Lanier tests contained algae which could have adhered to the walls of the manual bottles. In this test, replicates of samples were processed identically except that removal of one sample was accompanied with policing the bottle walls onto the filter. The results from the test did not show a significant difference between policed and nonpoliced samples.

Water samples from Lake Lanier in Georgia were collected by the FWQA at the two primary test sites and sent by air to Rockville for the tests.

Test 5 was used to determine the effect on growth that small concentrations of the detergent might have if

this were accidently incorporated into the sample in the APPI. Addition of detergent appears to have a negative effect.

None of the above tests provided an explanation to the problems with the Lanier tests.

3. Expert Opinions

Several noted experts were contacted to obtain their opinions about the results from the Lanier tests. The first of these was Dr. Robert Krauss of the University of Maryland. After briefly reviewing the factors involved, he expressed an opinion that if there had been sizeable errors in the quantity of isotope which were added to the samples, this would certainly be a reasonable explanation. Dr. Krauss further expressed his opinion that light and pressure shocks could have accounted for temporary reduction in the photosynthesis rates of the algae in the manual samples. Such shocks might occur when the samples for the manual assay are brought to the surface and manipulated in preparation for their resubmergence for incubation.

Late in November 1970, the Principal Investigator and Program Manager for the project had an opportunity to visit the National Marine Fisheries Service Laboratory of the National Oceanic & Atmospheric Administration at La Jolla, California and discuss the Lake Lanier data with two of the

staff members there (Mr. Michael Kruse and Dr. William Thomas). They stated that their experience with primary productivity measurements has shown that light shock produces an adverse effect on the organisms. Mr. Kruse gave us several examples of this effect which he had observed in data from their current ocean studies. Dr. Richard Eppley of Scripps Institution of Oceanography was contacted and he agreed to the possibility that this effect could contribute toward the Lanier data disagreement. Other factors which were discussed included:

(a) Differences in the samples acquired by the instrument and bottle for the manual technique.

(b) The high turbidity of the water body causes light intensity changes in the incubation chamber which have the same effect as though the samples had been obtained at slightly different depths.

(c) Techniques in handling the manual samples during preparation.

A recent article by Marvin Gibbs was reviewed (8) which offered another plausible reason for the data differences at Lake Lanier. This article is a review of literature and laboratory work which discusses the effect of

oxygen levels in a water body in inhibiting primary productivity. It also shows that this inhibition is greater when the bicarbonate concentrations in the water body are low.

The bicarbonate concentration measurements from our field tests have been compiled in Table 24. A review of this data shows that the Lake Lanier bicarbonate levels were in the range where Gibbs reported maximum adverse effect on photosynthesis by oxygen (see Figure 75, which is reproduced from reference 8). Although the bicarbonate levels at the Occoquan Reservoir are not much greater than those measured at Lake Lanier, an additional difference exists in that the Reservoir is aerated extensively near the location where the APPI tests were performed. Therefore, the manual samples taken here would undergo minimum oxygen concentration changes as a result of the pouring back and forth which occurs in adding the isotope to the sample and dividing it into light and dark bottles for resubmergence. Thus, in terms of this effect, it would be expected that the manual and APPI results should agree. This was the result from the Occoquan Reservoir tests.

Quite a different situation existed at Lake Lanier. There was no aeration, and as a matter of fact, the Flat

Table 24

Summary of Carbonate Data

Test No.	Date/Time	Location	pH	Water Temperature °C	Total Inorganic C mM/l	Bicarbonate mM/l
#38	Aug. 26 17:25	Occoquan	7.0	29.5°	1.14	.926
#39	Aug. 26 09:00	Occoquan	6.6	27°	1.42	.898
#40	Aug. 26 12:35	Occoquan	6.8	28°	1.12	.709
#41	Aug. 27 08:25	Occoquan	6.8	27°	1.07	.677
#42	Aug. 27 11:55	Occoquan	7.0	27°	.83	.675
#45	Sept. 8 07:00	Flat Creek	6.6	28°	.45	.284
#46	Sept. 8 12:05	Flat Creek	6.8	29°	.51	.323
#47	Sept. 9 11:15	Snug Harbor	7.1	28°	.35	.296
#48	Sept. 9 15:00	Snug Harbor	7.1	29°	.30	.252
#49	Sept. 10 08:32	Flat Creek	7.4	28°	.45	.413
#50	Sept. 10 14:30	Flat Creek	9.6	29°	.33	.270
#52	Sept. 11 08:10	Snug Harbor	8.2	27.5°	.30	.272
#53	Sept. 11 10:55	Snug Harbor	7.0	30°	.32	.26
#54	Sept. 12 08:50	Bald Ridge	7.0	27°	.44	.35

Carbon added in test = 0.003 mM/l, and at pH = 7.0, the $\left[HCO_3^-\right]$ added is only 0.0024 mM/l, an insignificant amount.

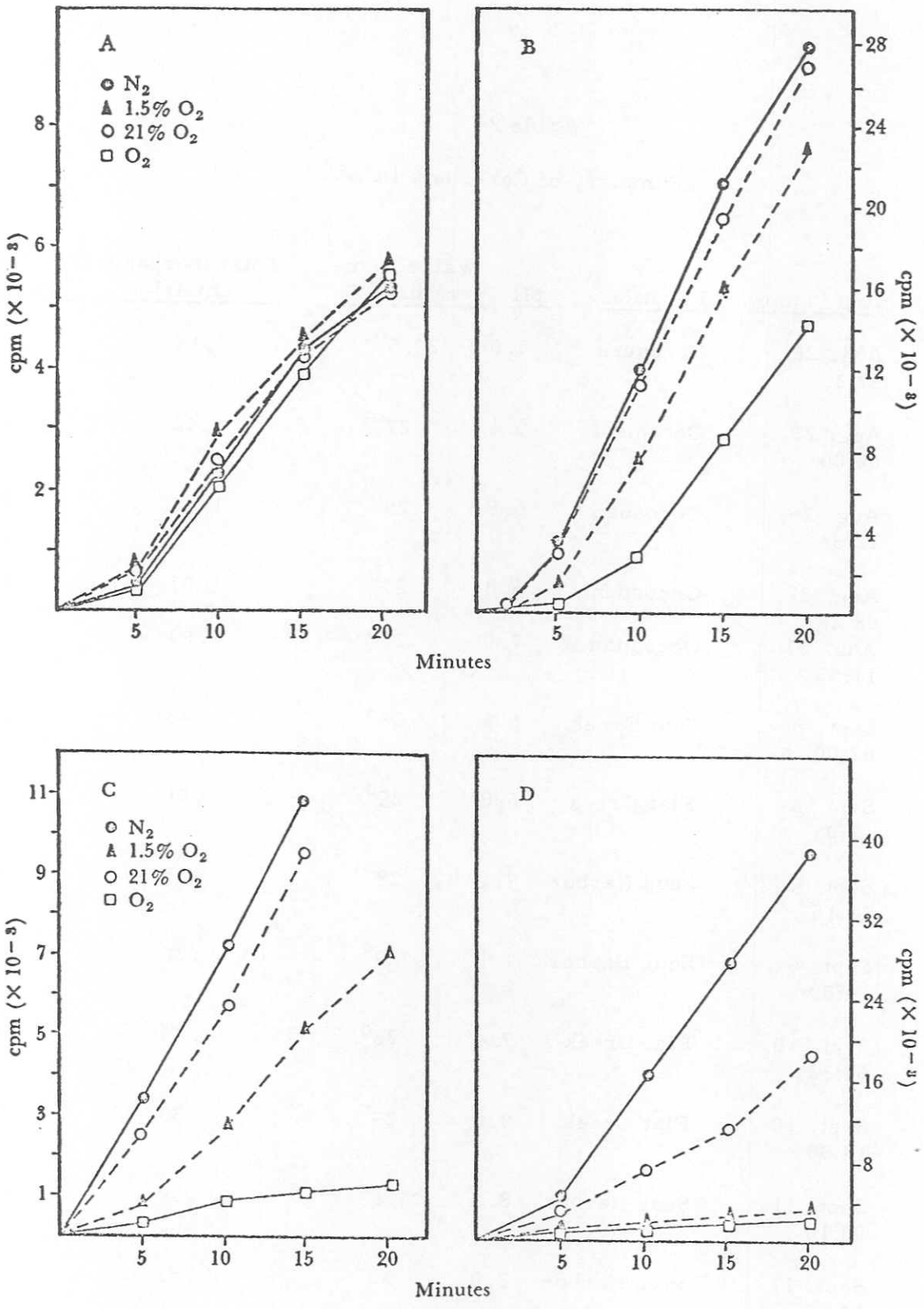


Figure 75
(from Reference 8)

The interdependence of oxygen and bicarbonate concentrations on photosynthesis measured as the uptake of radioactivity by spinach chloroplasts. In each case gas phases were 100% N_2 (●), 1.5% O_2 and 98.5% N_2 (▲), 21% O_2 and 79% N_2 (○) and 100% O_2 (□). Bicarbonate concentrations were: (A) 13 millimolar; (B) 10 millimolar; (C) 4 millimolar; (D) 1 millimolar. Note that at the highest bicarbonate level (A) used, the result was a complete elimination of oxygen inhibition.

