NYO-3993-1

AUTOMATED PRIMARY PRODUCTIVITY MEASUREMENTS IN WATER BODIES. PHASE I. FEASIBILITY AND PRELIMINARY DESIGN STUDIES

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RADIOISOTOPE AND RADIATION
APPLICATIONS (TID-4500)

AUTOMATED PRIMARY PRODUCTIVITY MEASUREMENTS IN WATER BODIES

Phase I - Feasibility and Preliminary Design Studies

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Biospherics Research, Inc. wishes to thank Dr. Theodore Sudia for his consultation on the Concept Literature Review and Dr. Constantine Sorokin for his aid during the seminar review of the biological phase of the APPI Project. The assistance of Gilbert Pascal, engineer, Margaret Redmond, biological technician, and Barbara Platte, technical writer, in the performance of this project and preparation of this report is also gratefully acknowledged.

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I. Introduction

The effort expended by Biospherics Research, Inc. during Phase I of AEC Contract No. AT(30-1)-3993, "Feasibility and Preliminary Design Studies, Automated Primary Productivity Measurements in Water Bodies," was primarily directed toward the following tasks: Concept Development, Literature Review, Biochemistry and Microbiology, and Engineering Design. These tasks have been successfully completed and are described in detail in this report. The ultimate objective of this program is the development of a fully operational Automated Primary Productivity Instrument (APPI).

The investigations and design work have led to a biochemical and engineering approach for the system which, following detail design, is ready to be fabricated into a breadboard instrument for component and concept testing and evaluation. It is felt that the present design using an automated radioactive isotope technique represents a practical and economical approach to the measurement of primary productivity in water bodies.

The biochemical literature review encompassed several hundred works dealing with all phases of activity associated with primary productivity analysis. This literature search supports the need for a simple method for determining primary productivity on a large scale. The literature search is presented as Appendix B.

The biological research involved the laboratory evaluation of several techniques applicable to automated primary productivity

determination with the use of ^{14}C.

The engineering effort during Phase I was directed toward the design of the instrument in accordance with the biochemical requirements of the determination of primary productivity. The design of the instrument and its systems is discussed in detail in the text of this report.

The first phase of the subject contract has reinforced the promise of the proposed approach. No significant biological or engineering problem areas have been revealed concerning the feasibility or the usefulness of the proposed program. In view of the successful results of Phase I and the demonstrated need for a simple automated determination of primary productivity, it is recommended that the program continue through Phase II and the development and evaluation of a prototype instrument.

II. Background

The potential use of the sea as a food resource for the world's expanding population is a major factor in the rapidly increasing interest in oceanography.

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 application of modern techniques such as sonar and remote sensing to commercial fishing are improvements, but they still 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 photosynthetically fixed to start the entire food chain in the seas. 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, and planktonic species and densities. To date, the measurement of primary productivity has been used only in limited research studies. Practical application to determining the 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 implications of successfully developing the proposed method and its instrumentation are impressive. The result would be the economic application of a basic scientific measurement to bring about greatly improved efficiency

in the fishing industry. A major step would thus be taken toward providing adequate nutrition for the World's increasing population.

Automated in the fashion proposed, the ultimate APPI would be housed in a submerged buoy and be programmed to conduct primary productivity measurements at desired times and depths. The instrument would be auto-cycling and would store the data obtained. The device could operate for considerable lengths of time as determined by on-board supplies of isotope, filters and power. In an extensive deployment of this type of instrument, the stored data could be collected upon maintenance visits, or could be retrieved by interrogation by ships, aircraft, or satellites. A grid pattern of automated buoys could provide the degree of information required to predict forthcoming fish crops, and critical times and locations. Subsequently, the primary productivity measurements could be augmented and integrated with the other important measurements, such as those of nutrient concentrations, light penetration, temperature, pH, and the like.

Another important application of the instrument is in studies of surface water pollution. Routine measurements of primary productivity in a water body would be helpful in assessing trends in eutrophication that may be caused by pollution. Despite the importance of these measurements, very few studies have been performed, primarily because the current methods of measurement are too complicated and time-consuming for routine use. Consequently, those concerned with water pollution also have an interest in this program.

III. Biological Considerations

A. Concept Literature Review

A literature search, Appendix B, was made to investigate the present state of knowledge and research techniques available for measuring the primary productivity of aquatic environments. The review of over 200 reports, with 50 percent more recent than 1955, encompassed the work of over 60 authors.

The literature search identified the array of radioisotope techniques for the determination of primary productivity which might be incorporated into the specific method developed for this program. Many of the important parameters associated with the instrument, its operation, and its sampling technique were obtained directly from the studies. The literature also provided background for the laboratory phase of the work which was required to test and complete the total concept developed.

Steemann-Nielsen (1951) demonstrated the reliability of the light-dependent incorporation of $C^{14}O_2$ (presently designated $^{14}CO_2$) as an indication of photosynthetic activity. Since then, several workers, including Goldman and Mason (1962), Ryther and Yentsch (1958), Saunders (1962), and Sorokin (1956), have modified and refined the procedure.

It was originally hoped that by measuring carbon dioxide fixation directly, respiratory activity could be ignored and a dark control would not be required. However, our investigation has shown that a significant amount of carbon dioxide may be fixed by heterotrophic and chemosynthetic pathways (Sorokin,

1965; and many others). Since the measurement of interest is primary productivity, and heterotrophic CO_2 fixation is a recycling of an already available organic food supply, a "dark bottle" control is required.

Approaches to sampling large bodies of water have been inadequate since the dependence upon manual procedures has severely limited the quantity of data normally collected. Most information is presented as gm $C/m^2/hr$ in the photic column or gm $C/m^3/hr$. To determine primary productivity, measurements are taken at several arbitrary depths between the surface and the compensation point. Integration of the photosynthesis-depth curve yields an estimate of the activity throughout the column (Saunders, 1962). Horizontal and temporal sampling have been limited by manpower and equipment available.

In applying the isotope dilution technique, it is necessary to know the carbonate content of the sample to which labeled carbonate is added. Therefore, a search for appropriate inorganic chemistry procedures for carbonate determination was made. The acid titration technique has been found most reliable and will be automated accordingly.

B. Biological Laboratory Work

In seeking to automate the ^{14}C primary productivity assay, it was necessary to study and evaluate the individual operations. Upon acquiring the skill and experience necessary for the manual determination of primary productivity by the labeled carbon method, a number of special laboratory

study tasks were undertaken. The studies were performed in close liaison with the engineering effort to provide answers to questions that arose during the design phase. Thus, the biological effort was of an applied type in that almost no fundamental research was required. The method has been given widespread acceptance as a research technique over the past decade or more so that additional effort to sustain the suitability of the method was unnecessary.

Of principal importance in constructing the program for the operation of the primary productivity monitor was the determination of the necessity for a dark bottle. Some reports found in the literature state that dark fixation might account for as much as 5% or 10%, and in one instance up to 25%, of the carbon assimilated in the primary productivity test. It was therefore decided to incorporate a dark bottle into the measurement procedure. In the interest of simplifying the instrument, the possibility of using only one photosynthesis chamber - alternating samples in it between light and dark regimens was considered. However, disadvantages of simultaneously measuring the activity of the dark bottle control and of the light chamber, coupled with the problems of developing an adequate shutter mechanism in a single chamber, discouraged this approach. Accordingly, it was concluded that one chamber maintained in the light and the other maintained in the dark would be required in the instrument.

In selecting the growth period for the measurement, samples were placed under light and dark regimens for periods ranging from 15 minutes up to 36

hours. All of the light exposure work was done using a combination of incandescent and fluorescent lights providing approximately 300 foot-candles to the test organisms. This light level is generally just above the compensation point. It was felt that using this light intensity challenged the system in its most difficult area of operation.

Experiments were undertaken to establish appropriate sample volume sizes, isotope concentrations, duration of procedures, sequence of events and readout sensitivity. In the course of these experiments, numerous water samples of widely different phytoplanktonic composition were used.

In applying the isotope dilution technique to the measurement of primary productivity, it is necessary to know the available carbon present in the seawater sample prior to the addition of the labeled carbonate. Several experimental approaches were attempted in the laboratory. In one, a known amount of labeled carbonate was supplied to a fixed seawater volume. A given quantity of barium ion was then added to precipitate that carbonate stoichiometrically. The radioactivity of the dried precipitate would then determine the dilution factor. Laboratory experiments with this method using distilled water were encouraging. However, when applied to seawater, the method failed because the sulfate content of the seawater competed with the carbonate making the stoichiometric determination of carbonate impossible. Ultimately, it was found most advantageous to automate the acid titration technique used in the manual determination of total alkalinity of water samples to measure the inorganic carbon content of the sample.

The inorganic carbon determination procedure is as follows:

1. A 100 ml sample of seawater is collected.
2. A trace of methyl orange indicator is added.
3. The pH and temperature of the sample are measured.
4. Sulfuric acid, 0.02N, is added until the methyl orange end point, pH 3.75, is attained.
5. The volume of sulfuric acid added is recorded.
6. Using a table of corrections based on pH and temperature measurements, the inorganic carbon content is readily determined.

This procedure was standardized in the laboratory and typical titration curves generated. A standard curve demonstrating the reliability of the assay procedure was developed.

In developing the automated program for the titration, various volumes and reagent concentrations were explored. The method was found to be accurate over two orders of magnitude of acid concentration. No critical volume or concentration parameters were found which would constrain the automation of the process.

A major problem confronting the continuous operation of the APPI over a period of months is that of fouling. Marine growths - on the window admitting light to the photosynthesis chamber, inside the photosynthesis and the dark chambers, and adjacent to the photosynthesis window, where long filaments might interfere with the ambient light intensity - would introduce artifacts into the primary productivity measurement. Marine fouling is a

most perplexing problem confronting all aspects of marine activity. Its impact ranges from the fouling of vessel bottoms to obscuring submarine periscopes. Investigation of this problem as encountered by others revealed that no simple solution has been found. The variety of approaches to prevent fouling considered in this study includes the use of toxic materials, the use of disposable growth chambers, mechanical cleaning of surfaces, ultrasonic cleaning, and chemical cleaning. A combination of mechanical cleaning of the instrument window by scraping, the use of toxic material for the scraper to prevent growths on it, and the use of dichromate solution to cleanse the inside of the growth chambers and the plumbing was the method selected.

After selection of the procedural steps and methods for the automated determination of primary productivity and upon completion of the engineering concept of the APPI, a seminar was held to review the process. The engineers and biologists associated in the program and Dr. Constantine Sorokin, noted algologist at the University of Maryland who was invited as a consultant, conducted a point-by-point review. A number of pertinent comments relevant to the design were made by this group. These points were incorporated into the final engineering concept of the instrument.

Appendix A contains much of the relevant data generated in the course of the biological laboratory program. The tables and figures presented contain the essential information explaining the nature of the experiments reported.

C. Biological Criteria

The purpose proposed for the APPI is the sampling and measurement

of primary productivity as it occurs in the water. Accordingly, it is important to maintain the sample under the ambient environmental conditions of the water body. The necessity of maintaining an instrument in the natural environment, isolating a sample of that environment for a fixed (even if limited) period of time, and the physical properties, i.e., refractive index of the light window, composition of the glass and all other parts in contact with the incubating sample, etc., will all exert some effects which modify the in situ environment. The best that one can hope to attain will be a minimization of these effects.

IV. Engineering

A. Responsibility

Mr. Chris Plakas performed the engineering research under the technical guidance and supervision of Dr. Gilbert V. Levin.

B. Marine Environment

1. Corrosion

Corrosion is of great importance to the designer of any device to be used in the marine environment. All materials used must be compatible with or protected from this environment if the device is to function properly for any length of time. The tremendous amount of activity in the water bodies - chemical, physical, and biological - will quickly reveal any weakness.

The form of corrosion of primary concern in the APPI design is galvanic corrosion caused by current flow between dissimilar metals. The amount of current flow is a direct function of the relative seawater galvanic potentials of the metals employed. In selecting component materials, therefore, it is desirable to select those with the same or very similar potentials. Additional considerations in lessening the effects of galvanic corrosion include the ratio of anode to cathode area, the presence of sharp corners and irregular stresses, the use of sacrificial non-functional parts and appropriate coatings.

For a given material, the rate of corrosion is quite naturally a function of the environment. The normal variations in seawater of pH, temperature, salinity, and pressure have relatively small effects on the corrosion rate.

However, the oxygen content of the water does have a significant effect, as does the rate of fluid flow past the exposed surface.

The highest corrosion rate is generally found in the zone from the water surface to a height of about 25 feet above the water. The high corrosion activity in this zone is caused by the high humidity and the alternating salt spray wetting and drying. Thus, superstructures protruding above the waterline are to be avoided if possible.

2. Pressure and Temperature

The structural integrity of a sealed device in the ocean is primarily a function of its resistance to the pressure. The ultimate APPI concept calls for possible operations to the limiting depth of photosynthetic activity - generally acknowledged to be approximately 300 meters.

At this operational depth, the external pressure is approximately 500 psi. Incorporating a safety factor of 2.0 requires that the buoy withstand approximately 1,000 psi compressive force. The structural strength of the shell is not the only consideration in working at this pressure, however, since all external dynamic and static seals, feed-through connections, and wiring harnesses must also maintain their integrity.

The APPI feasibility instrument fabricated in this program will be limited to operating for demonstration purposes in depths of water not exceeding 20 feet.

The temperature extremes encountered in unfrozen waters run from approximately 28°F to 120°F. The only significance of this range

from a design standpoint is in the effect on the seals and the electronic circuitry. Coefficients of expansion for the various materials used vary, and the dimensional changes resulting from temperature fluctuations can cause mismatches at seal points resulting in leaks.

3. Marine Life

Biofouling will probably constitute a problem in any waters where the APPI is deployed. Unfortunately, the general use of toxic materials or coatings would defeat the purpose of the experiment since the organisms under study would be adversely affected. However, in instrument parts where no contact with the organisms or sample is experienced its use may be permissible. Toxic structural materials include copper, mercury, and tin compounds. Antifouling paints are useful as coatings where the above material cannot be used due to undesirable mechanical characteristics.

In dynamic components where the motion is intermittent, additional precautions are necessary to prevent the freezing of parts by fouling or corrosion. This is equally the case with components such as valves where a tight pressure seal is required in the closed position. Fouling quickly degrades the performance of such components if proper precautions are not taken.

C. Materials of Construction

1. Reinforced Glass. The most common types of glass reinforcement are polyester and epoxy. These materials are highly resistive to the

ocean environment with the exception of their normal susceptibility to fouling.

While epoxy has a higher strength-to-weight ratio than polyester, epoxy occasionally tends to delaminate and absorb water under high pressure and is therefore used less frequently than the polyester.

2. Glass. Pure glass pressure vessels are assuming increasing importance in ocean engineering. They are being used for buoyancy devices and submersible hulls requiring high strength-to-weight ratios and corrosion resistance. Glass will generally remain transparent for 20-30 years before hazing occurs.

Problems involved with the use of glass include its notch sensitivity and brittleness. The primary causes of failure in glass structures are surface and internal flaws. Accordingly, quality control during fabrication is critical. The use of glass on the APPI will be limited to the photosynthesis structure.

3. Metals. The metals of primary importance in ocean engineering are the various high strength steel, titanium, copper, and aluminum alloys. Of the various alloys generally available, only titanium offers essentially complete freedom from corrosion. Titanium also offers good strength and weight characteristics. However, its cost and the difficulty of machining it precludes its use in all but the most critical applications. Table 1, compiled from several references, shows characteristics for some of the

Table 1

Characteristics of Common Marine Materials

		Steel						Titanium		Aluminum	Brass
	Material	Hy 80	Hy 100	Hy 150	12 Ni	4 Cu 9 Ni	Com.	6-4	6-2	7079-T6	Cu-Ni
Characteristics	Density Lb/in^3	0.28	0.28	0.28	0.28	0.28	0.28	0.16	0.16	0.16	
	Creep Resis.	Good	Good	Good	Good	Good	Fair	Good	Good	Good	Good
	Corros. Resis.	Poor	Poor	Poor	Poor	Poor	Poor	Exc.	Exc.	Poor	V. Good
	Stress Corros. Resis.	Exc.	Exc.	Exc.	Fair	Good	Fair	Good	V. Good	Good	V. Good
	Compressive Stress	80	100	150	180	180	50	120	100	60	40
	Relative Cost	Low	Low	Low	Med.	Med.	Low	High	High	Low	Med. to High

common oceanographic metals. The prototype instruments employed in a low stress environment will use commercial steel for the hull material.

V. Conceptual Design of the APPI

A. Responsibility

Mr. Chris Plakas performed the overall design engineering under the technical guidance and supervision of Dr. Gilbert V. Levin.

B. Components

1. Pumps

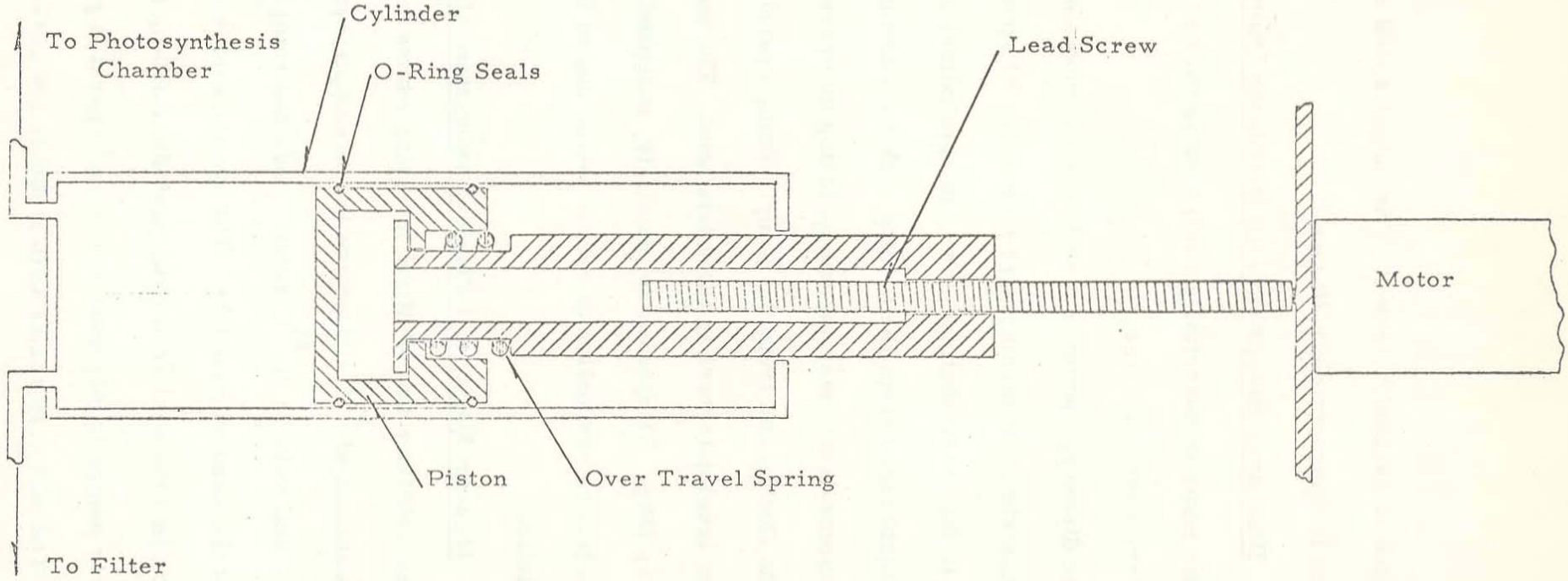
a. Sample Pump

A 100ml capacity sample pump is used to obtain the initial water sample, drive the sample through the various systems, and serve as a dark chamber during the two hour incubation period of a dark cycle.

The pump includes an internal stirrer to agitate the incubating sample. The ^{14}C inlet line that supplies the tagging medium to the sample feeds into the pump sample.

The filtration subsystem sample pump cylinder is fabricated from steel tubing coated with teflon. It possesses the same effective volume as the photosynthesis chamber and is designed to serve the dual function of pump and dark chamber.

The piston of the sample pump is driven by a geared motor through a lead screw mechanism (Fig. 1). The piston uses a double O-ring seal to prevent leakage between the piston and cylinder walls. These O-rings, as is the case with the free piston in the photosynthesis chamber, also serve to keep the pump interior free of growth. A limit switch governs the travel of the piston, and an overtravel spring is used as an additional precaution



Sample Pump

Biospherics Research, Inc.
Washington, D. C.

Fig. 1

against binding due to excessive travel. The output speed of the motor driving the piston is approximately 20 rpm.

b. Discharge Subsystem Hull Discharge Pump

This pump is essentially a bilge pump used to keep the hull interior free of accumulated water.

The discharge pump is a stainless cylinder and piston assembly driven by an eccentric shaft turning approximately 100 rpm (Fig. 2). The pump capacity, in the configuration shown, is approximately 330 cc/min., with the waste liquid taken from the holding tank and periodically ejected into the sea. Automatic one-way valves operating on pressure differential forces govern the flow to and from the pump. Pump operation is triggered with high and low level detectors in the waste tank. The pump and level detector circuitry (Fig. 3) operate continuously, independently of the programmer, to insure evacuation of excess water due to leakage or normal accumulation.

c. Medium Supply and Flushing Subsystem Metering Pumps

The APPI has two identical metering pumps to provide predetermined volumes of ^{14}C and poison to the sample system. The first pump dilutes a 1.0ml volume of ^{14}C solution into the sample pump concurrent with the entry of the seawater sample. The second metering pump injects a poisoned solution into the system for the periodic flushing operation.