Creek appeared to be septic, with bubbles constantly breaking on the surface. Hence, when the manual samples were brought to the surface at Lake Lanier and manipulated, considerable changes in oxygen tension in the samples occurred. These oxygenated samples were then introduced into light and dark bottles and placed beneath the surface for the incubation period. Thus, the combination of low bicarbonate and increased oxygen concentrations were present and now one would expect that the manual samples would suffer a significant diminution in primary productivity. The APPI, on the other hand, conducting its assay in situ would not have subjected its sample to this change in oxygen concentration.

IX. STATUS

A. Program

Phases I and II of the APPI program have now been completed. The instrument was developed to fulfill the operational requirements of interested user agencies. Various expert oceanographers and limnologists who were consulted during the program, or who wrote upon hearing of the program, supported the need for such an instrument in the conduct of both basic and applied studies.

The APPI has now been fabricated and tested under a variety of laboratory and field conditions. The objectives of the program executed to date - the design, fabrication, and demonstration of an engineering feasibility model - have essentially been achieved. However, a major mystery - that of the Lake Lanier test results - requires clarification. It is quite possible that the explanation may add proof to the fundamental advantage predicted for the APPI - the superiority of an in situ measurement. If, on the other hand, some electronic or mechanical defect is found to have caused the discrepancy, this can hopefully be fixed without requiring major redesign of the instrument.

The other problems which have developed during the course of the feasibility testing of the instrument are relatively minor and subject to straight forward

engineering solutions. These problems are detailed below.

B. Instrument

Except for the possible problem discussed in the preceding paragraph, the problems remaining in the APPI can be readily corrected to permit an unequivocal demonstration of feasibility of the APPI.

1. Detergent Contamination

The detergent system designed for internal cleansing of the APPI plumbing between samples permits the recycled detergent solution to become contaminated with radiocarbon and microorganisms.

2. Carbon Dioxide Scrubber

The scrubber to absorb $^{14}CO_2$ does not have the capacity for complete scavenging. As a result, the background level in the counting chamber slowly rises.

3. Photosynthesis Chamber

Cost considerations precluded the fabrication of a solid glass photosynthesis window and chamber. Fabrication of the original, all glass design would improve the optics of the system.

4. Sample Pump

Sample pump failure occurred as a combined

result of the drive screw design and inadequate motor torque.

5. Hull Leak

As the result of corrosion of the hull sealing flange, a slight leakage developed during a subsequent field test. A remachining of the flange surface has corrected the problem, but the potential for a repeat exists.

6. High Voltage Power Supply

During post-test evaluation of the instrument, the high voltage power supply burned out as the result of a hook-up error.

7. Filter Tape

Although development of the filter tape mechanism has been highly successful, the system can be somewhat improved by eliminating curl from the dry tape. When in the radioactivity counting chamber, the curl in the tape causes slight changes in the counting geometry.

C. Usefulness of Present APPI

1. The most direct application of the feasibility model of the APPI is to use it in determining the reason for the observed differences between the manual and APPI results.

2. Having successfully established the validity of the APPI field results, or having corrected the cause of the data discrepancy, the instrument will be of great use in the development of a true prototype.

3. In addition to its use in prototype development, the engineering feasibility model of the APPI can be used in the field for primary productivity studies. Such studies, of course, would be time-limited by the reagent capacity of the feasibility instrument. However, the several days of operation permitted would be suitable for some eutrophication studies and studies of the effects of temperature from cooling water discharges on, for example, the growth of algae. These uses might best be met by programming the instrument to conduct only one assay per day, thereby extending maintenance trips to a weekly basis. Another use of the instrument would be in making studies on the effect of nutrients or toxic materials on algal growth. For example, phosphate in

varying quantities might be added to natural or cultured algae populations to quantify the effect of that nutrient on primary productivity.

D. Deployable Model

A general design of a deployable model of the APPI has been made. A recoverable unit of this type could be developed for oceanographic or limnological studies.

E. Continuation of Project

A proposal to resolve the remaining problems on the APPI and to effect a complete field demonstration of the refurbished instrument has been submitted to the AEC. Upon satisfactory completion of this demonstration, Phase III of the project would begin. This phase would constitute the design, construction, and testing of a full prototype of the APPI.

F. Interested Potential Users

During the course of the project, the following potential user agencies have expressed an interest in purchasing commercial models of the APPI when these become available:

- Federal Water Quality Administration
- Bureau of Commercial Fisheries
- Atomic Energy of Canada, Ltd.

- Tennessee Valley Authority
- Scripps Institution of Oceanography
- Long Island Lighting Company
- State of Maryland
- University of Maryland
- State University of New York at Stony Brook

X. APPENDICES

I. Deployable Concept Instrument Design

II. Manual Primary Productivity Procedure Used for Comparisons with APPI Data

III. Programming Details for APPI Control of the Primary Productivity Assay

IV. APPI Test Plan

APPENDIX I

Deployable Concept Instrument Design

A. Background

Following the completion and submittal of the Phase I Report, extensive discussions concerning the conceptual design were held with several eminent marine scientists who felt that the direct development of an automated buoy system to monitor primary productivity in the ocean was a relatively large, and therefore risky, technological step. They recommended, and the AEC approved, an interim step in which a somewhat less sophisticated device could be built for shipboard deployment.

This is a description of the alternate design concept which embodies the advice received during our visits to The Johns Hopkins University, Scripps Institution of Oceanography, and the Bureau of Commercial Fisheries. These visits are also detailed in a report to the AEC. The preliminary design was submitted to the AEC on 29 March 1969, for an instrument that could be deployed over the side of a vessel and left unattended to take a single point measurement (rather than used as an autonomous, self-contained automatic buoy system). At some time later, the instrument must be recovered and the filters removed and assayed onboard the vessel.