Both pumps incorporate a solenoid operated piston with a spring return. The piston is sealed with a double set of O-rings similar to the

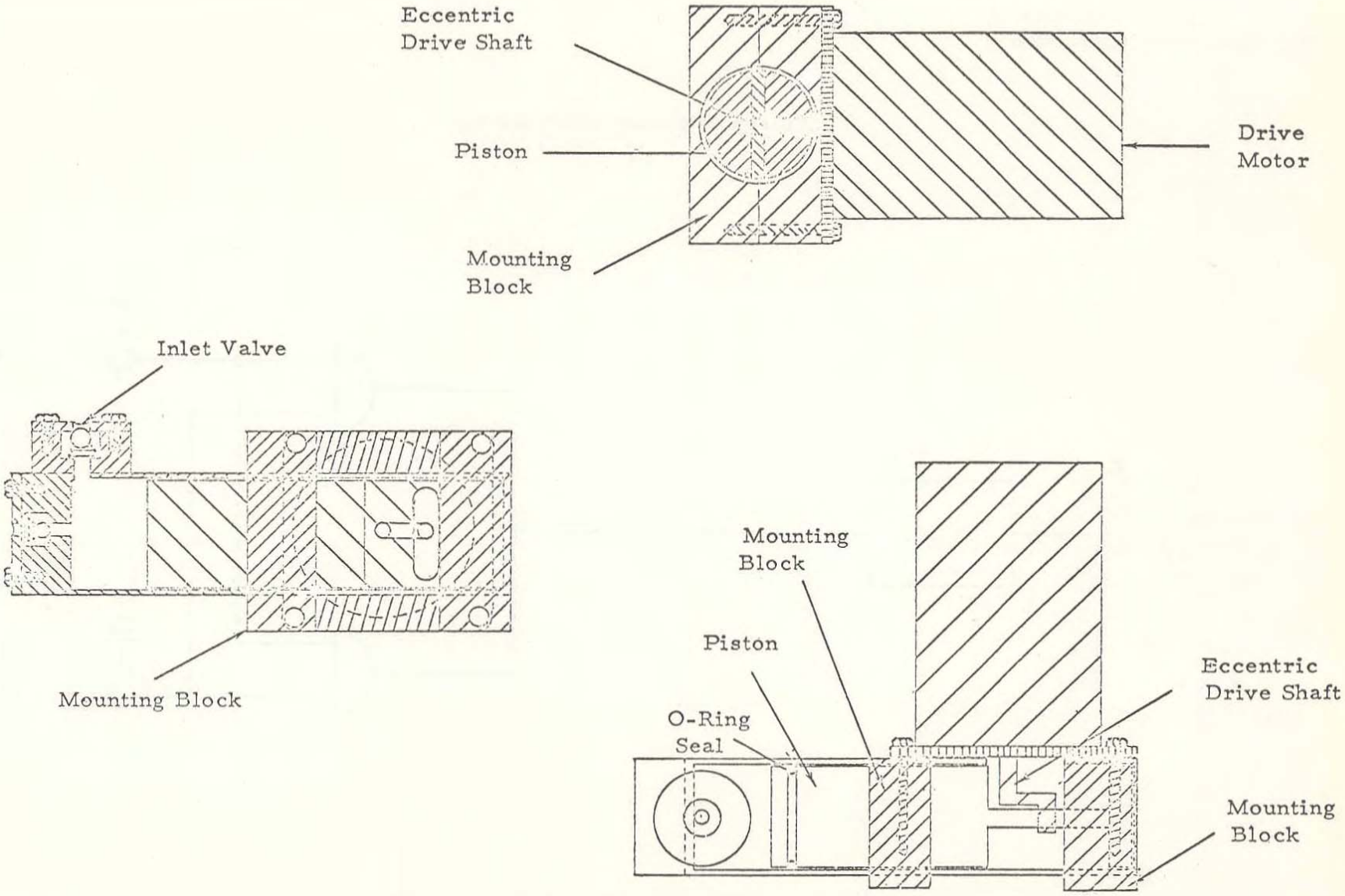


Figure 2.
Discharge Pump

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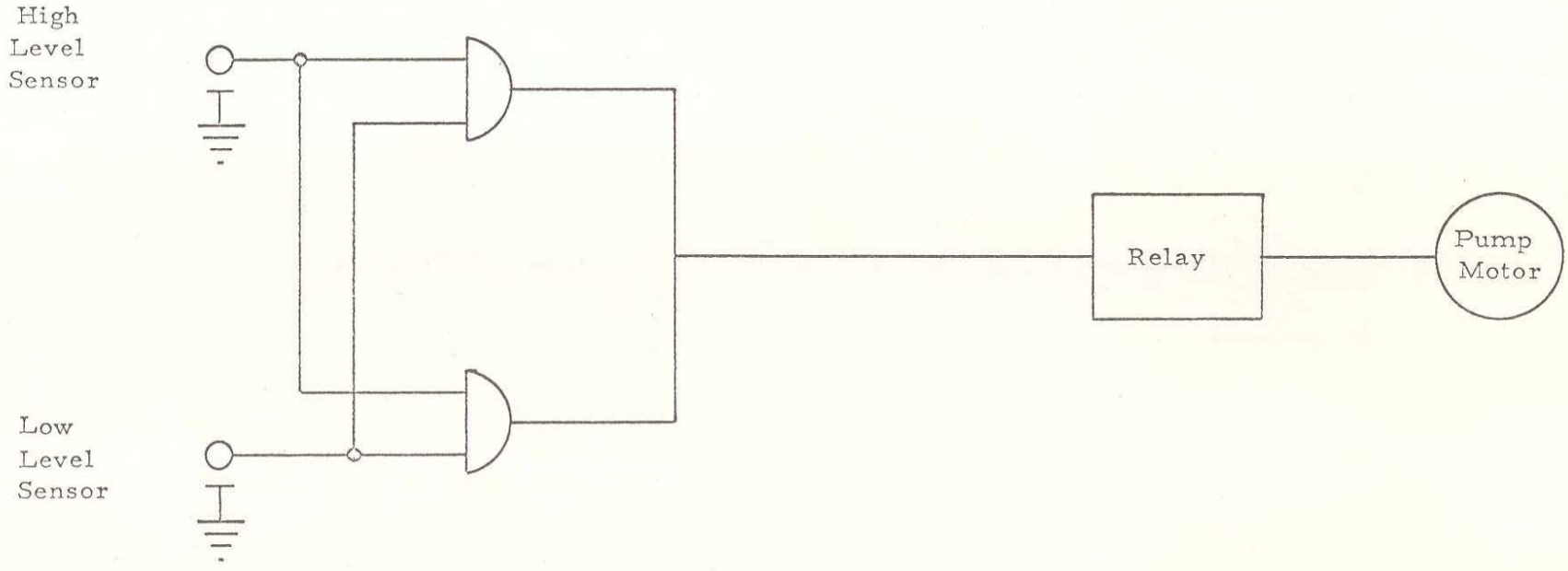


Figure 3.
Waste Level Detection Circuit

arrangement in the photosynthesis chamber and the sample pump. Fluid flow through each pump is governed by two automatic valves.

The ^{14}C metering pump is controlled through the sample programmer, while the poison pump is controlled with the flushing programmer. The basic design of the pumps is shown in Figure 4.

d. Titration Subsystem Metering Syringe

A small piston pump is used as a metering syringe for accurate injection of known amounts of sulfuric acid into the titration chamber during each carbonate determination. The syringe capacity is approximately 30 ml, enough to titrate any expected 100ml sample of seawater.

The arrangement of the metering syringe in the titration subsystem is similar to that of the sample pump, although on a much smaller and more precise scale. The motor in the syringe uses a greater gear reduction, giving a lower output speed. The reduced drive speed, coupled with a finer thread in the lead screw mechanism, gives very precise control of the displacement of this pump. The piston displacement is recorded to permit computation of the carbonate content of the natural sample water.

2. Photosynthesis Chamber

The photosynthesis chamber, a component of the filtration subsystem, is designed to operate under conditions as close to those of the adjacent natural water environment as possible.

The selection of the final photosynthesis chamber design involved an intensive study of the trade-offs of various possible approaches. The

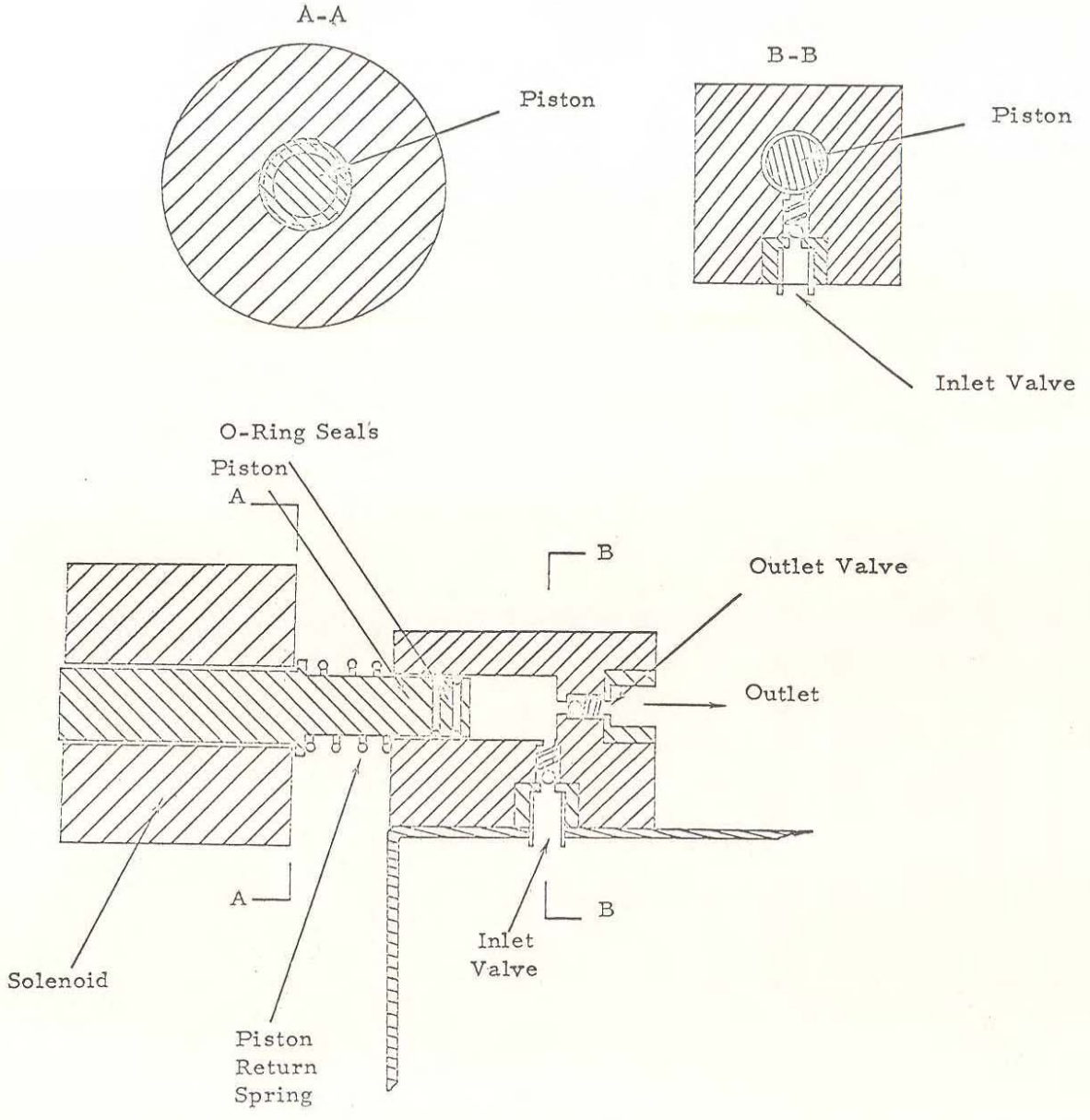


Figure 4.
^{14}C and Poison Metering Pumps

basic problem with any configuration involved the need for maximum transmission of ambient light into the chamber and the deleterious effects of marine growth which occurs on virtually any surface in contact with the water.

A search for transparent materials that are both anti-fouling and non-toxic to the organisms of interest turned up no possibilities. Efforts were then directed toward alternative chemical and mechanical methods of keeping the chamber free of growth, and the list of possibilities included mechanical covers, scrapers, retractable chambers, expendable shrouds, disposable incubation chambers and poison dispensers.

The method chosen (Fig. 5) is believed to offer the best compromise between reliability, simplicity, and effectiveness.

The amount of light lost passing from the water to the photosynthesis chamber is determined as follows:

Refractive Indices: water (n_1) = 1.33*
glass (n_2) = 1.60*

Fraction of light reflected at the water-glass interface = r_1

$$r_1 = \frac{(n_2 - n_1)^2}{(n_2 + n_1)^2} = \frac{(1.6 - 1.33)^2}{(1.6 + 1.33)^2} = \frac{0.073}{0.58} = 0.00852 \text{ or } 0.085\%$$

Fraction of light reflected at the glass-air (inside hull) interface = r_2

$$r_2 = \frac{(n_2 - n_1)^2}{(n_2 + n_1)^2} = \frac{0.36}{6.76} = 0.053 = 5.3\%$$

* for temperatures at 0°C to 40°C and wavelengths of 4,000 Å to 7,000 Å

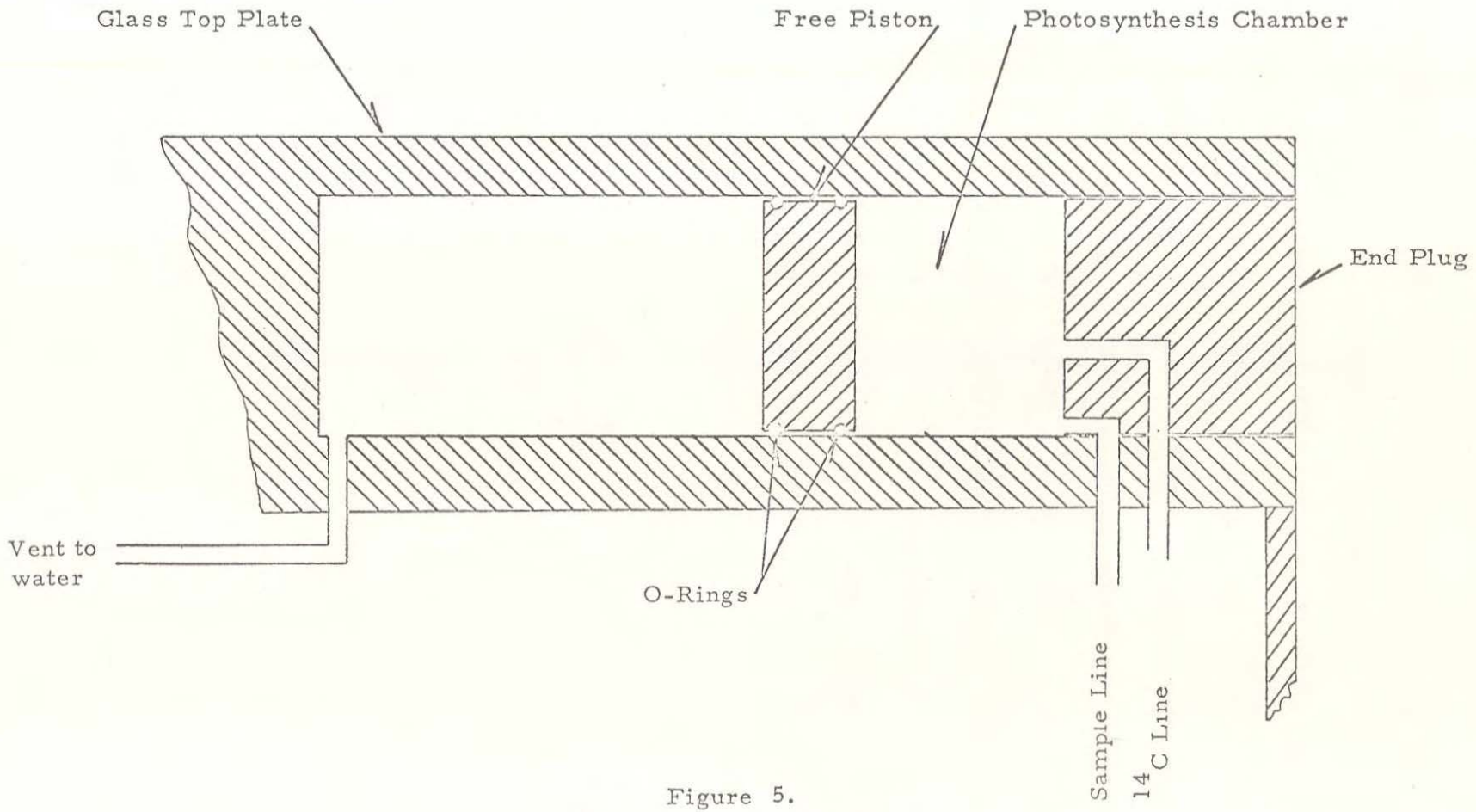


Figure 5.
Photosynthesis Chamber

The amount of light absorbed in the glass depends on the wavelength of the light and the thickness of the glass. Borosilicate glass has a coefficient of transmission of 0.993/cm (Chem. & Phys. Handbook) for wavelength from 4,000 Å to 7,000 Å.

Therefore, with a wall thickness of 1 cm, 0.007% of the light passing through the glass is lost. The total light loss due to reflection and absorption is:

Water to glass	0.085%
Glass to water	0.085%
Glass absorption	0.007%
Total loss	0.177%

The opening between the chamber and the edge of the glass formed during machining is plugged with a stainless steel insert containing portions of the chamber's inlet and outlet valves after insertion of the piston into the chamber.

A double-bladed wiper, spring loaded to keep a constant pressure on the glass surface, is used to sweep the top surface of the chamber free of sediment and growth (Fig. 6).

The wiper arm and drive shaft are fabricated from a Cu-Ni or stainless steel alloy that provides excellent corrosion resistance and anti-fouling characteristics. The two wiper blades are teflon, providing low frictional resistance, a poor surface for the adhesion of marine organisms, and insufficient hardness to scratch the glass. The blade length is sufficient

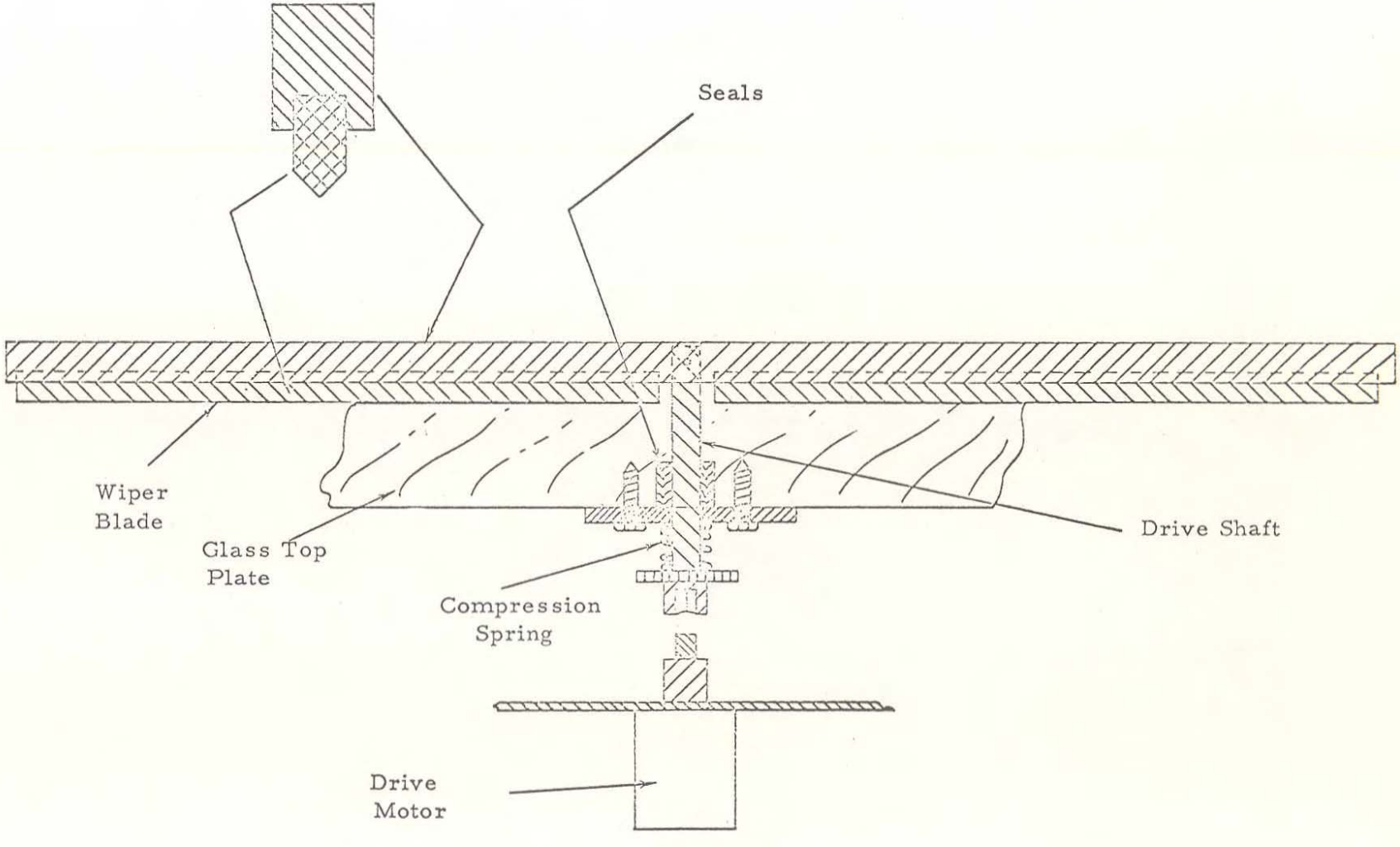


Figure 6.
Wiper Subassembly

to sweep almost the entire top of the instrument hull, thereby removing growth not only from the window but from other places from which shadows of filamentous growth could fall across the window.