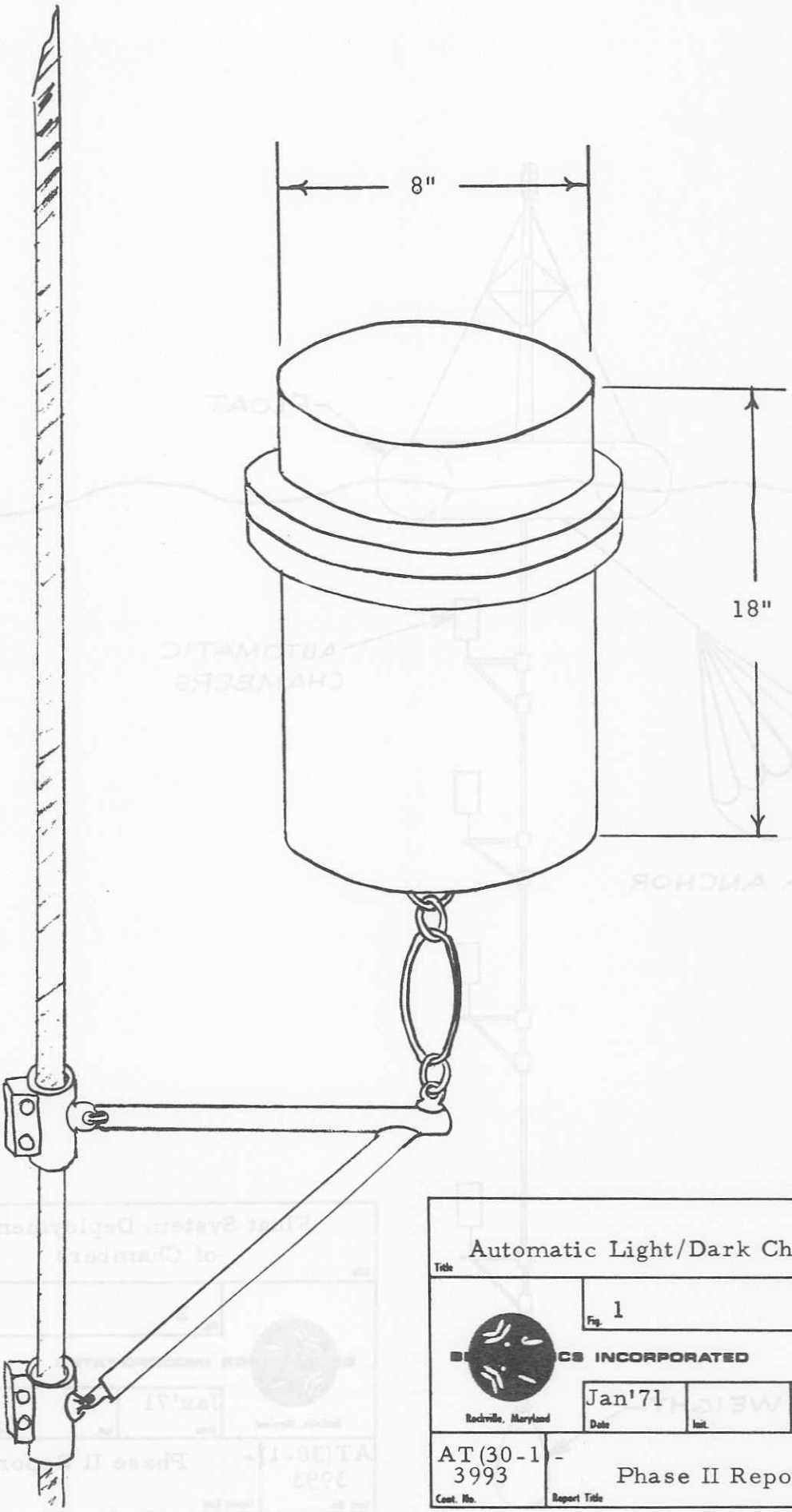
APPENDIX I
(continued - page 2)

B. System Concept

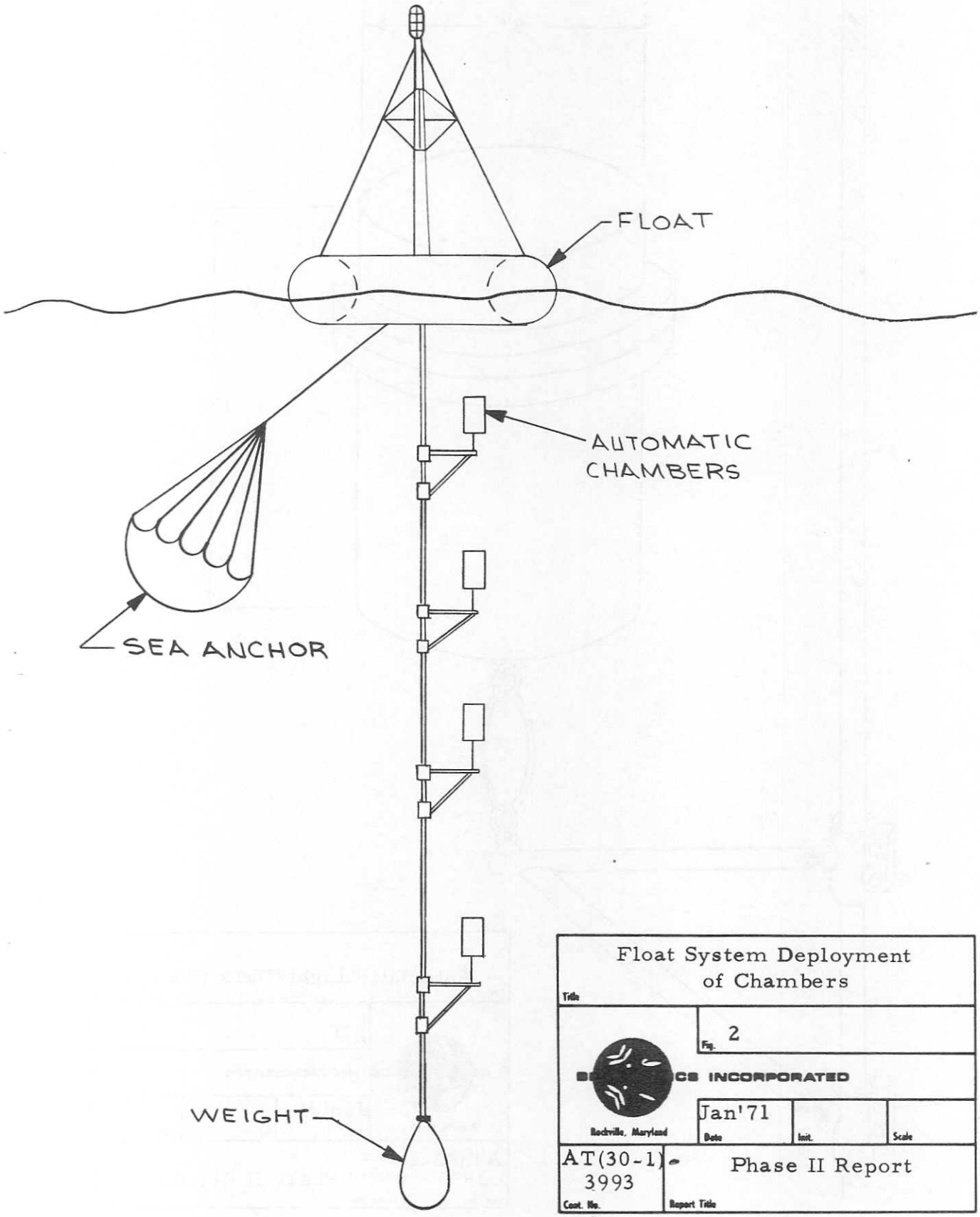
Figure 1 shows the automatic light/dark chamber which is a completely self-supporting instrument. These chambers can be fixed to a cable from a surface float singly or in a "string" with the float also serving to mark the location of deployment. Figure 2 shows a typical "string" of chambers which will automatically take samples from different depths for a parallel measurement of primary productivity in a water column.

In operation, the system is deployed overboard and the chambers sink to their preset depths. After a suitable preset equilibration time interval, the chambers collect their individual samples. ^{14}C is added and the samples are allowed to incubate in a light and dark chamber for two hours. At the end of the two hours, while still in situ, the sample is filtered by the actuation of the automatic chamber to collect the organisms, and they remain at depth until the float system is recovered.