The wiper is driven by a small 24 VDC geared motor turning approximately 2.5 rpm upon command from the programmer. The duty period of the wiper is approximately one minute prior to each light cycle.

The external portion of the wiper mechanism is shielded by the supports of the upper mooring frame which forms a cage around the top of the hull, protecting the wiper and glass plate from damage.

The interior walls of the chamber are cleaned by the scrubbing action of the O-rings used to seal the free piston against leakage. Since the piston travels the full length of the chamber each time a sample is added or discharged, the cleaning of the inner walls of the chamber occurs immediately before and after each sample acquisition. The chamber end surfaces are cleaned during flushing operations as no practical means of mechanically cleaning these areas could be found.

3. Liquid Tanks

Several operations of the instrument require long term storage of liquids for use over the entire on-station time of the buoy.

The holding tanks for the various solutions used in the buoy are made from urethane plastic, selected for its excellent corrosion resistance, insensitivity to chemical activity, and one piece construction which will provide a leak-free container. In addition, the advantages of light weight, ease of fabrication,

and the ability to assume complex shapes weigh heavily in favor of plastic.

The volumes of the various tanks are given below:

1. ^{14}C - 900ml
2. Poison - 250ml
3. H_2SO - 900ml
4. Waste - 3,000ml

These volumes will give the APPI the capacity required for about six months of continuous operation.

Tank location for the various functions depends primarily upon the need to keep all solution and sample lines as short as possible, thereby avoiding dead volumes and the resulting possibility of contaminating samples. For this reason, most tanks will be located immediately adjacent to the systems they serve.

4. Hull

During the evaluation of possible hull shapes for the APPI, spherical, parallelepiped disc, cylindrical, and toroidal configurations were studied. Considerations included such factors as pressure resistance, hydrodynamic stability, internal volume and configuration, strength, storage and handling characteristics, component availability, and fabrication ease.

It is felt that the requirements of this program are best met with a cylindrical configuration using flat end plates; the analysis that follows applies to this configuration.

While the ultimate APPI might be required to operate as deep

as 1,000 feet, the prototype unit will be used near shore or at dockside in shallow depths of up to approximately 20 feet. At 20 feet, the water pressure is about 10 psi. Allowing a safety factor of 2.5 for design purposes, the pressure differential seen by the hull is approximately 25 psi.

For a cylindrical shell with flat end plates under equal pressure from all sides, approximate collapse pressure of the cylinder is given by the following formula:

$$Pc = KE \left(\frac{t}{D}\right)^3$$

If we assume the walls are of steel 0.25 inches thick, and that the buoy is 14 inches high and 14 inches wide, the critical pressure can be calculated. The value of K, a coefficient dependent upon the cylinder length, thickness and diameter, is about 35 for this application. The modulus of elasticity, E, is taken to be 29.0×10^6 for steel. Thus,

$$Pc = 35 \left(29.0 \times 10^6\right) \left(\frac{0.25}{14}\right)^3$$

$$Pc = 5.8 \times 10^3 \text{ psi.}$$

This indicates more than ample strength for the prototype. However, since 0.25 inch thickness is about as thin as can easily be worked, no reduction in wall thickness will be made.

5. Valves

The overall system uses five controlled and six automatic valves

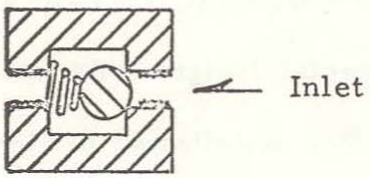
in the applications shown below.

Controlled Valves	Automatic Valves
1. Sample inlet	1. ^{14}C Pump (2)
2. Photosynthesis Chamber	2. Discharge Pump (2)
3. Sample Pump	3. Titration Syringe (2)
4. Drainage	
5. Titration Chamber	

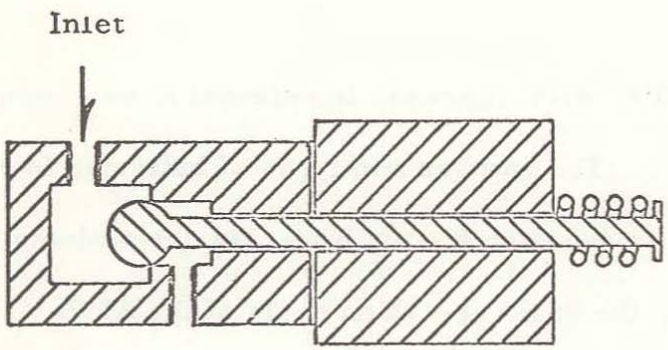
Valves have historically been a source of trouble in marine systems, primarily due to fouling and corrosion of moving parts. The problem of valve design on the APPI is made more complex due to the need for small sizes to keep dead volumes to a minimum.

An effort has been made to provide the maximum possible resistance to fouling through the configuration of the valve, using a sharp orifice sealed with a relatively soft oversize sphere (Fig. 7). In operation, the sharp edge of the orifice will provide the minimum surface area for growth and should penetrate any fouling occurring on the ball. If a particle becomes lodged between the ball and edge, the ball will deform and tend to seal around the particle.

As is the case with other components in the sample system, anti-fouling materials and coatings cannot be used due to their effects on the organisms under study.



Automatic Valve



Solenoid Valve

Figure 7

Valve Mechanisms

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6. Electronics

The prototype instrument will use a standard, commercially available power supply with a 115 V 60 cycle input and a 24 VDC output as the main power supply. No special design or fabrication effort will need to be directed to this unit. The distribution of electrical power to the various subsystems of the buoy is shown in Fig. 8 .

A high voltage power supply is used to provide the Geiger-Mueller tube with approximately a 600 V potential. This supply is a hybrid module capable of about a 10 ma output. A block diagram of the detector circuit is shown in Fig. 9 .

Operational APPI's, without access to external power, would rely on batteries for power. The number and types of batteries in the buoy would then determine the station time for the buoy. In determining the amount of power required, the basic operation cycle of the APPI, support devices such as position lights or radio transceivers, and any additional ancillary experiments must be considered in the analysis.

The two major categories of rechargeable batteries currently employed for long term or high capacity undersea instrumentation are the lead-acid and silver zinc types. The following table gives the significant characteristics of each type:

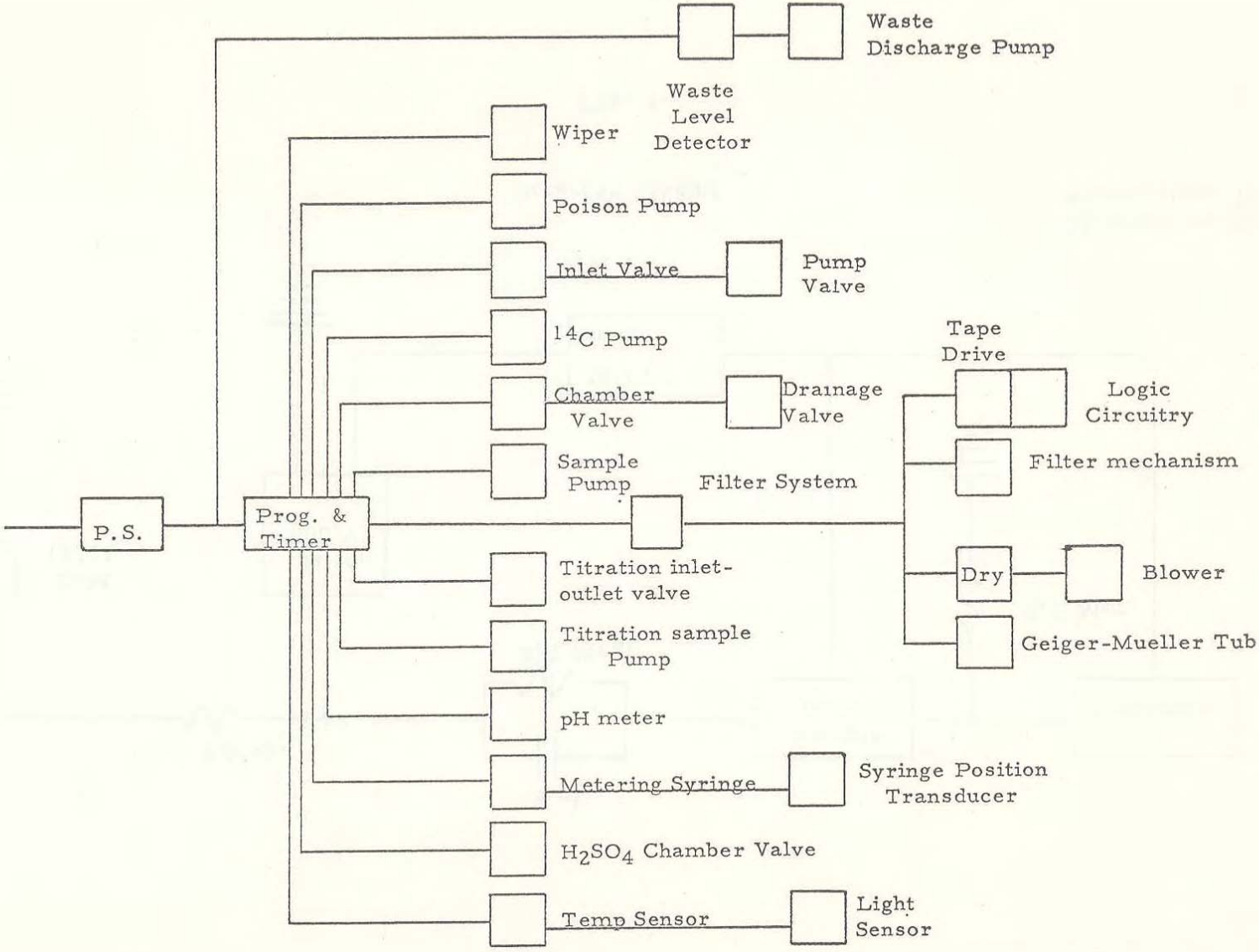

Fig. 8
Power Distribution

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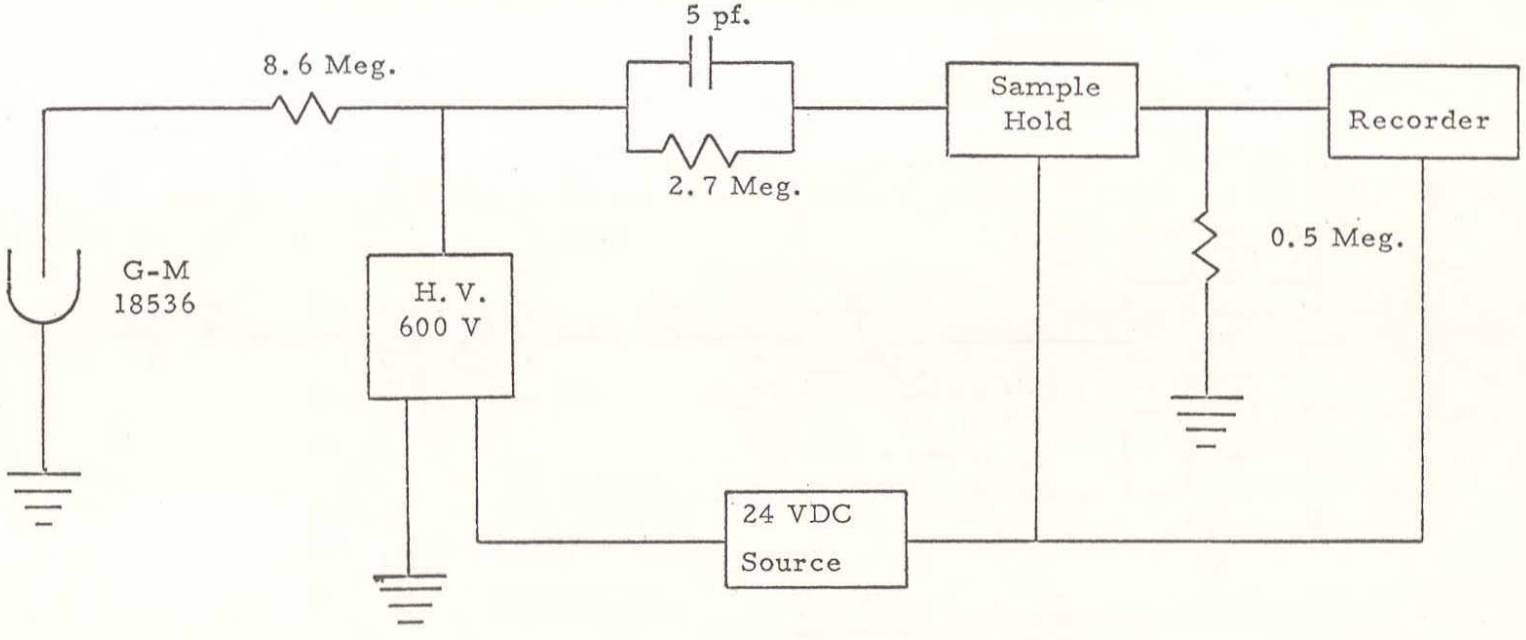

Detector Circuit

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Fig. 9.

Table 2

Battery Characteristics

Characteristic		Lead-Acid	Silver-Zinc
Weight	-	11.2 W-Hr/Lb.	35 W-Hr/Lb.
Volume	-	0.695 ft^3/KWH	0.26 ft^3/KWH
Recharge Life	-	500-2,000 cycles	100-150 cycles
Voltage Range	-	1.75-2.05 V/Cell	1.45 V/Cell
Temp. Tolerance	-	0-158°F	0-90°F
Cost	-	$65/KWH	$650/KWH

All electronic circuitry in the buoy is solid-state, for the obvious advantages of reliability, low power consumption, small size, and low heat production.

Circuit diagrams for the Geiger-Muller detector, waste tank liquid level detector, titration chamber, temperature measurement, and filter tape positioning are shown in Figures 9 , 3, 10, 11, and 12 respectively.

7. Recorder

At present, the system design concept requires the monitoring of five parameters: count rate, pH, light level, temperature, and quantity of titrant used. Two alternatives are applicable for making this information available to a recording device. The first is a remote selection of the individual parameter desired to be recorded. The second method is a

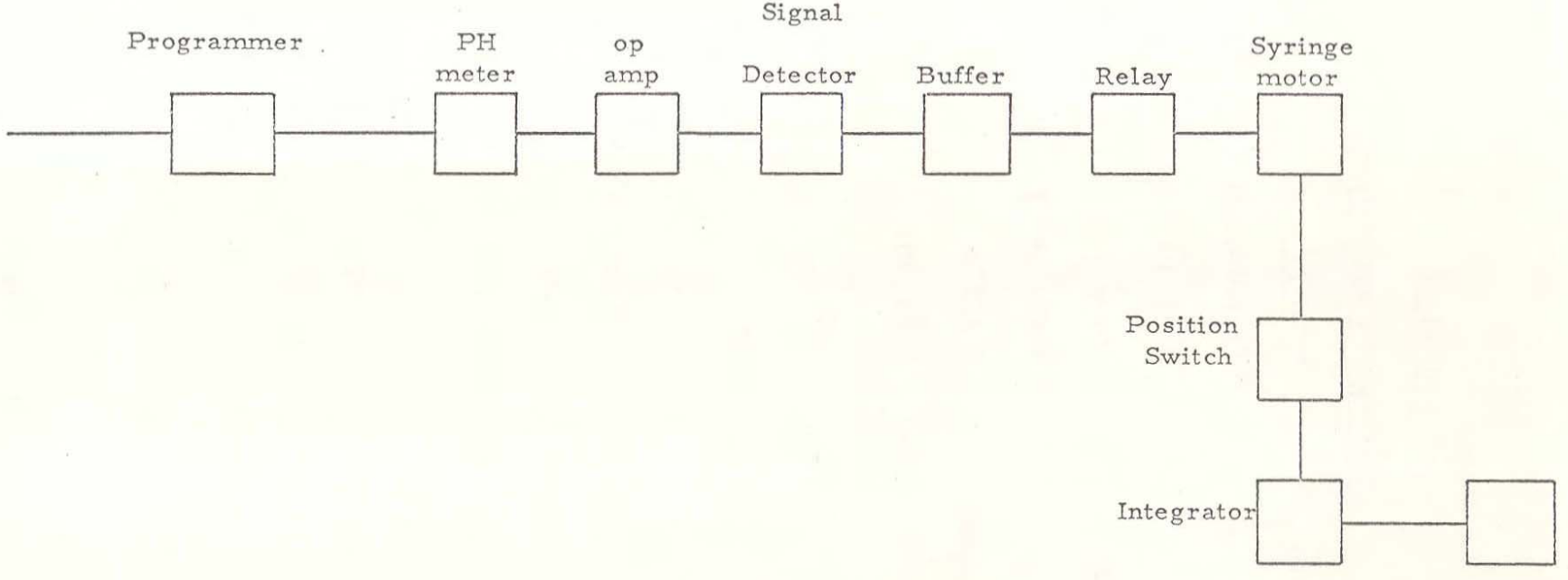

Figure 10.
Carbon Analyzer Block Diagram

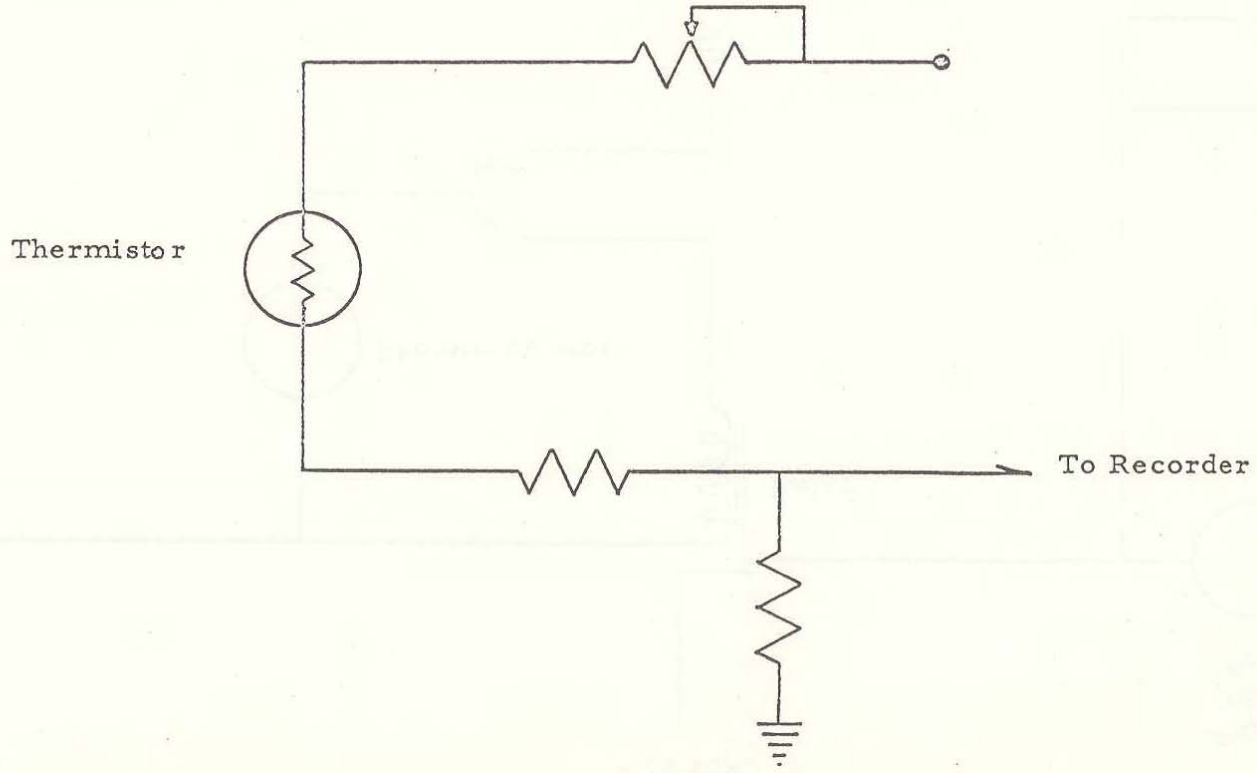

Figure 11.
Temperature Measurement
Circuit

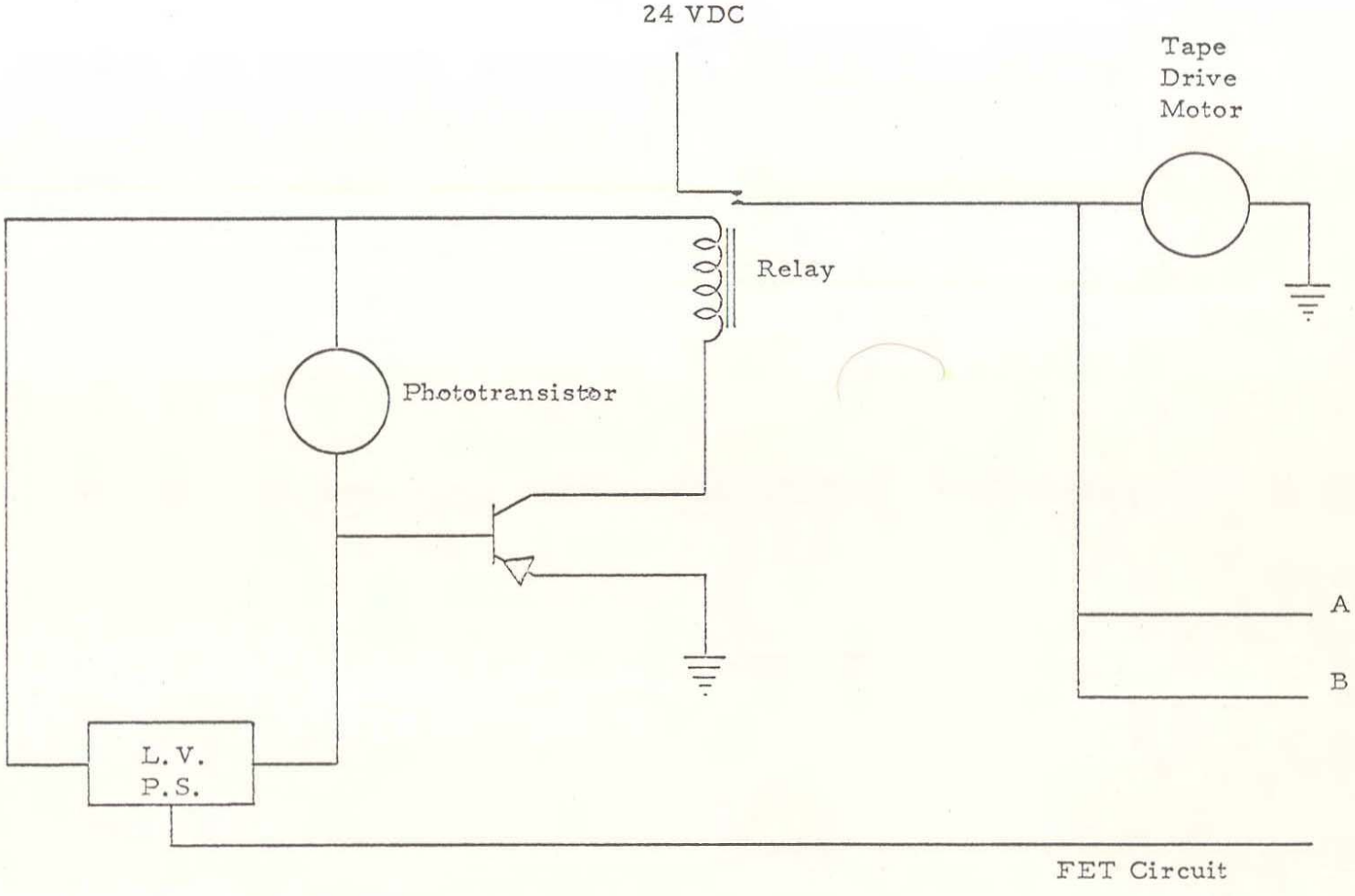

Figure 12.
Tape Positioning Circuit

programmed readout of all five parameters upon command. The latter is most applicable to the APPI system since it allows the greatest versitility within the instrument, and there are many commercial devices manufactured to perform this function. The programmed readout will consist of five equal time intervals comprising some predetermined total amount of time. These five intervals will occur in a set order and upon command will be monitored in the same order from an identical initiation point each time. If a particular time is chosen when one of the parameters is not in programmed operation, its readout will appear as the recorders reference level. This does not present any problem in data interpretation, since the time slot for each parameter in the programmed readout is known. A block diagram of the recorder circuit is shown in Fig. 13.