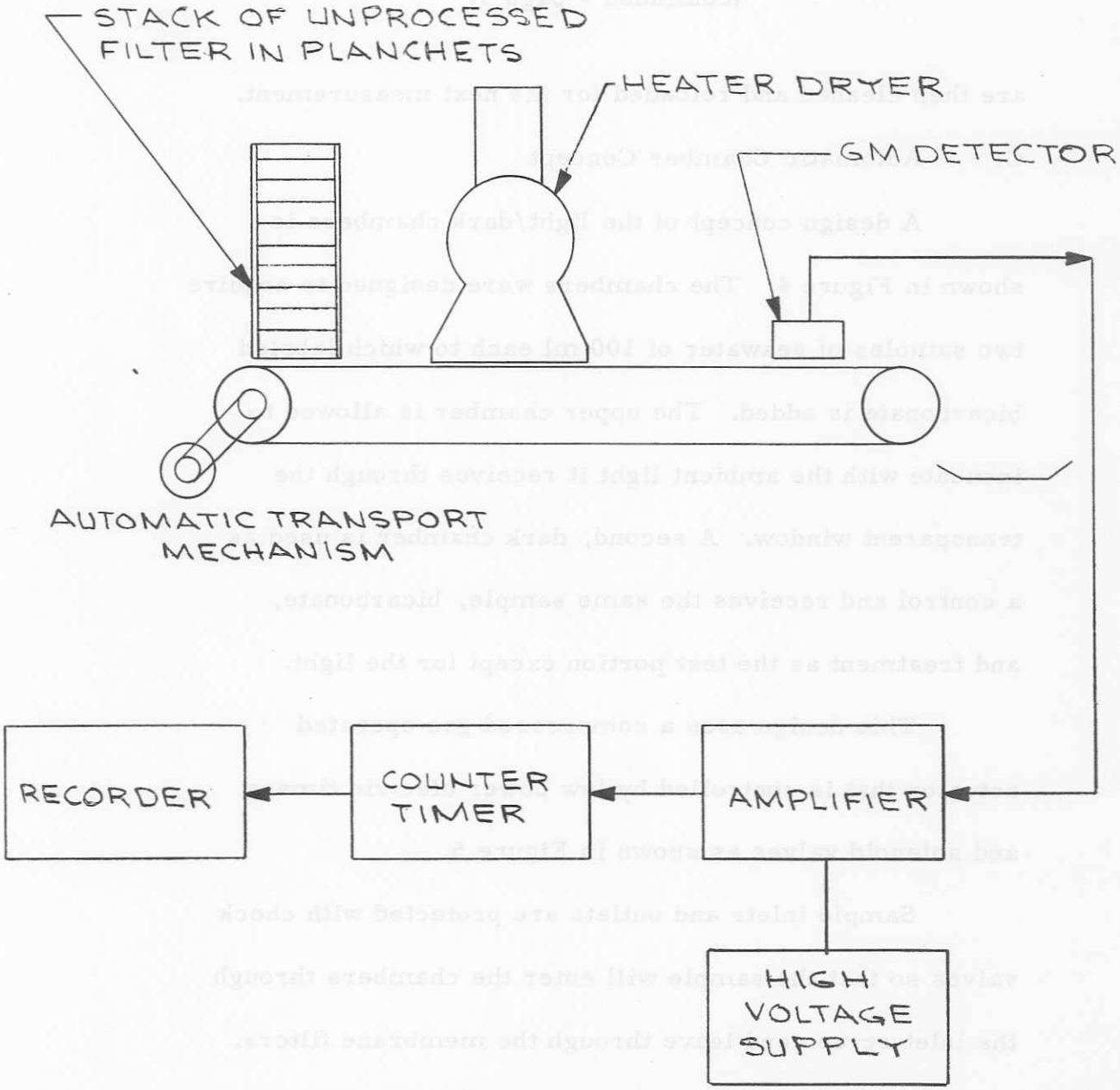
Upon retrieval, the filters are removed and processed by the automatic shipboard instrumentation shown in Figure 3, which dries and assays each filter for ^{14}C. The output data are recorded for permanent retention. The automatic chambers



Title	Automatic Light/Dark Chamber
Fig.	1
	CS INCORPORATED
Rockville, Maryland	Jan'71 (Date) / (Inst.) / (Scale)
Cont. No.	AT(30-1)-3993
Report Title	Phase II Report



Title: Float System Deployment of Chambers	
	Fig. 2
[illegible]CS INCORPORATED	
Rockville, Maryland	Date: Jan'71 / Init. / Scale
Cont. No.: AT(30-1) 3993	Report Title: Phase II Report



Title	Automatic Shipboard Filter Processing Apparatus
Fig.	3
	CS INCORPORATED
Rockville, Maryland	Jan'71 (Date) / Init. / Scale
Cont. No. AT(30-1)-3993	Report Title: Phase II Report

APPENDIX I
(continued - page 3)

are then cleaned and reloaded for the next measurement.

C. Automatic Chamber Concept

A design concept of the light/dark chambers is shown in Figure 4. The chambers were designed to acquire two samples of seawater of 100 ml each to which labeled bicarbonate is added. The upper chamber is allowed to incubate with the ambient light it receives through the transparent window. A second, dark chamber is used as a control and receives the same sample, bicarbonate, and treatment as the test portion except for the light.

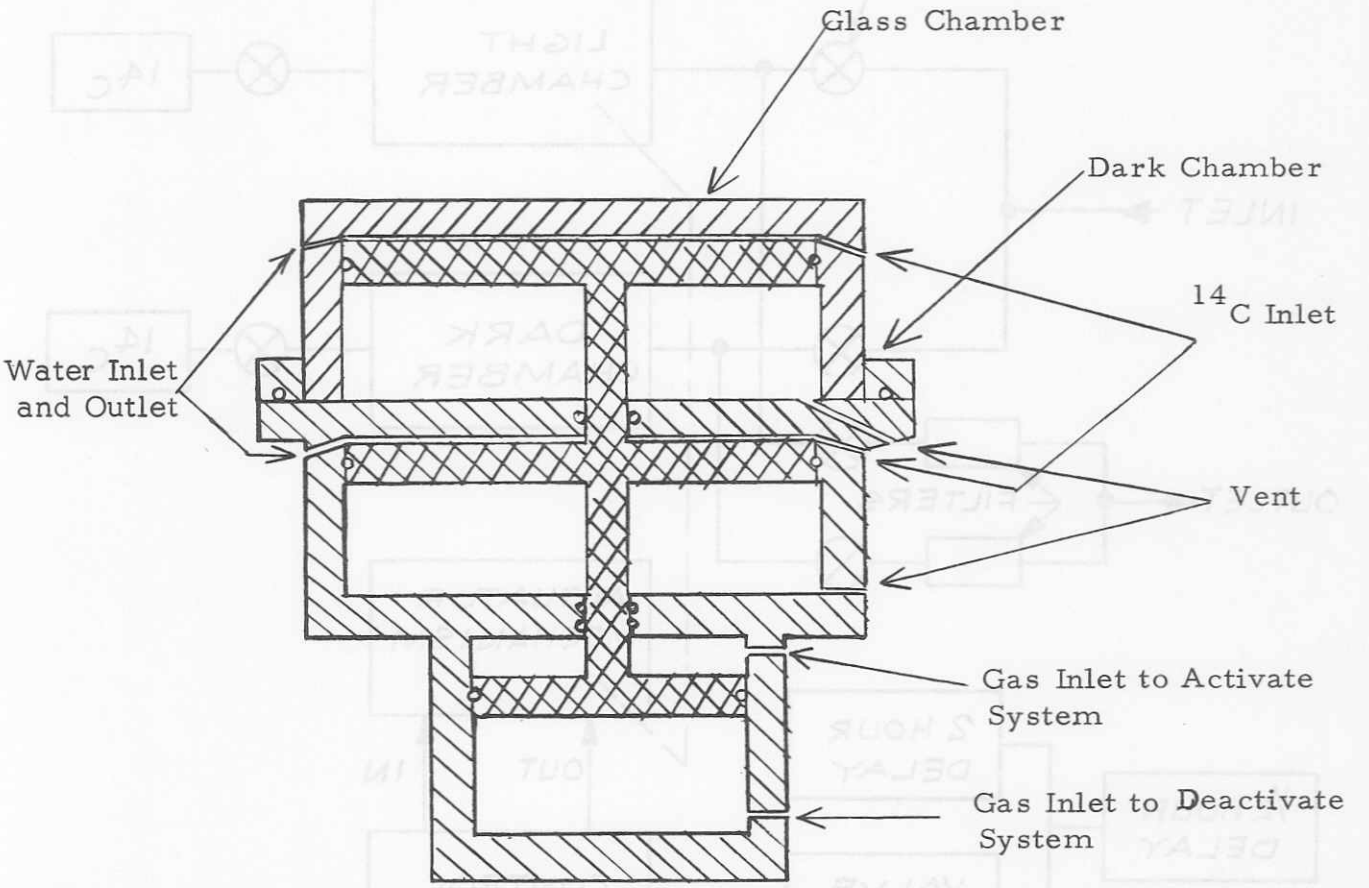
This design uses a compressed-gas-operated actuator that is controlled by low power electric timers and solenoid valves as shown in Figure 5.

Sample inlets and outlets are protected with check valves so that the sample will enter the chambers through the inlet screen and leave through the membrane filters.