C. Total Instrument Concept

The presently envisioned prototype APPI configuration is a cylindrical hull approximately 14 inches long and 14 inches in diameter containing all of the subsystem components except the main power supply, the programmer, and the recorder. The relative relationship of these components is shown in Fig. 14. These devices are separated from the hull and located on the shore or ship for ease of access during operation by the experimenters.

The instrument contains two independent fluid handling sections - the first containing components for the primary productivity determination subsystems (Figs. 15 and 16), and the second section containing components for the determination of the natural carbonate content of the sample water

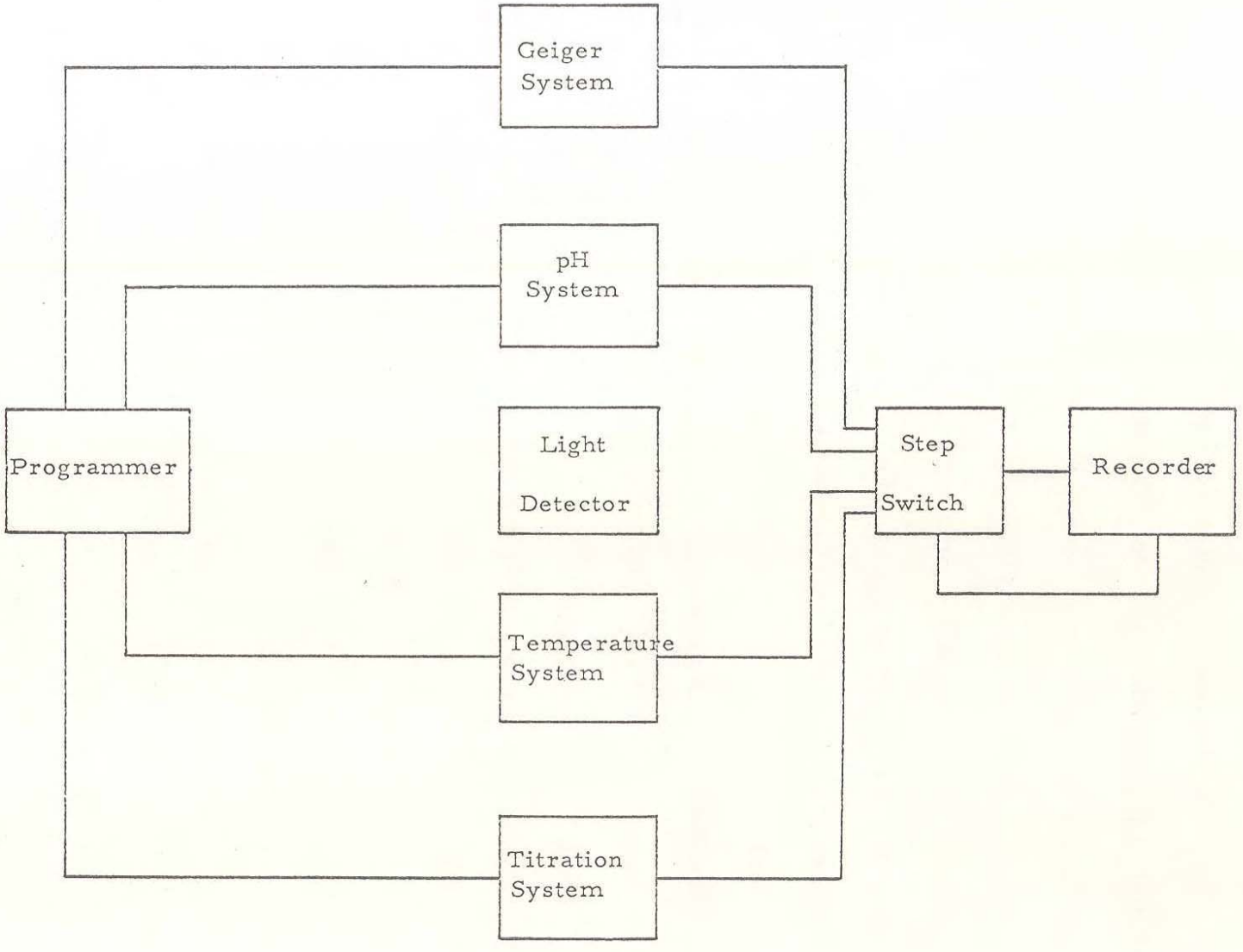

Figure 13.

Recorder Circuit Block Diagram

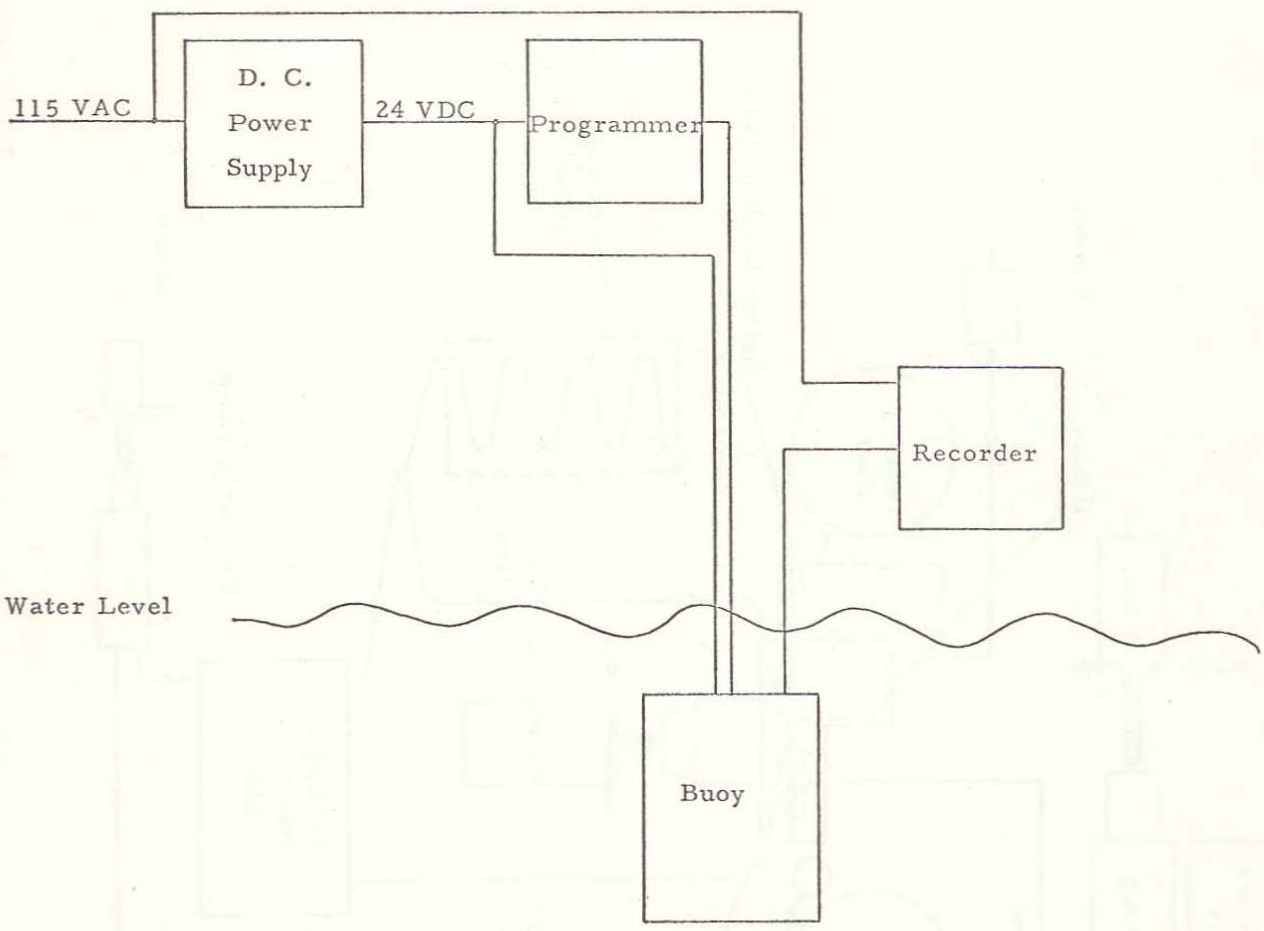

Fig. 14
APPI Prototype

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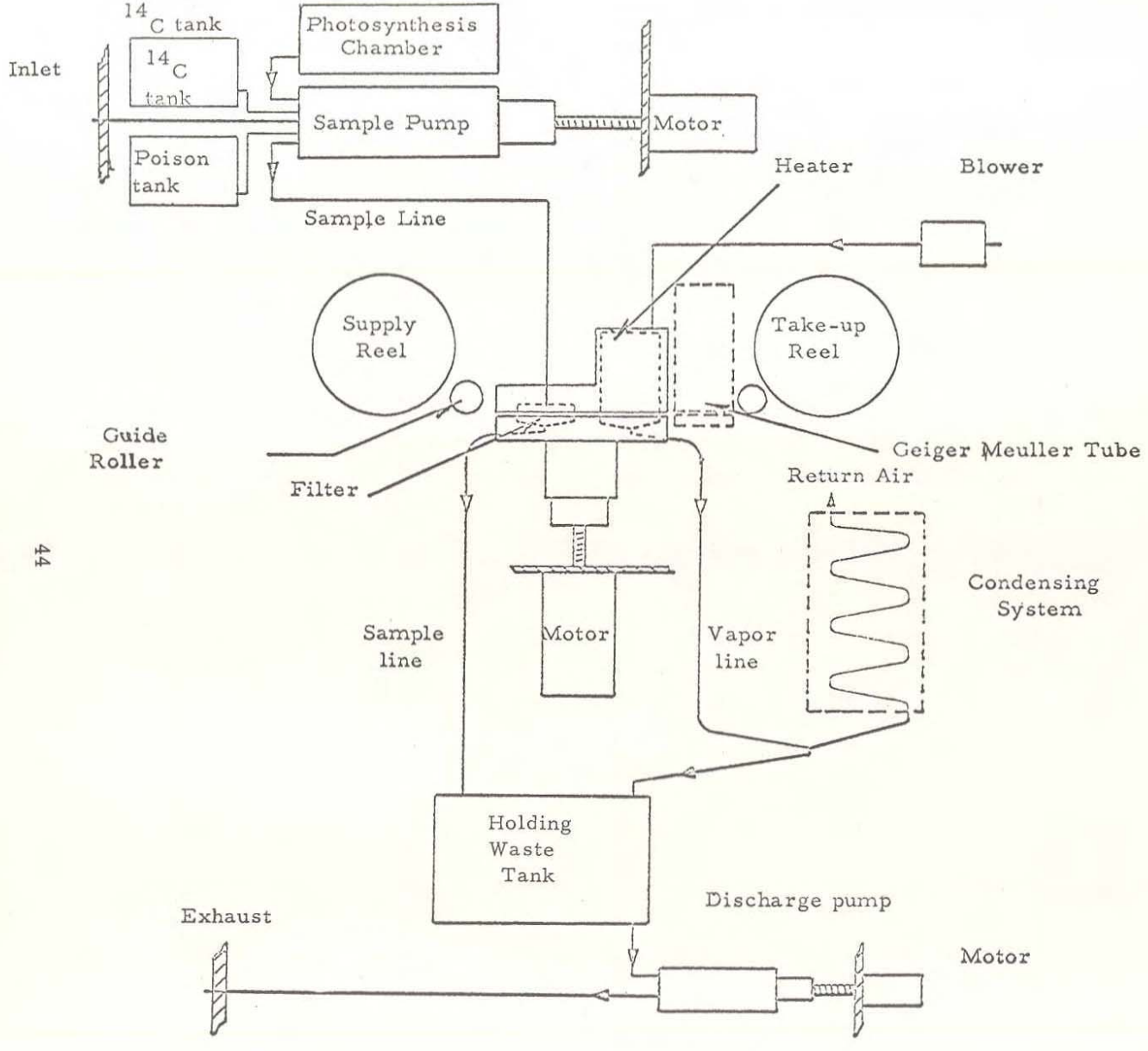

Figure 15.
Sample Flow Schematic

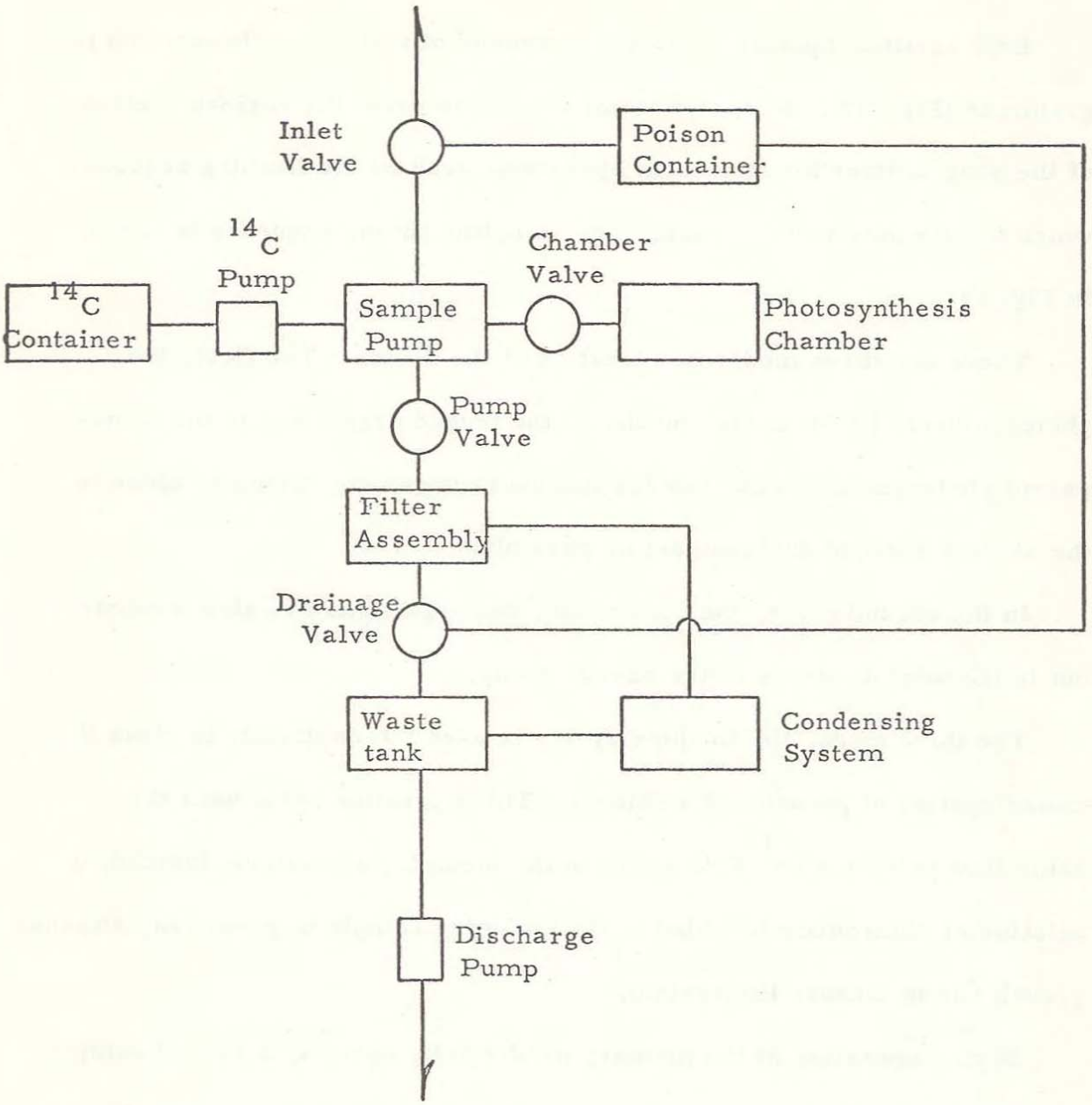

Figure 16.
Fluid Flow Path

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using a titration technique (Fig. 17).

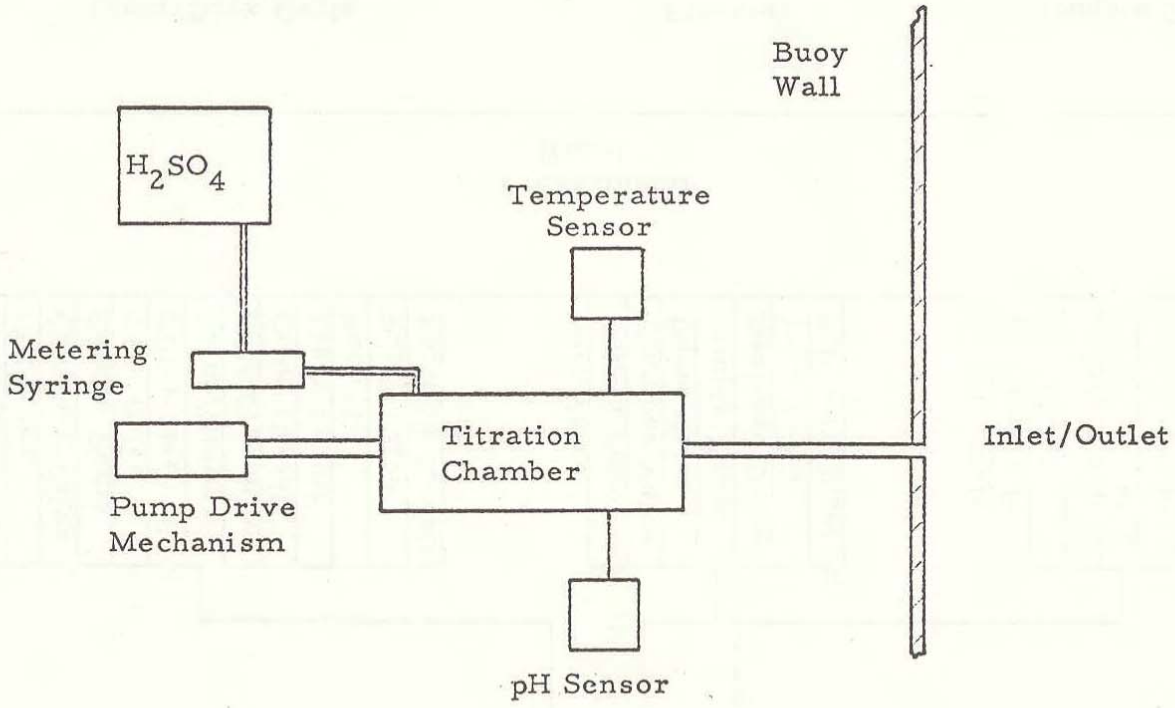
Both sections operate under the command of a single multi-section programmer (Fig. 18). A master timer is used to drive the various sections of the programmer for long term operations such as the flushing sequence which occurs only twice a week. The sampling timing sequence is shown in Fig. 19.

There are three modes of operation of the device. The first, the photosynthesis (light) cycle, incubates the tagged organisms in the transparent photosynthesis chamber for two hours under conditions as close to the natural daylight environment as possible.

In the second mode, the dark cycle, the organisms are also incubated, but in the total darkness of the sample pump.

The third mode, the flushing cycle, is used intermittently to clean the entire system of growth and sediment. This operation again uses the same flow path, but no ^{14}C is added to the incoming seawater. Instead, a solution of dichromate is added to the incoming sample to poison any attached growth and to cleanse the system.

During operation of the primary productivity system, a 100 ml sample of water enters the sample pump through the solenoid operated inlet valve. In a light cycle, the pump immediately transfers the sample to the photosynthesis chamber for two hours of incubation. Immediately upon entry into the pump, 1.0ml of ^{14}C solution is added to the sample for fixation by the



Carbonate Analysis

Figure 17.

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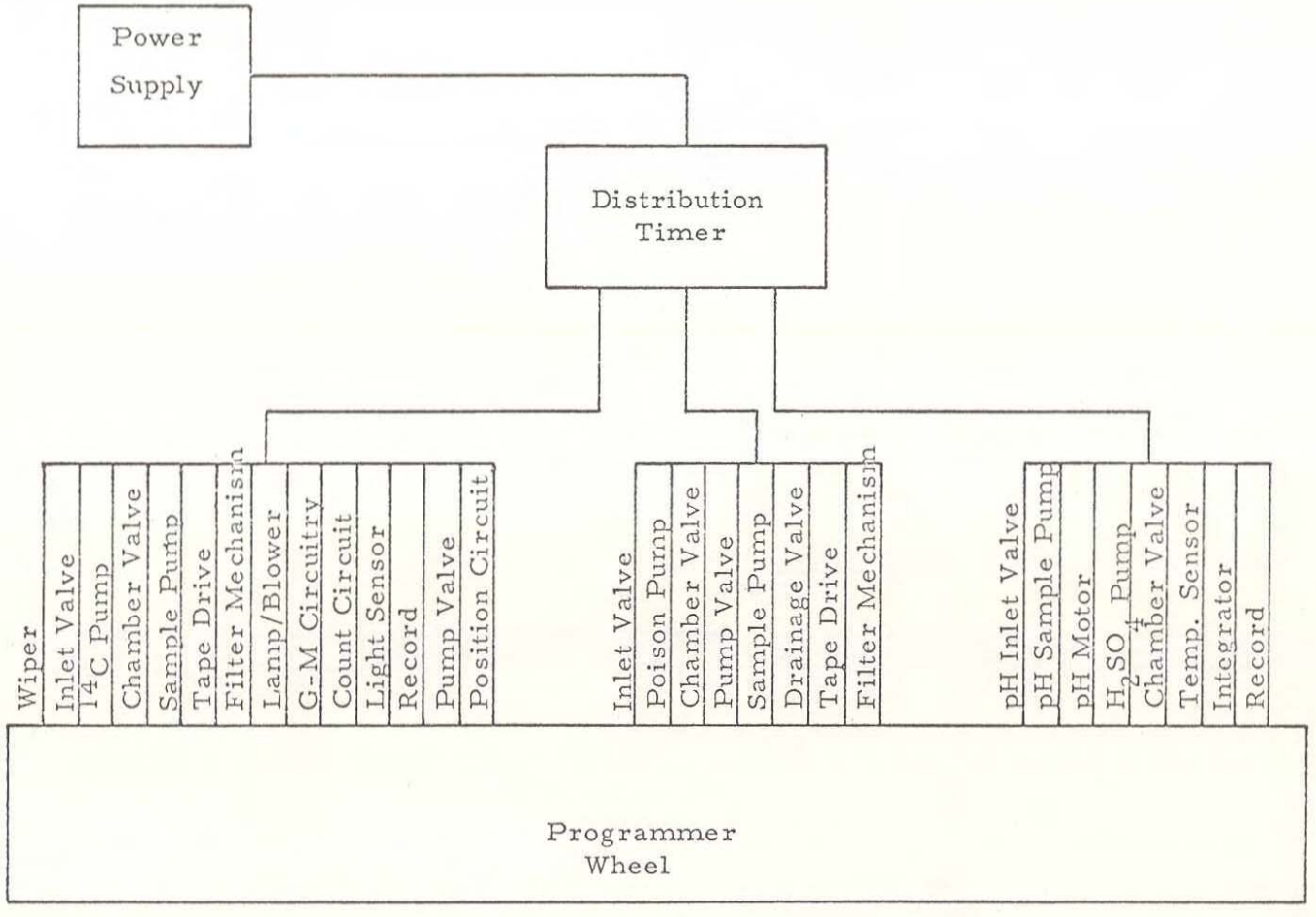

Figure 18.
Programmer
Arrangement

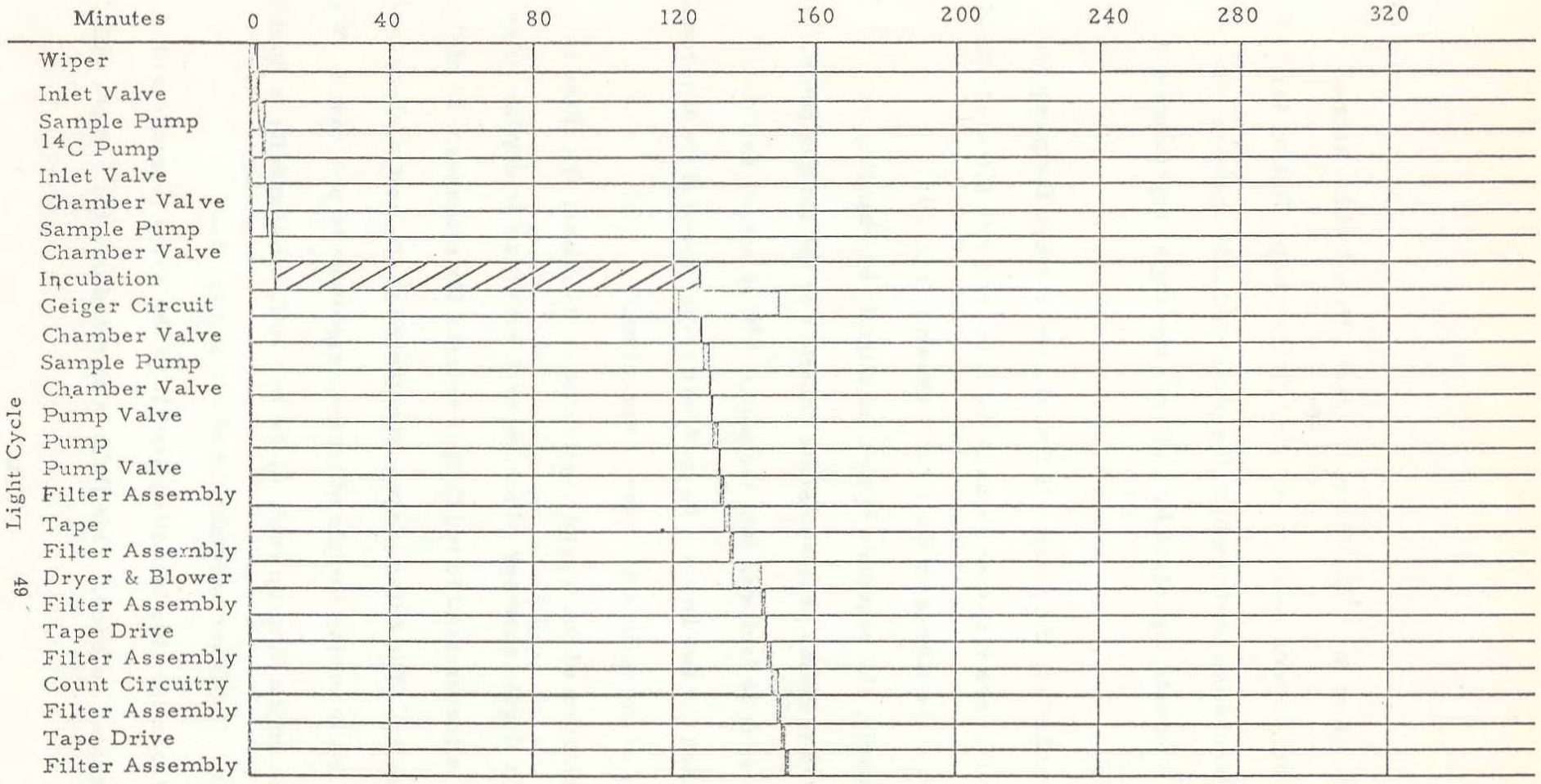

Figure 19.
Sampling Timing Sequence

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organisms. Addition of the ^{14}C solution is accomplished with a single stroke of a constant volume solenoid actuated piston pump. During the entire two hour incubation period within the photosynthesis chamber, the sample solution is constantly mixed by a stirrer turning at approximately 120 rpm.

Following incubation, the sample is transferred through the pump into the filter chamber where it passes through the filter and into the waste tank. The arrangement of the filters on the tape is shown in Fig. 20 .

In the dark cycle, the sequence is altered slightly by changing the incubation location from the photosynthesis chamber to the sample pump. In this case, the sample is drawn into the pump, ^{14}C is added, and the sample is incubated for two hours. Beyond this stage, there is no difference in the sequencing of the different modes of operation.

After accumulation of the tagged organisms on the filter, the filter is transferred to the drying chamber where several small lamps dry the filter. The dried filter is transferred to the Geiger counter for measurement of the level of radioactivity. The filter is then transferred to the next position where it remains until the following sample of tagged organisms is processed. Waste filters are stored on the take-up reel, which has sufficient capacity to hold the entire filter tape. The filter assembly is shown in Fig. 21.

In the flushing mode, the incoming sample of the water is mixed with a dichromate cleaning solution. This fluid is injected into the sample with

Fig. 22.

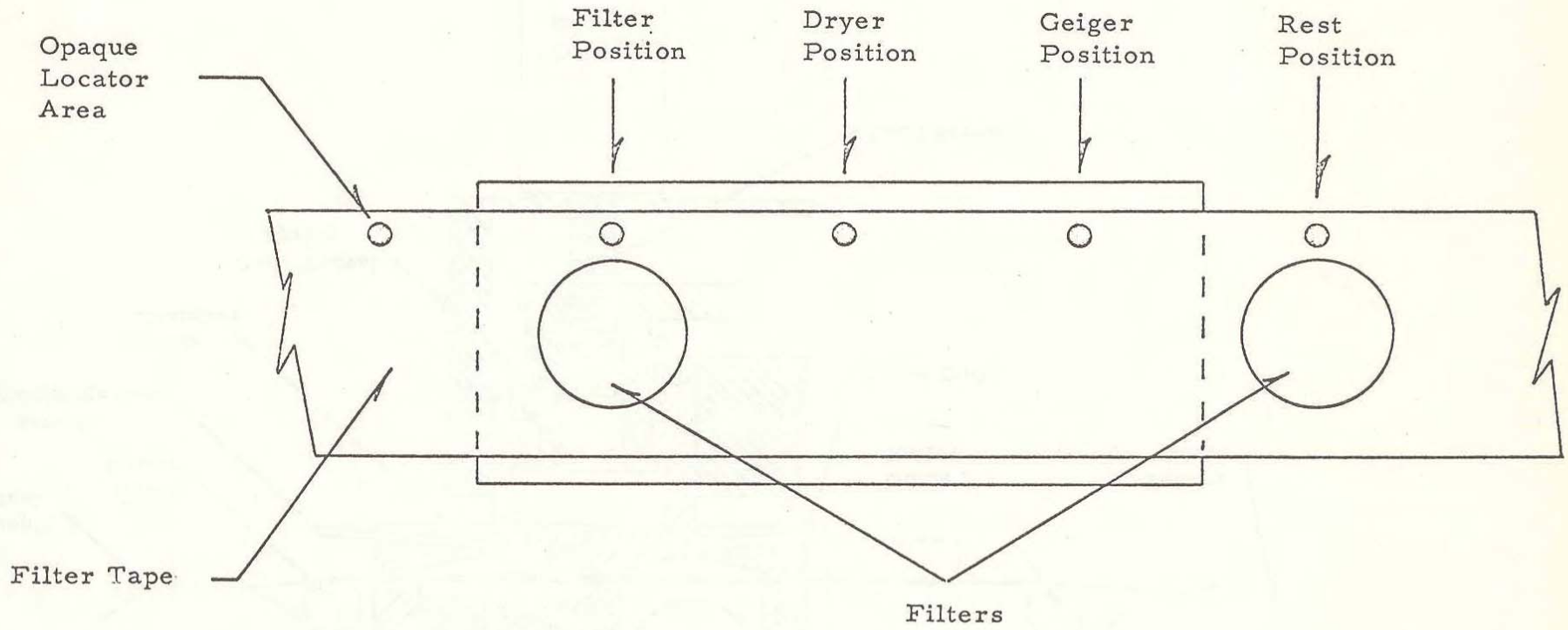
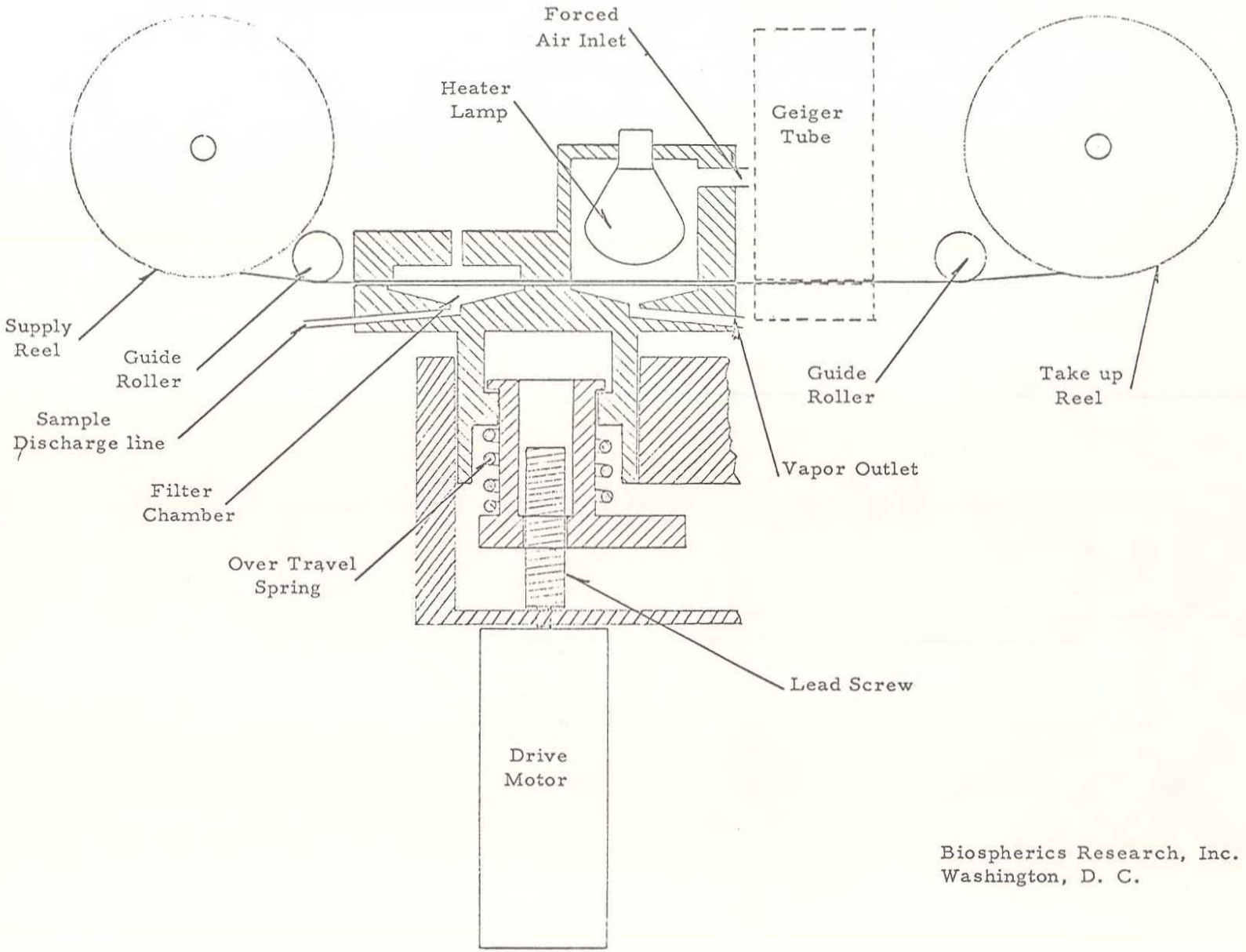


Figure 20.

Filter Tape



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Filter/Dryer Assembly
Fig. 21.

a solenoid pump similar to that used with the ^{14}C solution. The poisonous solution is then transferred into the photosynthesis chamber where it is held for a short time before passage through the remaining components of the system. After the complete passage of the poison, 20 sample volumes of pure water are rapidly passed through the system to remove all traces of the poison prior to the entry of the next sample.

The titration technique for natural carbonate analysis has been chosen as being the most suitable method to automate for our purpose. A block diagram of the carbonate determination system is shown in Fig. 18.

The titration system operates independently of all other components except for the power supply and programmer. Water is admitted into the chamber where a probe determines the natural pH, and the value is recorded. A metering syringe gradually mixes H_2SO_4 with the water sample until a pH of 3.75 is achieved and the syringe is stopped. The amount of H_2SO_4 required to attain pH 3.75 (displacement of the syringe) and the temperature of the sample in the chamber are recorded for use in the computation of the natural carbonate content. The system is shown in Fig. 17.

All functions involving the deposition and analysis of the sample acquired by the filter tape are performed at the filtration assembly (Fig. 21). Within the assembly, the filtration and dryer chambers are positioned side by side immediately above the filter tape. The sample is transferred from the pump under pressure to the filter chamber via a plastic tube. A liquid dispersal

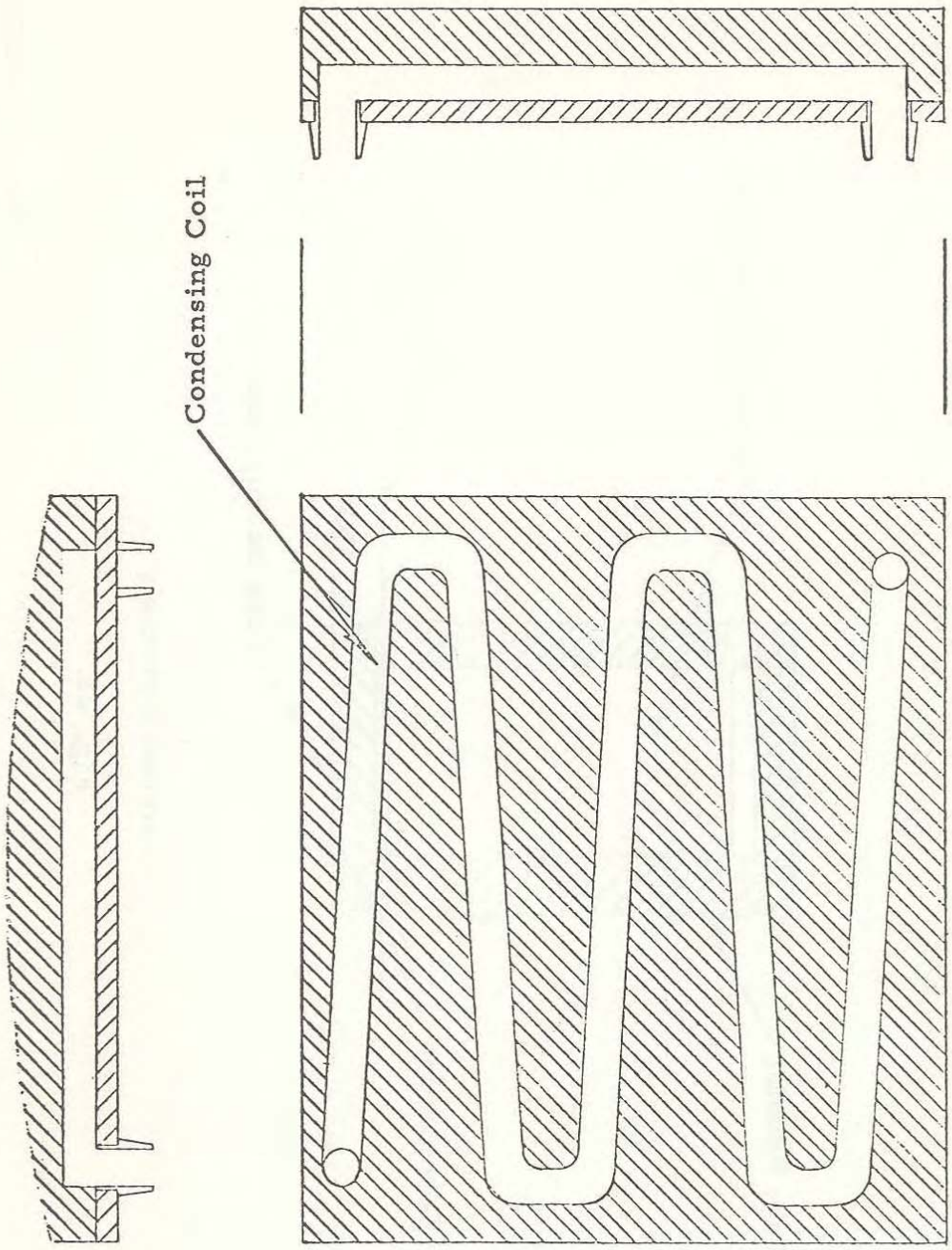
plate is located in the chamber to obtain an even distribution of sample over the filter.