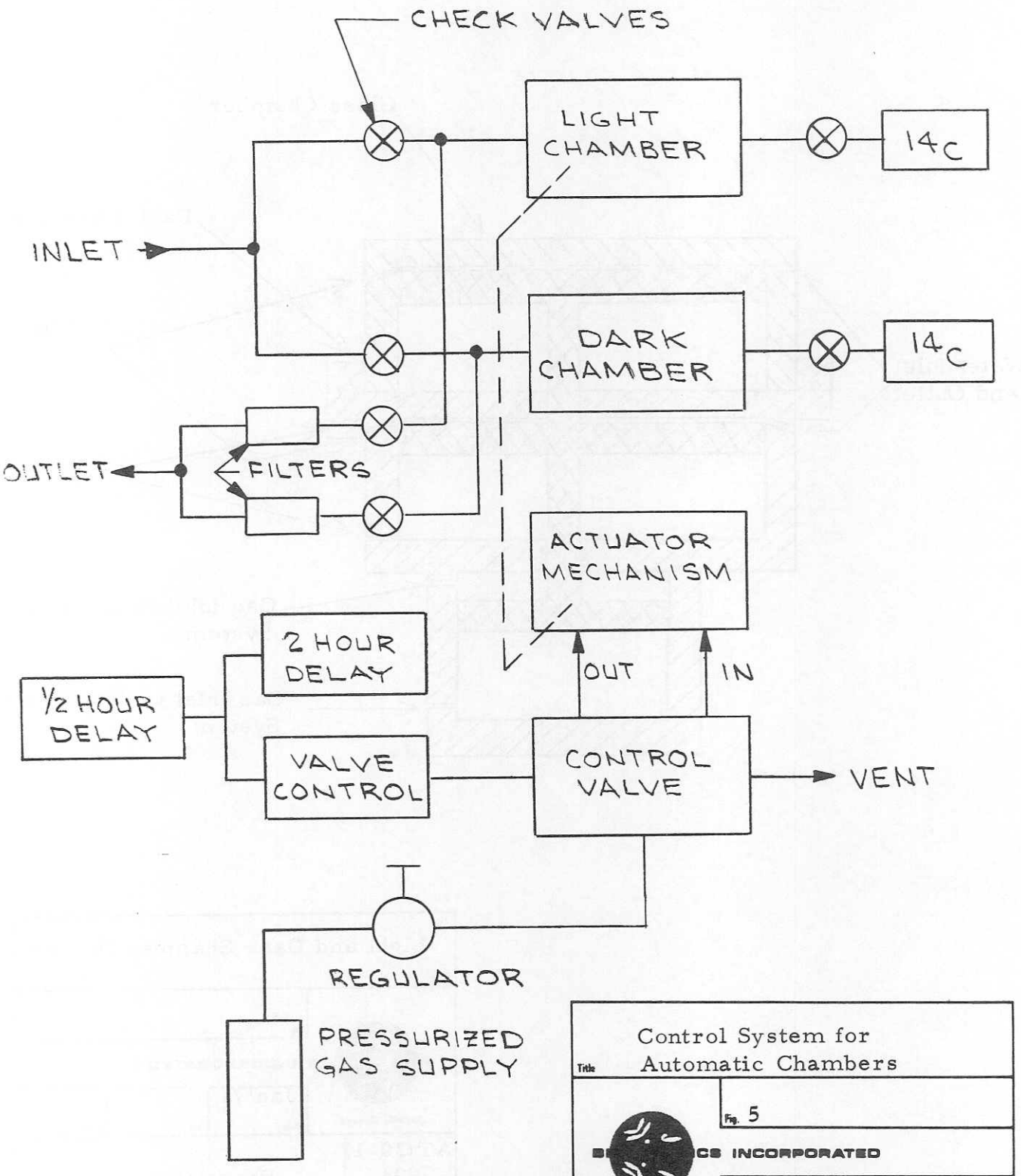
The outer housing used to contain the apparatus must be designed to withstand operating pressures to a depth of 200 feet with a closure mechanism designed to provide easy disassembly and removal of filters.

D. Detailed Design

Subsequent to the submission of the preliminary design for the deployable concept, the engineering effort



Light and Dark Chamber Details			
Title			
	Fig. 4		
BIOSERVICES INCORPORATED			
Rockville, Maryland	Jan'71 Date	Init.	Scale
AT(30-1)-3993 Cont. No.	Phase II Report Report Title		



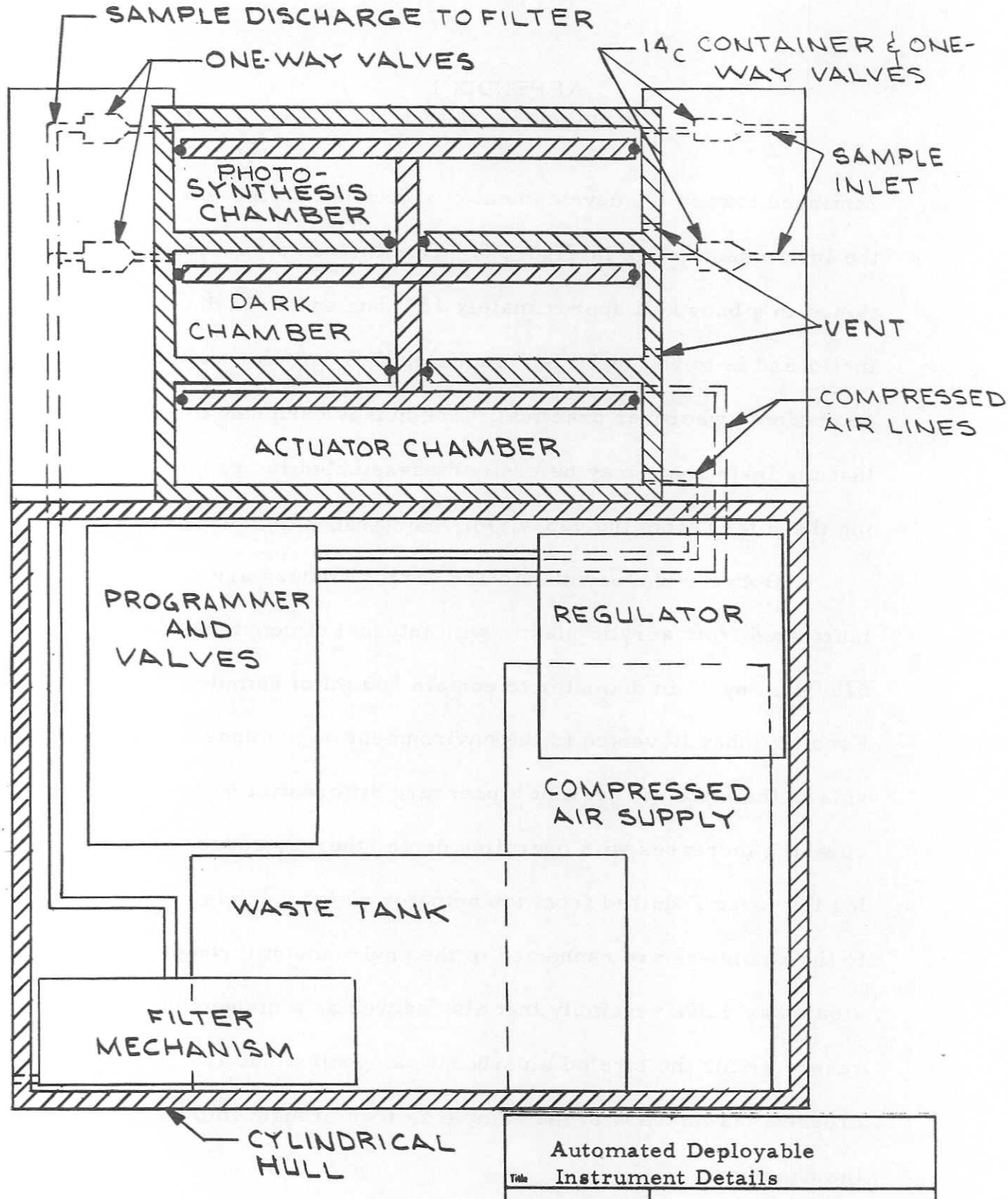
Title	Control System for Automatic Chambers
	Fig. 5
	BIOTICS INCORPORATED
Rockville, Maryland	Jan'71 (Date) / Init. / Scale
AT(30-1)-3993 (Cont. No.)	Phase II Report (Report Title)

APPENDIX I
(continued - page 4)

continued toward the development of a detailed design for the instrument shown in Figure 6. This instrument is contained in a buoy hull approximately 18" long and 8" in diameter and is to be fabricated from standard, off-the-shelf components wherever practical. The hull is designed so that the instrument may be easily disassembled for removing the filters from the assay, for maintenance and cleaning.

Both the photosynthesis and dark chambers are fabricated from acrylic plastic with internal dimensions of 5/8" high by 4" in diameter to contain 100 ml of sample. Each chamber is vented to the environment on the underside of the piston to prevent a pressure differential build-up which increases with operating depth, thereby reducing the force required from the actuator piston. Inlets to the chambers are connected to the environment through a one-way valve assembly that also serves as a dispensing reservoir for the labeled bicarbonate ampoules that are crushed and mixed with the sample as it is brought into the chamber.

The actuator piston is powered by a compressed gas system which has a supply tank capable of providing

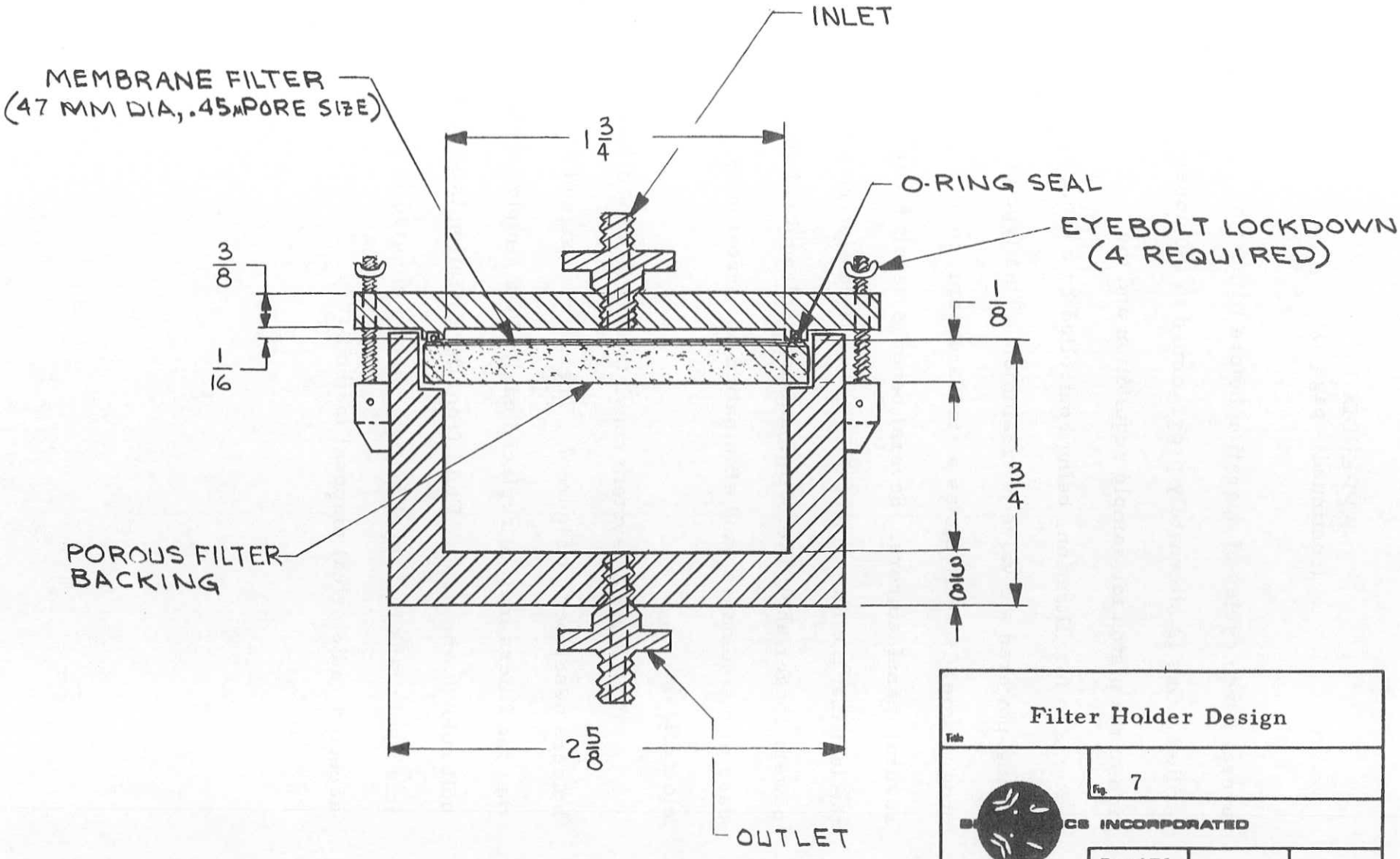


Title	Automated Deployable Instrument Details
Fig.	6
	BIOSCIENCES INCORPORATED, Rockville, Maryland
Date	Jan'71
Init.	
Scale	
Contr. No.	AT(30-1)-3993
Report Title	Phase II Report

APPENDIX I
(continued - page 5)

several dozen cycles of operation before it must be refilled. Gas is alternately programmed to the upper side of the piston for sample acquisition and the lower side for filtration, being controlled by a simple, spring-powered air valve programmer. This type of programmer is available as a standard item from several manufacturers. Several benefits result from the fact that the instrument does not require electrical power: longer shelf life, elimination of solenoid valves, ease of maintenance, and elimination of corrosion by electrolysis.

The filtration system consists of two of the filter holders described in Figure 7. These are designed so that the filters may be replaced easily by a simple toggle bolt hold-down clamp. Fluid from the filtration is stored in a waste tank that must be periodically emptied in an accepted radiological disposal method.



Title	Filter Holder Design
Fig.	7
	CS INCORPORATED
Rockville, Maryland	Jan'71 (Date) / Init. / Scale
AT(30-1)-3993 (Cont. No.)	Phase II Report (Report Title)

APPENDIX II

Manual Primary Productivity Procedure Used for Comparisons with APPI Data

A. Sample Acquisition

For laboratory tests, samples of local stream water were acquired in large volumes, stirred vigorously, and divided at the time of use for the APPI and manual determinations.

Samples for use during field tests were acquired with a Nansen sampling jar. This is a device containing a 1 liter volume which is opened at the sampling depth and location, closed, and returned to the surface with representative sample material.

B. Sample Allotment

100 ml of sample is set aside for titration to determine alkalinity.

776 ml of sample is inoculated with 24 ml of isotope solution (2 uCi/ml, $NaH^{14}CO_3$) and divided into three light and three dark bottles, each having a volume of 130 ml. Surplus material is disposed of as radioactive waste.

C. Incubation

Three light bottles and 3 dark bottles (taped and wrapped in foil) are placed in a glassware basket and

APPENDIX II
(continued - page 2)

lowered to the same depth as, and adjacent to, the APPI photosynthesis chamber. Incubation time is the same as the APPI light sample. All sample bottles are processed within approximately six minutes.

D. Filtration and Fixing

100 ml of each water sample is filtered through a 47 mm diameter, .45u Gelman "Acropor" AN-450 filter. This is identical to the filter material used in the APPI. Standard Millipore filter holders and vacuum flasks are used. A small vacuum pump is used to provide vacuum filtration. After filtration, the filter is acid washed with 25 ml of .01 N H_2SO_4. Filters are then set aside for drying.

E. Drying and Counting

The six filters are dried directly after filtration except when power is limited. In this case, drying may be delayed for several hours. Drying consists of exposure to a 75 watt floodlight placed 2" above the filters for at least 10 minutes.

The filters are mounted on a plastic slide and placed under a GM counter. The counter is identical to

APPENDIX II
(continued - page 3)

that used in the APPI (Tracerlab Type 11002T). The pulse amplifier is also identical and the scaler and printer shared with the APPI data recorder system (Canberra 1491/1487 and Mite 118A printer). The filter surface to counter distance ratio of the APPI to the manual is .976. The ratio of efficiencies of the APPI to manual procedures was determined experimentally to be 1.23. The counting period, as in the APPI, is 100 seconds.

F. Inorganic Carbon

Inorganic carbon measurement is achieved by measuring the sample temperature and then titrating 100 ml of sample to a pH of 3.75 with .02 N H_2SO_4. The number of milliliters of H_2SO_4 required adjustment by appropriate conversion factors from Saunders et al (7) to provide total inorganic carbon level in mg/l^3 for application to the primary productivity computation.

APPENDIX III

Programming Details for APPI Control of the Primary Productivity Assay

The operating cycle for the automatic assay of the APPI is controlled by an endless loop punched tape program. Format of this program follows standard ASCII coding which is described in Table 1. The tape reader senses the first 5 channels of an eight channel tape, which are decoded to actuate specific control relays for the various motors and timers.