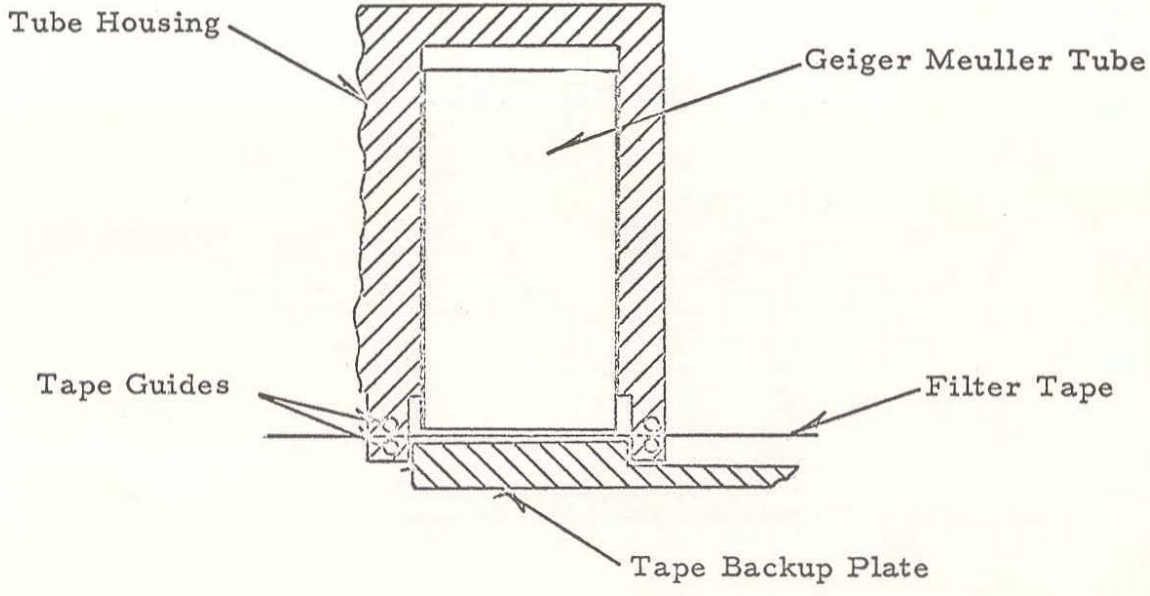
The drying chamber containing the heater lamps is necessary to obtain accurate radioactivity measurements of the weak ^{14}C beta emissions. Any liquid remaining in the samples during the counting period would absorb some of the signal emanating from the sample. A small blower provides forced air circulation, minimizing condensation and hot spots. A condenser coil is used in the exhaust line to dry the air leaving the chamber (Fig. 22).

Directly below the upper assembly and the tape, and normally in contact with the tape, are two drainage chambers for the filter and dryer sections. These two chambers are machined from one block of stainless steel. This block is driven by a lead screw mechanism similar to that of the sample pump. The motor withdraws the drainage block from the sample tape whenever the filter is stepped to the next position. Following filter transfer, the block is returned to its position in contact with the filter tape where O-rings on the upper and lower blocks seal the filter against leakage.

The motor used to drive this assembly turns about five rpm and the mechanism incorporates a limit switch and overtravel spring for control and protection.

The holder assembly (Fig. 23) for the Geiger-Muller tube is located adjacent to the dryer chamber. The holder incorporates tape guide rollers and a backup plate below the filter tape to insure a constant background radiation


Condensing Coil



Detector Assembly

Fig. 23.

count. The tube is positioned vertically within the holder with the tube window immediately above the sample filter.

The waste tank accumulates several days samples before a level is reached where the upper level detector will trigger the pump motor into operation and discharge the waste liquid . Approximately three minute's is required to empty the tank. Delaying the discharge of waste liquid permits the maximum separation of discharge and sample times.

D. Ambient Environment Simulation

The fundamental approach of the APPI experiment is to measure the primary productivity of water at a particular location under conditions as close to the natural environment as possible. The environmental parameters of primary concern are light intensity and temperature. Both of these parameters are measured and recorded for use in the primary productivity determination.

In support of this requirement, the incubation chambers (light & dark) are configured to provide conditions as close to that of the surrounding waters as possible. The photosynthesis chamber is fabricated from a glass or plastic material having light transmission characteristics similar to those of water The location of the two chambers as physically close to the water as possible insures temperature equilibrium by thermal conduction through the hull structure. No significant heat sources are located with the hull. The drying lamps used provide only localized heating and are insulated from the rest

of the instrument.

The motion of the buoy from external currents and the internal agitation from the stirrers within the photosynthesis chamber and sample pump prevent settling of the sample during incubation.

Frequent primary productivity determinations will be made to assure rapid detection of changes in ambient plankton populations. To prevent contamination of subsequent samples, the discharge of waste fluid occurs as far as possible from the sample intake opening. As an additional precaution, the waste is discharged at the end of the several days sampling activity, thereby providing an 8-12 hour interval between discharge and the next sample acquisition. The poisonous flushing solution is recycled through the instrument to eliminate the possibility of contamination from this source.

VI. Program Planning - Phase II

The anticipated work for the next phase of the program includes the following areas.

1. Completion of detail design and specifications based on the present design approach - Phase II Biochemistry/Microbiology Instrumentation Design Support.
2. Mechanical and electrical detail design of the buoy's logic system.
3. Joint AEC, BRI preliminary design review of biological and engineering design.
4. Preparation of final engineering drawings and specifications.
5. Quality assurance and control over incoming items procured from commercial sources.
6. In-house fabrication of remaining items.
7. Assembly and testing for functional operation of electronic and electromechanical subsystems.
8. Prototype assembly.
9. Debugging and functional testing of breadboard instrument.
10. AEC/Biospherics Research, Inc. review of prototype and design.
11. Completion of recommended changes.
12. Final laboratory checkout of breadboard instrument
13. Environmental checkout on-site.

In addition, Biospherics will revise and update the engineering manpower requirements and the original estimates of parts and service procurement from commercial sources.

The development priorities assigned to the various systems of the buoys are as follows:

1. Detection System - Includes the Geiger-Mueller tube and circuitry, filter interface, and required power supplies.
2. Photosynthesis System - Includes chamber configuration, cleaning technique, and mixing requirements.
3. Anti-fouling Requirements throughout the buoy.
4. Filtering System - Includes the filter, filter tape, drive mechanism, and locator circuitry.
5. Drying System - Includes heaters, blowers, and insulation requirements.
6. Data Reduction System - Includes recorder and output sequencing and control circuitry.
7. Programmer System - Includes timers, programmer, and switching circuits.
8. Valve Mechanisms and Actuators.
9. Pumping Mechanisms
10. Carbonate Determination System - Includes titration chamber, metering syringe, and pH and temperature sensors.
11. Hull and Mooring System - Includes hull structure, internal arrangement, and mooring provisions.

The above information provides all necessary data for the computation of primary productivity.

Phase I of the contract study has brought to light no information that would suggest significant modification of the Phase II plan of work. The major areas requiring additional effort are the problem of marine fouling and demonstration of genuine in situ conditions within the photosynthesis chamber. Several alternative solutions to these problems exist and the goal of Phase II biology research should be directed toward optimization of instrument reliability by assuring true ambient conditions in the sample chamber and by selection of the best method of scraping and poisoning to eliminate the anticipated undesirable growths. Therefore, the results of the work accomplished in Phase I of this effort support strongly the recommendation that Phase II be continued as planned. Accordingly, approval of the instrument concept herein presented is requested along with authorization for Phase II to begin in March, 1969. The intervening month between submittal of this report and the March approval will be spent in continued pursuit of the engineering and biological tasks in Phase I.

APPENDIX A

CONCEPT MICROBIOLOGY - BIOCHEMISTRY REVIEW

The research described in Appendix A has been developed in the laboratories of Biospherics Research, Incorporated, to establish the feasibility of and assist in the design of the APPI, an automated instrument for the determination of primary productivity in water bodies, in fulfillment of Phase I, AEC Contract # AT (30-1)-3993, Task 220.

		Replicates	Light (CPM)	Dark (CPM)
Filtered Volume:	10 ml	1	6,345	374
Incubation Time:	161 hours	2	6,657	330
Filtered Volume:	5 ml	1	3,633	177
Incubation Time:	36 hours	2	3,603	125
Filtered Volume:	10 ml	1	8,724	160
Incubation Time:	36 hours	2	8,275	201
Filtered Volume:	10 ml	1	9,864	431
Incubation Time:	4 hours	2	10,274	358
Filtered Volume:	10 ml	1	9,864	238
Incubation Time:	1 hour	2	10,068	282

Table 1. ^{14}C Uptake by C. vannielii

Preliminary determination of required incubation period and reproducibility of carbonate uptake measurement.

Assay Conditions:
Cell Density ------ 1×10^6 organisms/ml
Light Intensity ---- 300 ft-candles
Temperature ------ 30° C
Incubation Chamber - 200 ml
Isotope ---------- 1 μCi $NaH^{14}CO_3$

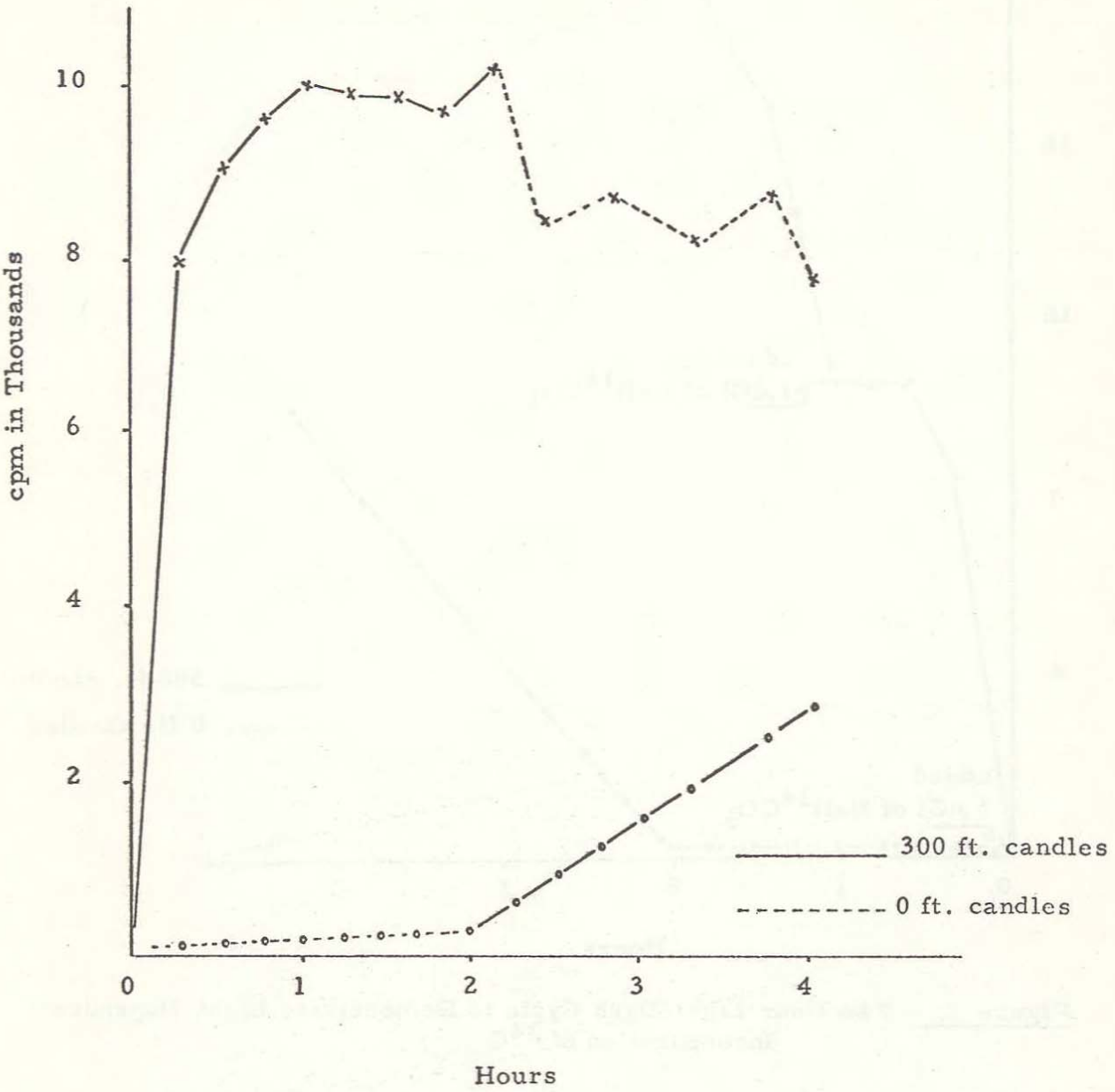


Figure 1. Two Hour Light/Dark Cycle to Demonstrate Light-Dependent Incorporation of ^{14}C

A 200 ml sample of Chlorella sorokiniana was incubated under the conditions shown, in the presence of growth medium supplemented with 1 μCi (10 μgm) of $NaH^{14}CO_3$. At 15 minute intervals, 10 ml aliquots were removed and assayed for radioactivity.

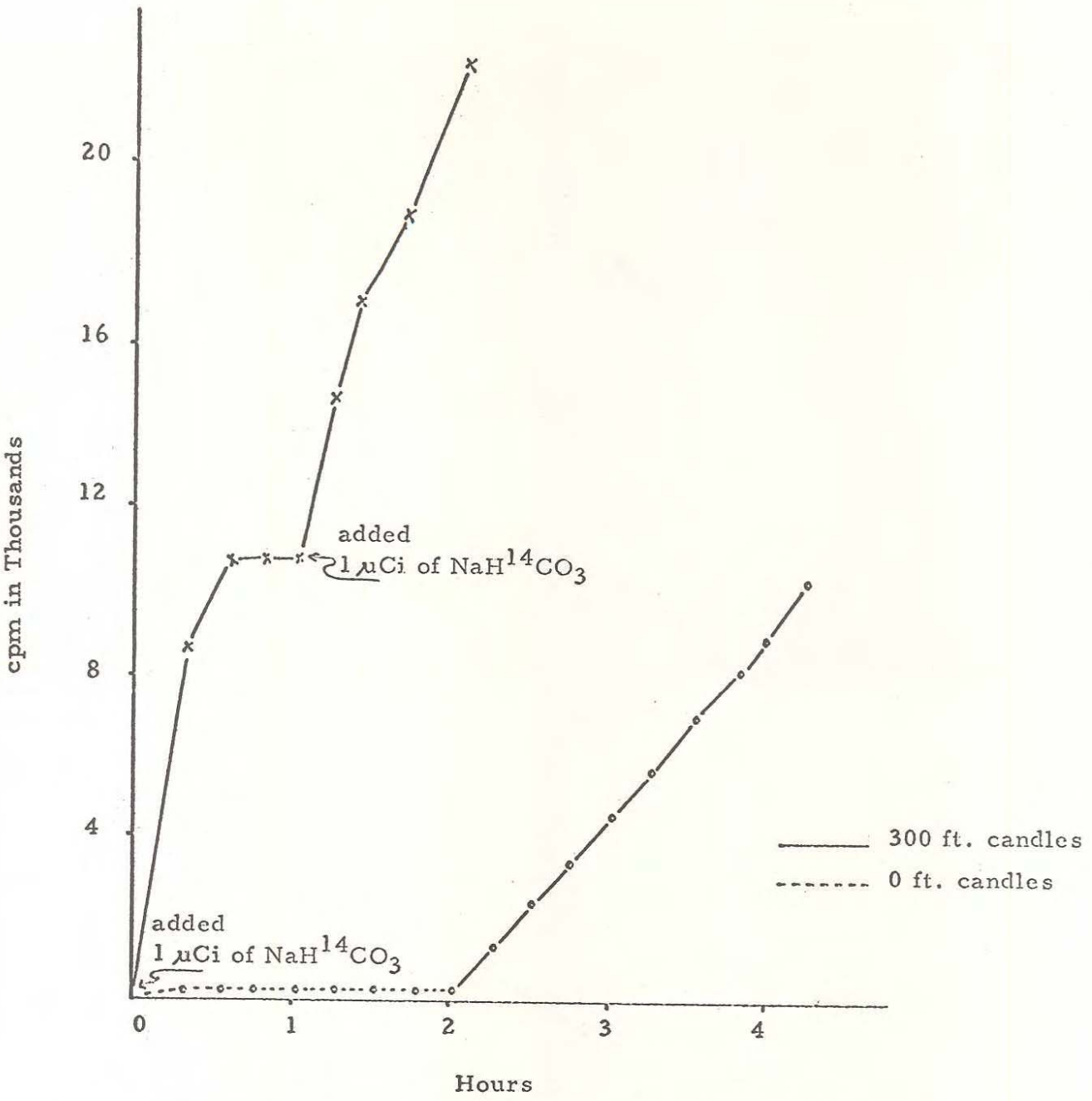


Figure 2. Two Hour Light/Dark Cycle to Demonstrate Light-Dependent Incorporation of ^{14}C

A 200 ml sample of Chlorella sorokiniana was incubated under the conditions shown, in the presence of growth medium supplemented with 1 μCi (10 μgm) of $NaH^{14}CO_3$. At 15 minute intervals, 10 ml aliquots were removed and assayed for radioactivity. To demonstrate that the plateau occurring in the previous figure resulted from exhaustion of radioactive substrate, an additional ampoule containing 1 μCi (10 ugm) $NaH^{14}CO_3$ was added as shown.

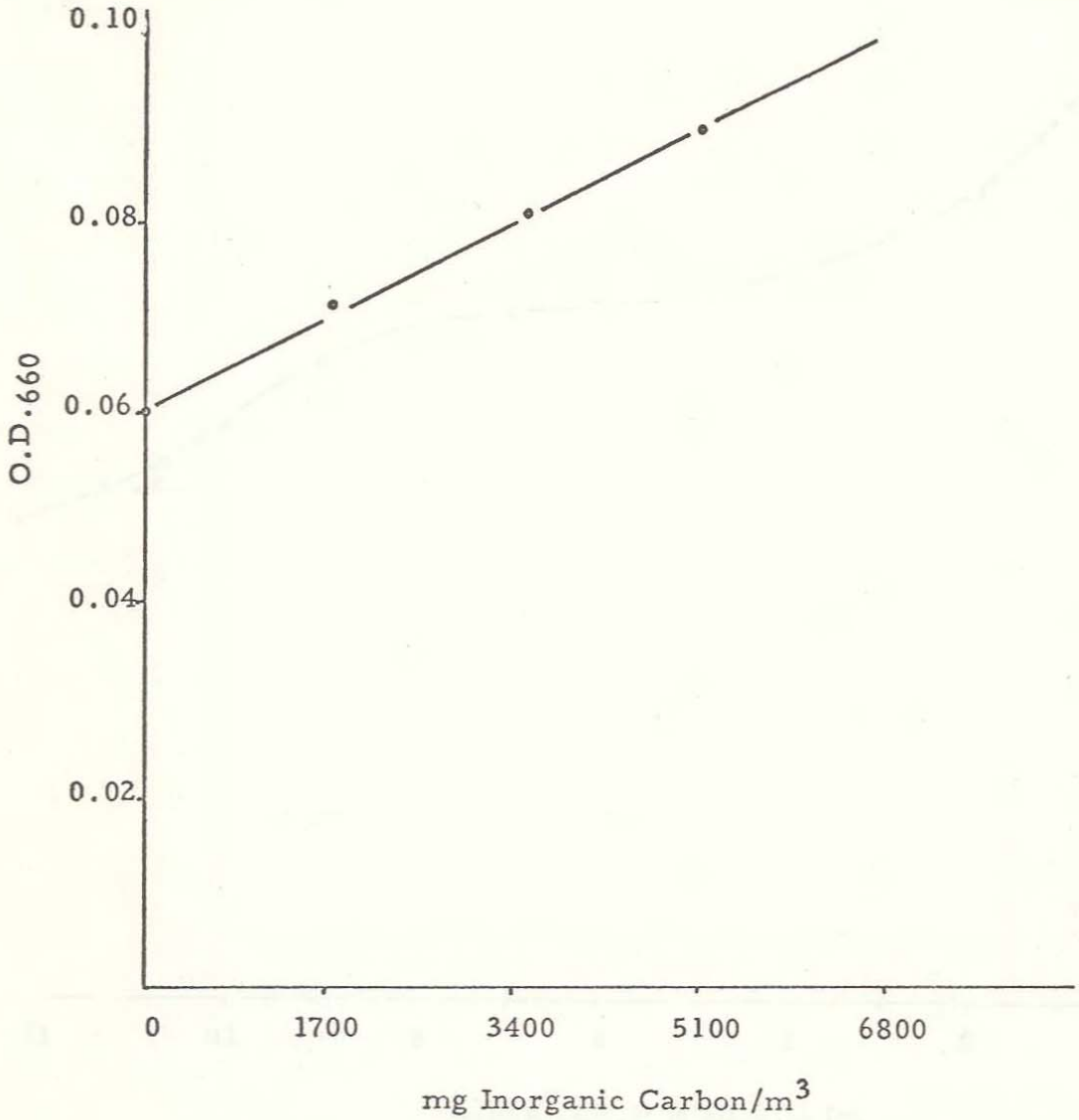


Figure 3. Turbidimetric Determination of Inorganic Carbon Standard Curve

Each sample is acidified to release all carbonate as CO_2. The gas is collected by precipitation of $BaCO_3$ in a half-saturated $Ba(OH)_2$ solution and the resulting turbidity is measured at 660 mu.