A printout of the final version of the tape program (designated OCCO XIII) is shown in Figure 1 and described in Table 2.

Table 1

ASCII Codes for APPI Programmer Control

ASCII Code	Operation	Component Function
X	Open Valve A	^{14}C Metering
H	Close Valve A	" "
Q	Open Valve B	^{14}C Injection
A	Close Valve B	" "
R	Open Valve C	Sample Inlet
B	Close Valve C	" "
S	Open Valve D	Photo Chamber
C	Close Valve D	" "
$\overline{L}$ (\ )	Open Valve F	Wash Solution
L	Close Valve F	" "
U	Open Valve G	Rinse Solution
E	Close Valve G	" "
V	Open Valve H	Filtration
F	Close Valve H	"
$\overline{M}$ ( ])	Fast Fill	Sample Pump
M	Fast Empty	" "
I	Slow Empty	" "
G	Open Clamp	Filter Tape
W	Close Clamp	" "
O	Advance	" "
K	2 Minute Operation	Window Wiper
D	2 Minute Operation	Drier
$\overline{O}$ (←)	2 Minute Operation	Vacuum Pump

Table 1
(continued)

ASCII Code	Operation	Component Function
J	Turn ON	Readout Electronics
$\overline{N}$ (↑)	5 Seconds	Delay
N	1 Hour	"
$\overline{P}$ (@)	Indefinite	"

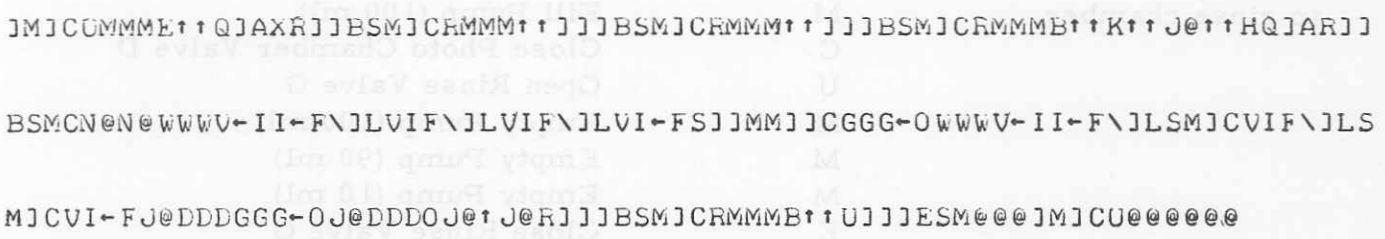


Figure 1

Printout of Program Number OCCO XIII

Table 2

Description of Typical Control Program
for APPI Assay Cycle

Operation Description	ASCII Code	Component Function
	M	Fill Pump (100 ml)
Rinse and return rinse	M	Empty Pump (100 ml)
to rinse chamber	M	Fill Pump (100 ml)
	C	Close Photo Chamber Valve D
	U	Open Rinse Valve G
	M	Empty Pump (100 ml)
	M	Empty Pump (90 ml)
	M	Empty Pump (10 ml)
	E	Close Rinse Valve G
	N	5 Second Delay
	N	5 Second Delay
Prime Meter Chamber	Q	Open ^{14}C Injection Valve B
with ^{14}C	M	Fill Pump (10 ml)
	A	Close ^{14}C Injection Valve B
	X	Open ^{14}C Meter Valve A
	R	Open Sample Valve C
	M	Fill Pump (90 ml)
First Sample Rinse	M	Fill Pump (100 ml)
	B	Close Sample Valve C
	S	Open Photo Chamber Valve D
	M	Empty Pump (100 ml)
	M	Fill Pump (100 ml)
	C	Close Photo Chamber Valve D
	R	Open Sample Valve C
	M	Empty Pump (100 ml)
	M	Empty Pump (90 ml)
	M	Empty Pump (10 ml)
	N	5 Second Delay
	N	5 Second Delay
	M	Fill Pump (10 ml)
	M	Fill Pump (90 ml)
	M	Fill Pump (100 ml)
	B	Close Sample Valve C
Second Sample Rinse	S	Open Photo Chamber Valve D
	M	Empty Pump (100 ml)
	M	Fill Pump (100 ml)
	C	Close Photo Chamber Valve D

Table 2
(continued)

Operation Description	ASCII Code	Component Function
Second Sample Rinse	R	Open Sample Valve C
	M	Empty Pump (100 ml)
	M	Empty Pump (90 ml)
	M	Empty Pump (10 ml)
	N	5 Second Delay
	N	5 Second Delay
	M	Fill Pump (10 ml)
	M	Fill Pump (90 ml)
	M	Fill Pump (100 ml)
Third Sample Rinse	B	Close Sample Valve C
	S	Open Photo Chamber Valve D
	M	Empty Pump (100 ml)
	M	Fill Pump (100 ml)
	C	Close Photo Chamber Valve D
	R	Open Sample Valve C
	M	Empty Pump (100 ml)
	M	Empty Pump (90 ml)
	M	Empty Pump (10 ml)
	B	Close Sample Valve C
	N	5 Second Delay
	N	5 Second Delay
Wiper on to clean photo chamber	K	Wiper 2 Minutes
	N	5 Second Delay
	N	5 Second Delay
Re-count last filter under GM counter	J	Readout Electronics ON
	P	Wait (Readout Stop Advances)
	N	5 Second Delay
	N	5 Second Delay
Admit ^{14}C to pump chamber	H	Close ^{14}C Meter Valve A
	Q	Open ^{14}C Injection Valve B
	M	Fill Pump (10 ml)
	A	Close ^{14}C Injection Valve B
Admit 190 ml of sample	R	Open Sample Valve C
	M	Fill Pump (90 ml)
	M	Fill Pump (100 ml)
	B	Close Sample Valve C
Load photo chamber with sample	S	Open Photo Chamber Valve D
	M	Empty Pump (100 ml)
	C	Close Photo Chamber Valve D

Table 2
(continued)

Operation Description	ASCII Code	Component Function
Incubate first hour	N	Start 1 Hour Timer
	P	Wait
Incubate second hour	N	Start 1 Hour Timer
	P	Wait
	W	Close Filter Tape Clamp
Filter dark sample	W	Close Filter Tape Clamp
slowly	W	Close Filter Tape Clamp
	V	Open Valve H to Filter
	O	Vacuum Pump 2 Minutes
	I	Empty Pump Slowly (90 ml)
	I	Empty Pump Slowly (10 ml)
	O	Vacuum Pump 2 Minutes
	F	Close Valve H to Filter F
	L	Open Wash Valve F
First acid wash	M	Fill Pump (10 ml)
	L	Close Wash Valve F
	V	Open Valve H to Filter H
	I	Empty Pump Slowly (10 ml)
	F	Close Valve to Filter H
	L	Open Wash Valve F
Second acid wash	M	Fill Pump (10 ml)
	L	Close Wash Valve F
	V	Open Valve to Filter H
	I	Empty Pump Slowly (10 ml)
	F	Close Valve to Filter H
	L	Open Wash Valve F
	M	Fill Pump (10 ml)
Third acid wash	L	Close Wash Valve F
	V	Open Valve to Filter H
	I	Empty Pump Slowly (10 ml)
	O	Vacuum Pump 2 Minutes
	F	Close Valve to Filter H
	S	Open Photo Chamber Valve D
	M	Fill Pump (10 ml)
Unload photo chamber	M	Fill Pump (90 ml)
	M	Empty Pump (90 ml)
	M	Empty Pump (10 ml)
	M	Fill Pump (10 ml)
	M	Fill Pump (90 ml)
	C	Close Photo Chamber Valve D

Table 2
(continued)

Operation Description	ASCII Code	Component Function
	G	Open Filter Tape Clamp
	G	Open Filter Tape Clamp
Advance filter tape	G	Open Filter Tape Clamp
	O	Vacuum Pump 2 Minutes
	O	Advance Filter Tape
	W	Close Filter Tape Clamp
	W	Close Filter Tape Clamp
	W	Close Filter Tape Clamp
	V	Open Valve to Filter H
Filter light sample	O	Vacuum Pump 2 Minutes
	I	Empty Pump Slowly (90 ml)
	I	Empty Pump Slowly (10 ml)
	O	Vacuum Pump 2 Minutes
	F	Close Valve to Filter H
	L	Open Wash Valve F
First acid wash	M	Fill Pump (10 ml)
	L	Close Wash Valve F
	S	Open Photo Chamber Valve H
	M	Empty Pump (10 ml)
	M	Fill Pump (10 ml)
	C	Close Photo Chamber Valve D
	V	Open Valve H to Filter
	I	Empty Pump Slowly (10 ml)
	F	Close Valve H to Filter
	L	Open Wash Valve F
	M	Fill Pump (10 ml)
Second acid wash	L	Close Wash Valve F
	S	Open Photo Chamber Valve D
	M	Empty Pump (10 ml)
	M	Fill Pump (10 ml)
	C	Close Photo Chamber Valve D
	V	Open Valve H to Filter
	I	Empty Pump Slowly (10 ml)
	O	Vacuum Pump 2 Minutes
	F	Close Valve H to Filter
Count background	J	Readout Electronics ON
radiation	P	Wait (Readout Stop Advances)
	D	Drier 2 Minutes
Dry dark sample	D	Drier 2 Minutes
filter tape	D	Drier 2 Minutes
	G	Open Filter Tape Clamp

Table 2
(continued)

Operation Description	ASCII Code	Component Function
Advance filter	G	Open Filter Tape Clamp
	G	Open Filter Tape Clamp
	O	Vacuum Pump 2 Minutes
	O	Advance Filter Tape
Count dark sample	J	Readout Electronics ON
radiation	P	Wait (Readout Stop Advances)
	D	Drier ON 2 Minutes
Dry light sample	D	Drier ON 2 Minutes
filter tape	D	Drier ON 2 Minutes
	O	Advance Filter Tape
Advance filter Tape	J	Readout Electronics ON
Count light sample	P	Wait (Readout Stop Advances)
radiation	N	5 Second Delay
	J	Readout Electronics ON
Re-count light sample	P	Wait (Readout Stop Advances)
radiation	R	Open Sample Valve C
	M	Fill Pump (10 ml)
Rinse photo chamber	M	Fill Pump (90 ml)
and pump with sample	M	Fill Pump (100 ml)
	B	**Close Sample Valve C**
	S	Open Photo Chamber Valve D
	M	Empty Pump (10 ml)
	M	Fill Pump (100 ml)
	C	Close Photo Chamber Valve D
	R	Open Sample Valve C
	M	Empty Pump (100 ml)
	M	Empty Pump (90 ml)
	M	Empty Pump (10 ml)
	B	Close Sample Valve C
	N	5 Second Delay
	N	5 Second Delay
	U	Open Rinse Valve G
Load photo chamber	M	Fill Pump (10 ml)
and pump with rinse	M	Fill Pump (90 ml)
solution	M	Fill Pump (100 ml)
	E	Close Rinse Valve G
	S	Open Photo Chamber Valve D
	M	Empty Pump (100 ml)
End of Cycle		

Table 2
(continued)

Operation Description	ASCII Code	Component Function
	$\overline{P}$	Indefinite Wait
	$\overline{P}$	Indefinite Wait
	$\overline{P}$	Indefinite Wait
	$\overline{P}$	Indefinite Wait
	$\overline{P}$	Indefinite Wait
	$\overline{P}$	Indefinite Wait

APPENDIX IV

APPI Test Plan

Testing of the APPI in the environment in compliance with the requirements of the program Task 370 was guided by the attached documents.

1. Copy of Test Plan sent to U. S. Atomic Energy Commission on 21 April 1970

2. Copy of letter from U. S. Atomic Energy Commission's Review of 19 May 1970

AEC Automated Primary Productivity Instrument
Test Plan - Task 370

Submitted to AEC on 21 April 1970

I. Background

A. Test Plan Purpose and Preparation

The purpose of this plan is to define the objectives and procedures of a test program that will be performed for the evaulation of the Engineering Test Model (ETM) of an Automated Primary Productivity Instrument (APPI).

In the preparation of this test plan, Biospherics personnel have communicated with representatives of the Atomic Energy Commission, Federal Water Quality Administration, and several other organizations who have an interest in monitoring primary productivity. Their suggestions have been incorporated in this plan.

B. Test Plan Objectives

The objectives of this testing program are:

1. To verify the technical approach used to develop an instrument in the automation of the ^{14}C primary productivity assay, and

2. To provide an opportunity to demonstrate the operation and capabilities of the APPI instrument to all interested parties.

II. Test Program

The test program consists of two test cycles that will be

AEC Automated Primary Productivity Instrument
Test Plan - Task 370
(continued - page 2)

performed at a local open water site, possible the WSSC's Dalecarlia Reservoir located immediately in the western corner of Washington, D. C. at Little Falls on the Potomac River.

The first test cycle is planned for a two-day period to accomplish the following:

a. Allow the examination of instrument operation under field-test conditions.

b. Verify the laboratory calibration of the device on an in situ sample.

c. Allow the identification of modifications of the instrument or testing techniques that may be desirable for the successful operation of the second demonstration cycle.

A second test cycle is planned with a five-day duration to accomplish the following:

a. Operate under field conditions for one, and possibly two, continuous two-day cycles.

b. Allow the observation and participation of interested persons in the demonstration of the device.

Performance verification will consist of a comparison of the primary productivity values obtained from the automated instrument's data and the standard light-dark bottle assay technique conducted in situ. The light-dark bottle assay will

be performed on site by acquiring a sample of water at the same time and location as the instrument sampling. The standard sample will then be immediately brought to the surface, injected with ^{14}C, and lowered to the test depth for incubation. At the end of incubation, the sample will be returned to the surface for filtration, drying, and counting by standard techniques.

The instrument system under test is self-contained with the exception of the data recorder and power supply. Power may be either 12V storage batteries or line voltage. Since only minimal external support is required for this test program, Biospherics intends to handle details concerning site arrangements, equipment procurement, and transportation and operation of equipment.

Following the completion of the second test cycle, the data acquired will be analyzed and reported to interested parties.

UNITED STATES
ATOMIC ENERGY COMMISSION
WASHINGTON, D.C. 20545

May 19, 1970

Dr. Gilbert V. Levin, President
Biospherics Incorporated
4928 Wyaconda Road
Rockville, Maryland 20853

Dear Dr. Levin:

This is in reply to your letter of April 21, 1970 requesting our review of the test plan for Task 370 in Contract No. AT(30-1)3993. My reply has been delayed to permit a detailed discussion of the plan with Dr. Walter Sanders and Miss Pat Kerr at the FWQA, Athens Laboratory.

Under Test Plan Objectives, Item 2 should be "to assure that the instrument works and that the recorded data is reasonably comparable to primary productivity measurements made by other accepted methods." The third objective should be "to provide an opportunity to demonstrate the operation and capabilities of the APPI instrument to all interested parties."

Under Test Program, we have no objection to preliminary tests at Dalecarlia reservior on the Potomac. However, it has always been our desire that the Engineering Test Model be tested in collaboration with FWQA scientists at a suitable water body in Georgia or Florida. Drs. David Duttweiller and Walter Sanders told me during the recent visit that they would be glad to cooperate in carrying out these tests.

It is our understanding that a 2-day test cycle means that the instrument operate continuously for 2 days. Likewise, a 5-day test cycle means that the instrument operate continuously for 5 days duration to accomplish: (a) a longer period of automatic operation under field conditions, (b) a reverification of the calabration, (c) an operational demonstration of the device to interested persons, (d) involvement of FWQA personnel in the operation of the device, and (e) the mapping of long-term plans for the system. These plans and objectives were

discussed with Mr. Lindgren on November 12, 1969, and made a part of the contract plan.

In the discussions with Dr. Sanders, he suggested that we vary the test cycles, if possible, so that tests are made at different times of day, such as: (a) 5 a.m. to 9 a.m., (b) 10 a.m. to 2 p.m., (c) run one or two tests after dark, with one stopping as late as 11 p.m., and (d) run one right through dusk.

Miss Kerr emphasized that one must know water temperature, pH, and availability of inorganic carbon (CO_2, bicarbonate, and carbonate) in order for the radioactivity fixation measurements to be meaningful. For these tests with the engineering test model, I requested that the FWQA scientist assist us with these additonal measurements, and provide independent primary productivity data against which to compare the radioisotope device.

Dr. Duttweiller invited me to go back to Athens for these field tests, and I hope to do so.

Sincerely,

Oscar M. Bizzell

Oscar M. Bizzell, Program Manager
Environmental and Ocean Sciences
Division of Isotopes Development

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