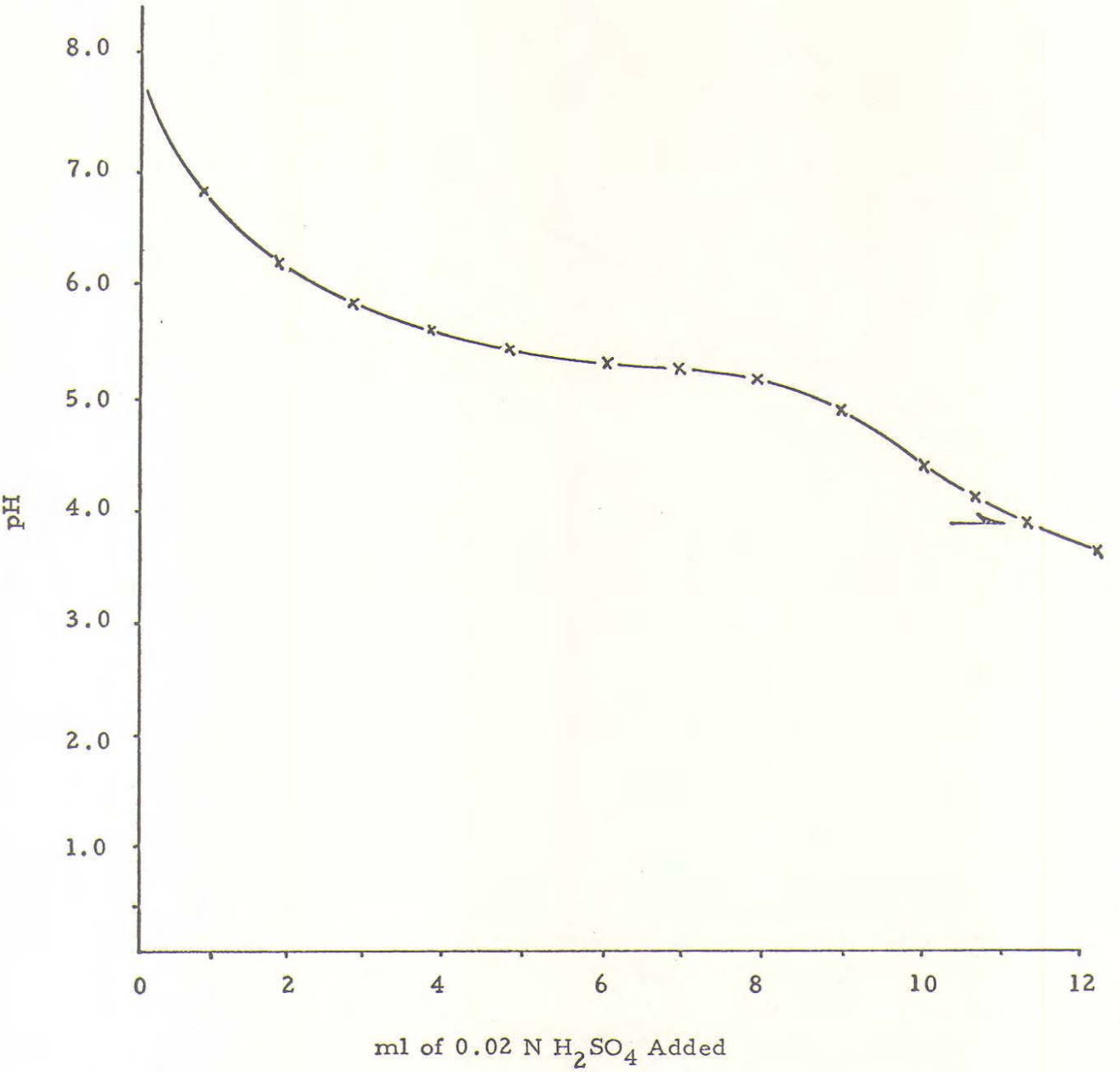


Figure 4. Potentiometric Titration of a Sample of Seawater

Assay: Sample --- 100 ml Seawater
Indicator - Methyl Orange
Titrant --- 0.02 N H_2SO_4

Arrow indicates Methyl Orange Endpoint

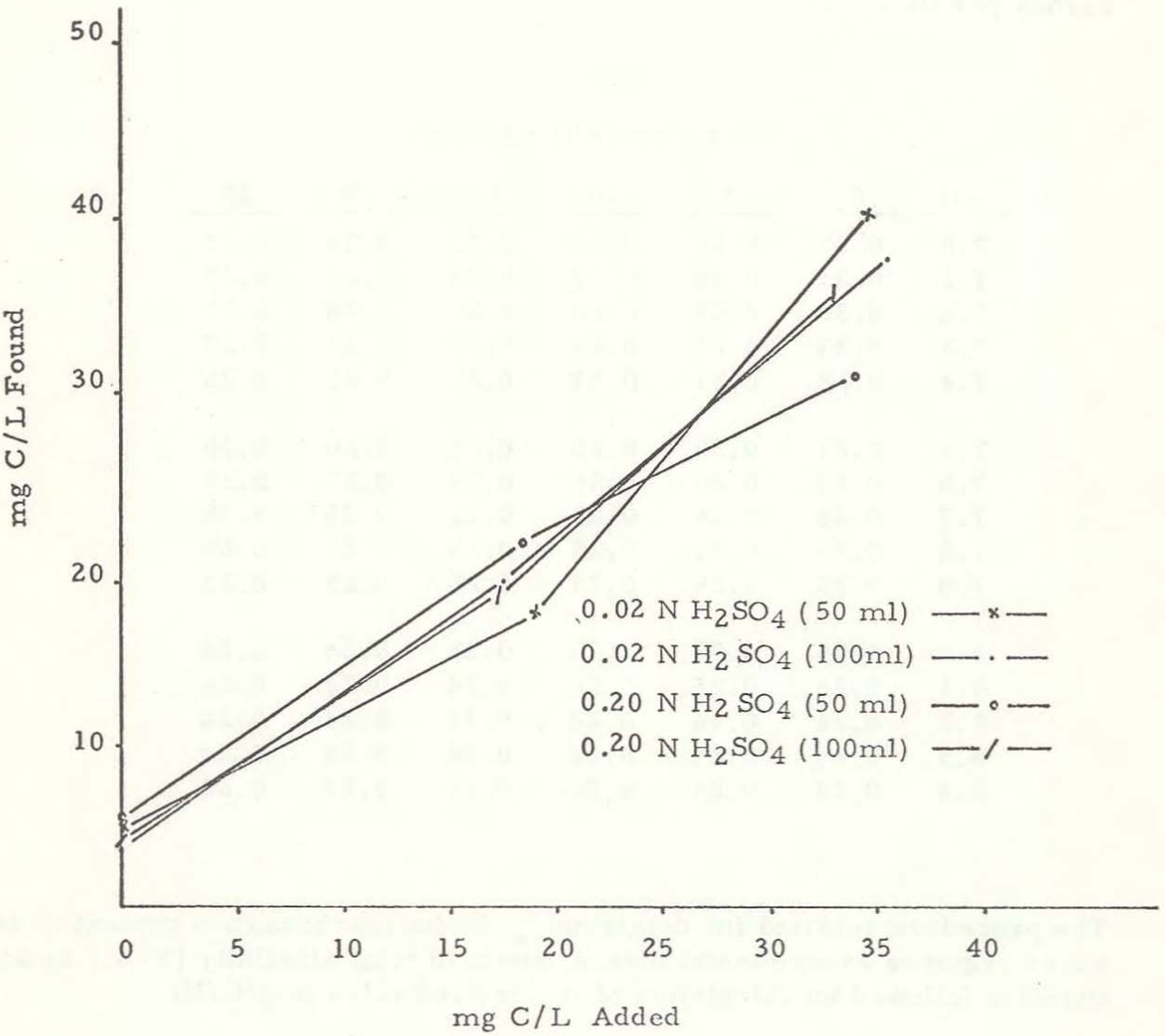


Figure 5. Acid Titration for Total Alkalinity (T.A.) Determination

Determination of the effect of varied sample volume and normality of titrant upon reliability of standard assay for inorganic carbon content of seawater.

Table 2.

Factors for the conversion of total alkalinity to milligrams of stable inorganic carbon per liter, (k).

	TEMPERATURE (°C)					
pH	0	5	10	15	20	25
7.0	0.33	0.32	0.31	0.30	0.30	0.29
7.1	0.31	0.30	0.29	0.29	0.29	0.28
7.2	0.30	0.29	0.28	0.28	0.28	0.27
7.3	0.29	0.28	0.27	0.27	0.27	0.27
7.4	0.28	0.27	0.27	0.26	0.26	0.26
7.5	0.27	0.26	0.26	0.26	0.26	0.26
7.6	0.27	0.26	0.26	0.25	0.25	0.25
7.7	0.26	0.26	0.25	0.25	0.25	0.25
7.8	0.25	0.25	0.25	0.25	0.25	0.25
7.9	0.25	0.25	0.25	0.25	0.25	0.25
8.0	0.25	0.25	0.25	0.25	0.24	0.24
8.1	0.25	0.25	0.24	0.24	0.24	0.24
8.2	0.24	0.24	0.24	0.24	0.24	0.24
8.3	0.24	0.24	0.24	0.24	0.24	0.24
8.4	0.24	0.24	0.24	0.24	0.24	0.24

The procedure selected for determining the inorganic carbon content of seawater requires experimental measurement of total alkalinity (T.A.) by acid titration followed by calculation of the desired value (mg/C/l).

$$\text{mg C/l} = \text{T.A.}(k)$$

Where T.A. = 10 (t), t = ml of 0.02 N H_2SO_4 used in titration of 100 ml sample to pH 3.75.

k = conversion factor

PRIMARY PRODUCTIVITY CALCULATION METHOD

$$P = \frac{r}{R} \times C \times f$$

where P = photosynthesis in mg C/m^3

r = radioactivity taken up in/cpm

R = total available radioactive carbon in cpm

C = total available stable inorganic carbon in mg/m^3

f = isotope correction factor

Uptake of Radioactive Carbon

$$r = \text{count/min (for volume filtered)} \times \frac{\text{volume experimental chamber}}{\text{volume filtered}}$$

Total Available Radioactive Carbon

R = microcuries of radioactivity used x efficiency of counter (in percent) x disintegrations per minute per microcurie = count/min.

Figure 6. Method used for the calculation of gross photosynthesis from experimental values obtained, (taken from Saunders et al, 1962). Determination of primary productivity is then obtained as the difference between light bottle and dark bottle response (see Literature Search).

APPLICATION OF PRIMARY PRODUCTIVITY EQUATION

Using this equation, an experiment was run with a laboratory strain of green algae, Chlorella sorokiniana, to measure the level of primary productivity and the results have been calculated as follows:

Experimental Chamber = 200 ml
Illumination = 300 ft. candles
Incubation = 1 hour

$$P_L = \frac{r}{R} \times C \times f$$

where P_L = gross uptake of inorganic carbon occurring in the light

$$r = \frac{10{,}000 \text{ cpm}}{1} \times \frac{200 \text{ ml}}{10 \text{ ml}} = 200{,}000 \text{ cpm}$$

$$R = (1.0\,\mu\text{Ci})\ (0.30)\ (0.838)\ (3.7 \times 10^4 \times 60 \text{ dpm}) = 558{,}108 \text{ cpm}$$

$$f = 1.06$$

P_L = gross photosynthesis in light = $\frac{200{,}000 \text{ cpm}}{558{,}108 \text{ cpm}}$ (8.5) mg C/m^3 (1.06)

$P_L = 3.604$ mg C/m^3 for 1 hour

$$P_D = \frac{r}{R} \times C \times f$$

where P_D = gross uptake of inorganic C occurring in the dark

$$r = \frac{300 \text{ cpm}}{1} \times \frac{200 \text{ ml}}{10 \text{ ml}} = 6000 \text{ cpm}$$

C = 10μg $NaHCO_3$/200 ml 8.5μg C/m^3

P_D = gross photosynthesis in dark = $\frac{6000 \text{ cpm}}{558{,}108 \text{ cpm}}$ (8.5) mg C/m^3 (1.06)

$P_D = 0.0085$ mg C/m^3 for 1 hour

$PP = P_L - P_D = 3.6040 - 0.0085 = 3.5955$ mg C/m^3/ hour

CALCULATIONS OF PRIMARY PRODUCTIVITY OF NATURAL WATER SAMPLE

Experimental Chamber = 200 ml
Illumination = 300 ft. candles
Incubation = 2 hours
Sample = Rock Creek, Washington, D.C.
at Northern District Boundary

$$P_L = \frac{r_L}{R} \times C \times f$$

where P_L = gross uptake of inorganic C occurring in the light

$$r_L = \frac{40 \text{ cpm}}{1} \times \frac{200 \text{ ml}}{10} = 800 \text{ cpm}$$

R = 558,108 cpm

$$\frac{r_L}{R} = \frac{800 \text{ cpm}}{558{,}108} = 0.0016$$

C = 52,000 mg C/m^3 as $NaHCO_3$ = 8.84×10^3 mg C/m^3

$$P_L = 0.0016 \times 8.84 \times 10^3 \times 1.06 = 14.9920 \text{ mg } C/m^3/2 \text{ hrs.}$$

CALCULATIONS OF PRIMARY PRODUCTIVITY OF NATURAL WATER SAMPLE

Experimental Chamber = 200 ml
Illumination = 0 ft. candles
Incubation = 2 hours
Sample = Rock Creek, Washington, D.C.
at Northern District Boundary

$$P_D = \frac{r_D}{R} \times C \times f$$

where P_D = gross uptake of inorganic C occurring in the dark

$$r_D = \frac{25 \text{ cpm}}{1} \times \frac{200 \text{ ml}}{10 \text{ ml}} = 500 \text{ cpm}$$

$R = 558,188$ cpm

$$\frac{r}{R} = \frac{500 \text{ cpm}}{558,108 \text{ cpm}} = 0.001$$

$C = 8.84 \times 10^3$ mg C/m^3

$$P_D = 0.001 \times 8.84 = 10^3 \times 1.06 = 9.3700 \text{ mg } C/m^3/2 \text{ hrs}$$

Primary productivity is the difference between the light and dark bottle values:

$$PP = P_L/2 - P_D/2 = 7.49 \text{ mg } C/m^3 - 4.60 \text{ mg } C/m^3$$

$$PP = 2.81 \text{ mg } C/m^3/hr$$

Potomac River Site	Collection Date	Assay Date	PP mg C/m^3/hr.
Great Falls, Va.	1/9/69	1/16/69	2.46
(Northwesternmost)	1/9/69	1/17/69	24.33
	1/9/69	1/22/69	8.88
	1/23/69	1/24/69	4.91
Washington, D. C.	1/9/69	1/16/69	1.15
(Chain Bridge)	1/9/69	1/17/69	7.90
	1/9/69	1/22/69	4.66
	1/23/69	1/24/69	0.00
Washington, D. C.	1/9/69	1/16/69	12.88
(Boat Center)	1/9/69	1/17/69	9.84
	1/9/69	1/22/69	1.17
	1/23/69	1/24/69	0.00
Roaches Run, Va.	1/9/69	1/16/69	15.84
	1/9/69	1/17/69	193.75
	1/9/69	1/22/69	---
	1/23/69	1/24/69	75.01
Alexandria, Va.	1/9/69	1/16/69	7.46
City Dump	1/9/69	1/17/69	13.65
(Southeasternmost)	1/9/69	1/22/69	4.00
	1/23/69	1/24/69	2.23

Table 3. Primary Productivity Determinations of Samples Collected at Sequential Downstream Sites in Rock Creek.

The established procedure for calculating primary productivity permitted determination of the level of photosynthetic activity in the laboratory for a variety of samples. From the above results, one can observe both the relative levels of activity on two winter collection dates and the effects of laboratory (25°C) storage of samples prior to assay. Changes in activity reflect modification of the available nutrient supplies in isolated samples.

Sample	Site	Collection Date	Assay Date	PP mg $C/m^3/hr.$
Marine	Delray Beach, Fla.	12/29/68	1/3/69	16.80 mg
			1/6/69	14.89 mg
Freshwater	Lake Ontario - 10 miles West of Genesee River	12/23/68	1/3/69	86.60 mg
			1/6/69	30.36 mg

Table 4. Calculation of Primary Productivity from Marine and Freshwater Samples Returned to Laboratory. Repetitive measurements on samples following 72 hour incubation demonstrates correlation between initial activity and rate of exhaustion of non-carbon growth factors.

APPENDIX B

A. Literature Search

1. Responsibility

Dr. Daniel J. Simons performed the Concept Literature Review under the technical guidance and supervision of Dr. Gilbert V. Levin.

2. Literature Search

In order to develop a more accurate and efficient approach to automatically measuring primary productivity in water bodies, a literature search has been undertaken to establish the current state of biological and engineering research in the carbon-14 method. This literature search has the additional objectives of providing guidelines for the design of an automated instrument, the APPI, for primary productivity measurement, ascertaining all pertinent factors that must be considered in the design of such an instrument, and serving as a basis for planning the laboratory phase of the project.

3. The Determination of Primary Productivity

Primary productivity - the rate at which carbon is photosynthetically fixed by marine phytoplankton - is a measurement of the fundamental rate of production of organic matter in water bodies. A direct relationship exists between the density of phytoplankton and the abundance of commercially desirable fish (Uda and Ishino). In inland water bodies, excessive growth of phytoplankton can lead to pollution eutrophication. Determination of primary productivity in the sea thus permits an extrapolation to establish the productivity of the fish crop which can then be used to predict quantities, timing,

and locations of fish harvests. In smaller water bodies, it can be used to predict the buildup of conditions leading to serious water pollution.

The primary productivity parameter integrates the effects of all factors upon which food production depends - such as light intensity, turbidity, temperature, nutrient concentration, inhibitory substances, planktonic species and densities. The concept is subdivided into "gross primary productivity," the gross rate of photosynthetic increase in the plant crop before any corrections for respiration or excretion are made; and "net primary productivity," the net rate of autosynthesis of the plant material in water. Throughout the text, the term primary productivity is used to refer to net primary productivity. Other frequently used terms appear in the glossary.

An examination of the characteristics of primary productivity by marine forms reveals some factors which must be considered in making an appropriate measurement. Findenegg (1966) has demonstrated that the rate of primary productivity of a given quantity of marine algae varies even as frequently as from hour to hour. The productivity may be reduced by either an increase or decrease of light intensity (Ryther, 1956), and the photosynthetic rate oscillates with the time of day as well as weather conditions. Local biomass has a direct effect upon the primary

productivity per unit of fresh weight of algal cells. Where small quantities of algae are present, significantly higher rates of carbohydrate formation are observed. A correlation exists between the photosynthetic rate and cell size. Per unit of biomass, nannoplankton generally photosynthesize far greater quantities of carbon than large diatoms or blue-green algae (Riley, 1957). As a result, lakes which have undergone extensive eutrophication and are therefore rich in blue-green and green algae produce surprisingly little quantities of organic matter per unit of phytoplankton present.

The information usually presented in measuring metabolic activity refers to the fixation of carbon dioxide per unit of algal cell. Therefore, in determining the true level of carbon fixation, one must also have information on the density of active cells as well as the fixation on a per cell or per unit "CO_2 fixing material" basis. This means that although in the presence of extensive eutrophication each individual cell may be functioning at a suboptimal level of efficiency, the total amount of carbon dioxide fixed in that region may still be extremely high. To determine this, it is important to obtain precise knowledge of the amount of carbon dioxide fixed photosynthetically in a clearly defined region of the ocean. Therefore, within any region in which sampling devices are placed, a careful selection of sampling sites is necessary to insure an adequate sample.

"Basic data" such as the dry weight, ash, and carbon content of the organisms (Lund, 1964), as well as seasonal physical changes in the environment, seasonal variation in biomass, and the ecological microclimate under study have been used in determining primary productivity. Other criteria used in previous investigations include the volume, fresh weight, fresh organic weight, dry organic weight, oxygen or carbon dioxide exchange, energy content and chlorophyll content. Complementary analyses include ashing, protein, carbohydrate and fat content determinations and measurement of lipid content. An analysis of these parameters shows that each suffers from some basic inadequacy as a measure of photosynthetic activity.

Because of variation in the specific gravity of marine organisms, volume is generally considered to be a poor measure of biomass. In cases where this measure has been used, centrifugation to compress air spaces and remove interstitial fluids in a relatively constant fashion is employed to provide some uniformity of results. The best of these procedures includes removing adherent water in a spin dryer, determining plant volume by displacement of water, and repeating several times for adequate sampling (Westlake, 1966). By extrapolation from the decreasing volume upon repetitive centrifugation, it is possible to get an estimate of dry weights for several algal species. However, correlation between

dry weight and fresh volume for numerous species ranges over a wide variety of values and is probably not indicative of any interspecies constant. Forsburg (1960) reports values ranging between 11 and 17 cm^3/gram for noncalcareous plants but as low as 6 cm^3/gram for the calcareous Chara fragilis. Values as low as 1.06 cm^3/gram have been reported for other species.

An alternative method utilized for determining biomass is the fresh weight of living organic material in a given volume of water. In practice, fresh weight is determined as wet weight of the sample collected; the remaining residual adherent water is considered negligible.

A somewhat more tedious but more accurate measure of the biota of a test sample is achieved by the determination of dry weight of organic matter. This is accomplished by drying each sample to a constant weight in an oven at 105°C. Alternatively, fresh samples may be dried to constant weight in air equilibrated with atmospheric moisture. In general, air-dried samples are 5 to 10% heavier than those dried in the oven. Each of these weight determinations suffers from shortcomings in accuracy and significance. Although the measurements reflect the amount of organic material in a sample, they give no indication of the metabolic nature of the sample from which they were taken.

Chlorophyll abundance can be used as a measure of the standing crop of phytoplankton (Yentsch et al., 1959 and Saunders, 1962). However, extensive study of the parameter has shown it to be inadequate since frequently photosynthetic pigments remain long after photosynthetic activity has ceased (Soeder, 1966).

Measuring the phenomenon of short-term constant light photosynthetic activity is also an inadequate method of determining of primary productivity for several reasons. Under artifical conditions, photosynthetic organisms are capable of physiological adaptation to their new environment in relatively short periods of time. Hence the measurements will differ from those which would have been obtained in the natural environment.

A method of determining a photosynthesis contour map of oceanic regions uses a very clever but somewhat outdated technique involving mechanical sampling. The classic tool for this purpose is the Hardy Continuous Plankton Recorder which may be towed behind any ship. The device works by collecting samples of the plankton population as it is being towed, depositing them on filters, and fixing the filters in a solution of formalin for later investigation. The filter is located on a continous drive drum so that sequential collecting may be recorded. In this manner, the distribution of plankton over the path of the towing vessel may be reconstructed. However, the procedure is extremely tedious since it entails the microscopic analysis of the recovered sample, and at best

can only give a reconstruction of the planktonic abundance at the time of the sampling. Since only a general correlation exists between primary productivity and numbers of phytoplankton, this method leads only to an approximation.

One of the earliest methods of determining primary productivity that is still most commonly used is the measurement of the oxygen produced during the photosynthetic process. This method was successfully used by Gaarder and Gran as early as 1927, and since that time has been utilized by numerous workers including Marshall and Orr (1961), Steemann-Nielsen (1952) and many others.

In the oxygen-measuring experiments, sealed bottles containing phytoplankton populations collected at various depths are lowered to their original depths in the ocean following the determination of the oxygen content in those bottles. The phytoplankton population then is allowed to photosynthesize for a known period of time and following recovery of the bottles, the new oxygen content is then determined. In the upper few meters of the illuminated zone, a distinct increase in oxygen owing to the photosynthetic activity of the green plants occurs. At levels where insufficient light for photosynthetic activity is available, respiration causes a consumption of the available dissolved oxygen in the sealed bottle and therefore upon recovery, a decrease in the oxygen content of those bottles is observed. At a certain depth however, no

change will occur; that is, the oxygen produced by photosynthesis will exactly balance the amount absorbed by respiration. This depth is defined as the compensation point. It should be emphasized that the compensation point does not represent the depth at which photosynthesis can no longer occur. Photosynthetic activity has been clearly demonstrated below this depth. However, no net production can occur below this level, and therefore it should not be necessary in determining primary productivity to take measurements below this depth. Oster and Clark (1935) have demonstrated that in the Gulf of Maine in the summertime the compensation point lies between 25 and 30 meters, but some degree of photosynthesis was detectable as deep as 40 meters.

For better understanding of the balance between photosynthetic assimilation of carbon dioxide and non-photosynthetic assimilation based on autotrophic and chemosynthetic energy sources, the light and dark bottle technique is also applied. A series of duplicate bottles similar to those used in the oxygen bottle method are prepared, but dark bottles are covered with an opaque material and placed at the same depth as the light bottles. The change in the oxygen concentration of the dark bottles is also determined and this gives a measure of the respiratory activity of the organisms present. If one then combines the oxygen consumed in the dark bottle with the oxygen evolved by photosynthetic activity in the light bottle, a measure of the total photosynthetic activity,

can be determined (Raymont, 1963).

Radioactive carbon has been utilized in numerous studies of the gross photosynthetic rate of phytoplankton (Steemann-Nielsen, 1951; Doty, 1958; Goldman, 1963). The application of this radioisotope technique for detecting small quantities of carbon dioxide has been well documented (Levin et al.) as an acceptable method for the detection of even low levels of metabolic activity. The automation of this assay technique for marine studies would eliminate many of the major drawbacks presently encountered in other methods of detecting metabolic activity.

One of the principal advantages of the radiocarbon method is that it is a simplified procedure which measures directly rather than indirectly the material being photosynthetically fixed. It is also more sensitive than other techniques and the analytical method is more accurate. Another advantage of this technique is that it is the most easily automated method for determining primary productivity. An automated method will make it possible to obtain high-frequency samplings at numerous oceanic sites with a minimal expenditure of equipment and manpower.

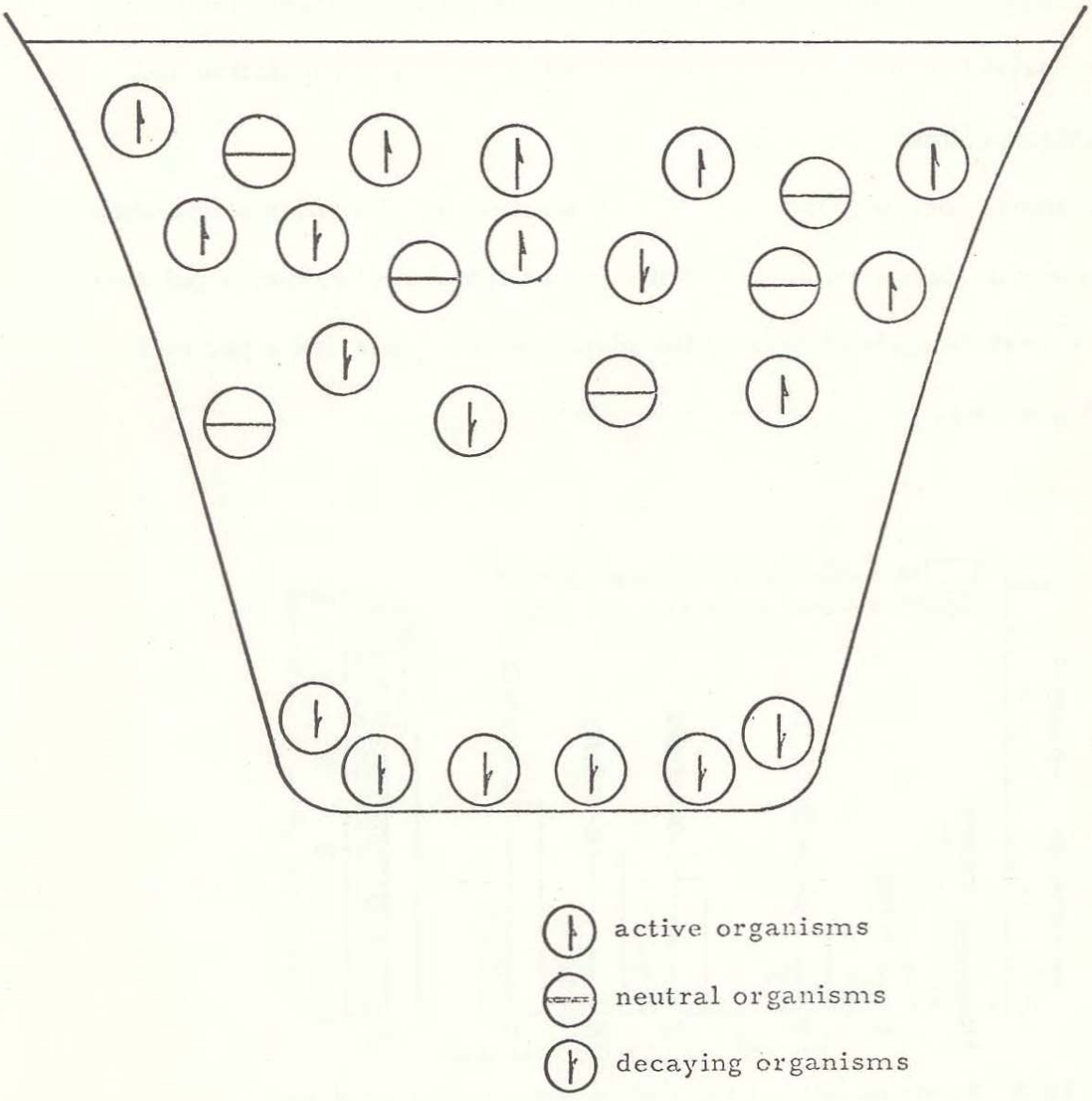
In a study by Elster (1966) the absolute and relative rates of $^{14}CO_2$ assimilation in phytoplankton populations have been examined

and the resulting evidence indicates that the non-photosynthetic incorporation of CO_2 into living cells must be substracted from the total. The effects of temperature, water depths, volume of available population, light transmitting properties of the aqueous environment, phytoplankton distribution as well as depth and total illumination, have also been studied for their effects on carbon dioxide photosynthesis by algae in several European lakes (Findenegg, 1966).

For all the lakes studied, the vast majority of fixation of carbon occured in the top ten meters below the surface. Several physical parameters were correlated with this phenomenon. For example, in the top ten meters the water temperature had dropped more than 15°C. Similarly, in 10 meters the light intensity dropped to below 10% of the surface value. As might be expected, the algal population as determined by both wet cell volume and dry weight was reduced to almost zero at depths of 15 meters. Moreover, when samples were collected at these depths and exposed to surface illumination and the uptake of $^{14}CO_2$ measured, the results indicated that algal cultures at these depths were already damaged or in some state of decay (Elster, 1966). This phenomenon is apparently quite common, with the generalized case summarized in Figure 1 (Soeder, 1966).

The relative assimilation rate at the surface of a body of water containing a limited algal population could reach a high rate of as much as 32 micrograms of carbon per hour for 10^9 cubic microns of algal volume. Other

Figure 1. Scheme of the "activity structure" of a phytoplankton community. The overall activity in the upper layers of the water body is supposed to be anabolic. Catabolic activity prevails at the bottom.



oligotrophic lakes were investigated and the graph below shows an approximate range of response that has been observed for several varying conditions (Figure 2).

It should be recognized that it is impossible to generalize concerning algal response under varying conditions. A limited correlation does, however, exist between the assimilatory rate of an algal population and phytoplankton volume.

The figure below (Elster, p. 83) shows results of studies undertaken for a variety of European lakes. Note that as the phytoplankton population exceeds a certain optimal density the photosynthetic rate, on a per cell basis, decreases.

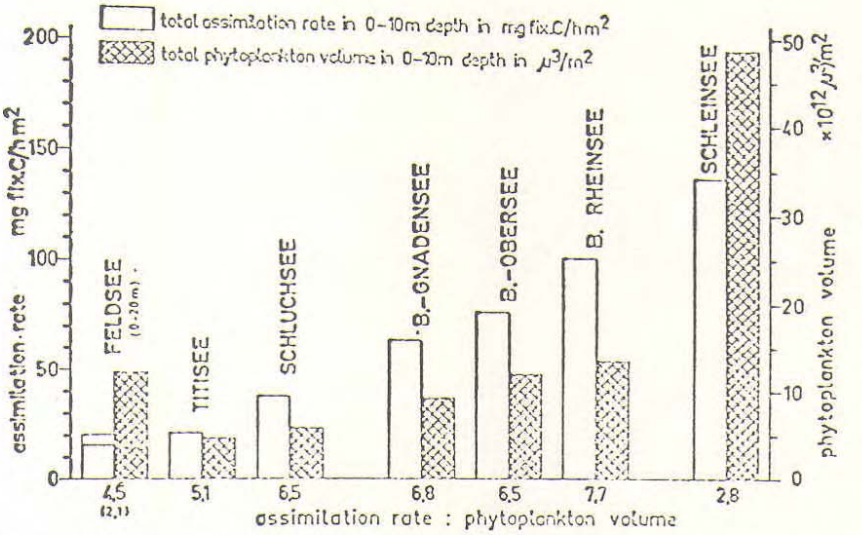


Fig. 2. - Assimilation rate and phytoplankton volume under 1 m² of lake surface in the different lakes. Thin-lined columns for the Feldsee indicate the values between 0 and 20 m depth.

4. The Measurement of Primary Productivity by the Carbon-14 Method

The original carbon-14 method of determining photosynthetic activity conceived by Steeman-Nielsen, with a limited number of modifications introduced in the past several years, is still the presently accepted procedure. In principle, the $^{14}CO_2$ incorporation measurement of primary productivity differs from the oxygen concentration, light-dark bottle procedure, because the amount of carbon fixed by the organism is measured directly.

One modification of the Steeman Nielsen carbon-14 method, Sorokin's procedure (1956), attempts to extrapolate the primary productivity measured in a sample to the entire column of water from which the sample was taken. This method is consistent with the goals of the APPI program and will be described in detail. In the Sorokin method, three factors must be determined: (a) the absolute photosynthesis of a reference sample of water, generally a surface sample, (b) the effective light attenuation on photosynthesis with depth, and (c) a correction factor for non-uniform phytoplankton distribution. The steps of the method are as follows:

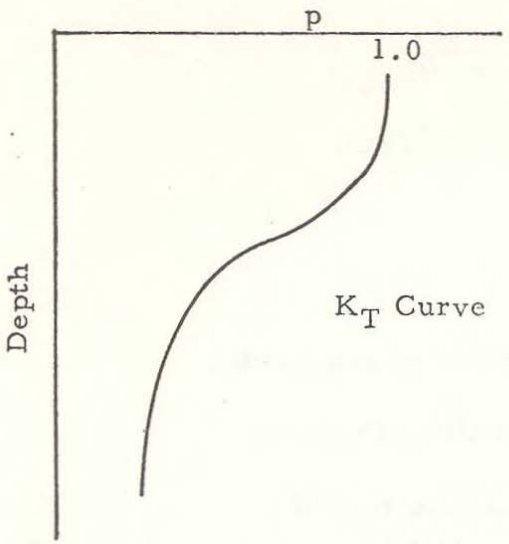
a. Total daily photosynthesis of reference sample

Samples of surface water are placed in an aquarium onboard ship or resuspended at the ocean surface for one day. Gross photosynthesis

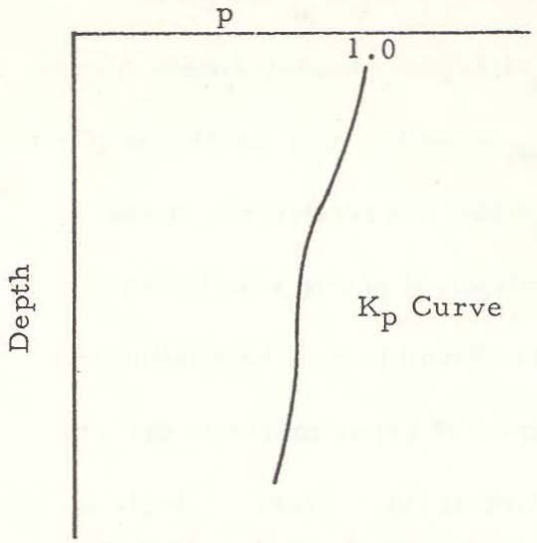
in grams carbon per liter is calculated from this sample.

b. Correction for effect of water transparency on integral photosynthesis

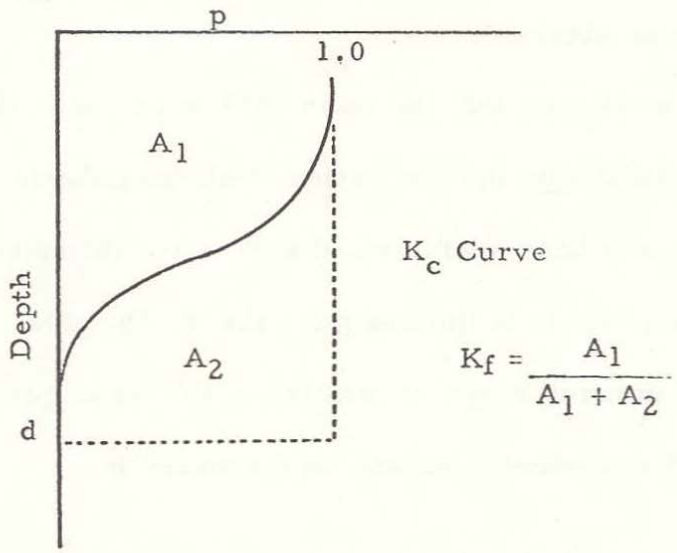
Samples from any single depth (but preferably from surface water) are resuspended at various depths in the body of water under investigation. Photosysthesis in these samples is measured over intervals of one half of the daylight period. The phytoplankton populations are the same and therefore differences in photosynthesis with respect to depth are a result of the temperature and light acting on each sample. Assuming that the lake is isothermal, temperature will not vary and differences observed in photosynthetic activity are simply a result of the different levels of illumination. In this manner, a ratio of the photosynthetic activity at any depth to photosynthetic activity at the surface may be determined. The resulting values yield a curve of the effect of illumination upon photosynthesis. Intergal photosynthesis of a uniformly distributed phytoplankton population may thus be defined relative to the same conditions under no attenuation of the light. The results thus obtained are defined as the transparency curve, (K_T) (Figure 3).



Transparency curve: relative photosynthesis versus depth for a constant density phytoplankton population.



Relative phytoplankton density curve.



Total correction curve K_c ($K_T \times K_p$) and K_f, relative integral photosynthesis correction coefficient.

Figure 3.

using the formula: $C_f = C_{ph} \times K_f \times D$

where: C_f=integral photosynthesis (Grams carbon/m^2/day_{24})

C_{ph}=absolute photosynthesis (Grams carbon/m^3/day)

K_f=final correction coefficient

D=depth of photic zone/meters

This formula may be applied to any body of water and, with a limited amount of experimentally determined information (the rate of ^{14}C fixation at the surface of the body of water and the rate of ^{14}C fixation at several depths, each determined in the above manner) a good estimate of the photosynthetic activity in that body of water for any period of time can be obtained.

An extensive work was undertaken in 1962 to compare this standard method with the in situ determination of photosynthetic activity. The results have been summarized in treatise (Saunders et al, 1962) which is applicable to the design of the APPI. The method of Saunders is in many respects similar to the technique as conceived for the APPI instrument and will be discussed in detail below:

1. Preliminary Procedures

A variety of physical and chemical determinations are made at the site of each assay. Water temperatures are obtained with bathythermographs at the beginning and termination of every experiment. The surface water temperature is also determined with a stem thermometer. General weather conditions are noted including degree

of cloudiness, haze, etc. The pH of water samples of each depth and the total alkalinity are determined in accordance with procedures in Standard Methods.

2. Standardization of the Radioisotope

Glass ampoules containing 2 ml of $Na_2{}^{14}CO_3$ solution containing 0.94 uCi of sodium carbonate of specific activity 0.54 mCi/mM are prepared. The activity of each ampoule is standardized by counting in reference to National Bureau of Standards ^{14}C material.

3. Sampling Procedure

Surface water is obtained by dipping and is placed in a five gallon carboy to insure a uniform sample. The water is then transferred by rubber tubing to the experimental bottles. Other samples are taken from 5, 10, 15, 20, and 25 meters successively with a single two gallon plastic tube sampler especially designed for the purpose. The experimental bottles are wide mouth, ground glass, stoppered bottles sealed with paraffin of approximately 270 ml capacity. After the sample water is placed in these containers, an ampoule is submersed and broken in each bottle which is then immediately stoppered and shaken.

4. Experimental Design

Four different types of experiments were conducted with the above procedure:

(a) in situ estimation of photosynthesis

(b) a transparency series

(c) a constant light series

(d) a surface sample of daylight aquarium

Both light and dark bottle ^{14}C samples were examined and all individual determinations were replicated to insure reliability of the results.

The In Situ Estimation of Photosynthesis

Replicate samples from several depths were taken and treated with $Na_2{}^{14}CO_3$ in sealed containers and resuspended at the sampling depths. The experiments were initiated at sunrise and terminated 24 hours later. A dark control was provided by wrapping one of the bottles in aluminum foil to exclude light.

Experiments were conducted from sunrise to sunrise to permit full daylight photosynthesis with subsequent dark respiration to yield the considered best estimate of carbon-14 fixation for a 24-hour period. The bottles were suspended in a lake in a horizontal position on racks especially designed for this purpose.

Transparency Series

Replicate samples taken from the test surface depth were resuspended at six different experimental depths. Assuming the temperature remains constant with depth and population collected at the surface is constant, the resulting photosynthesis measured at each depth is directly related to the light attenuation by the water itself. For this series of experiments the procedure as described above was followed.

Constant Light Series

Replicate samples from each experimental depth were collected and placed in a constant light aquarium designed for this purpose on the ship itself. Surface water was continuously pumped through the aquarium and a temperature was maintained with a $\pm$ 2°C variation. Samples containing labeled carbon were exposed to a constant light intensity of 500 foot-candles. The results of this measurement of uptake yielded information on the gross photosynthetic activity and provided an index of the metabolic population density.

Daylight Aquarium

An aquarium identical to that described above was prepared with illumination provided solely by exposure to the sun at the level of surface water. Runs of 24 hours were carried out and the results served as a measure of the gross photosynthetic activity at surface illumination. Dark bottles were provided in this case to measure the non-photosynthetic fixation by the plankton sample.

Procedure for Analysis of Samples

Upon termination of the incubation period, 100 ml was pipetted from each experimental bottle and suction filtered through a Millipore type AA filter, rinsed several times with distilled water, and finally rinsed with a weak formaldehyde solution. The Millipore filters with the phytoplankton sample were then thoroughly dried and

and cemented to aluminum planchets. The samples were stored in a desiccator prior to counting.

Radioactivity incorporated into the organisms collected on the Millipore filter was counted in a D-47 gas flow counter with a micromil window installed. Appropriate Nuclear Chicago Corporation support equipment (sample changer, printer, scaler, etc.) was used. Suitable procedures were undertaken for the standardization and determination of the efficiency of this apparatus. The activity of the isotope samples was determined and found to agree to specifications within 10%. Absorption of the radiation emitted by the samples by the Millipore filters themselves was measured and evaluated, with the finding that the Millipore membranes absorbed approximately 16% of the radiation being emitted by the samples. This served as a correction factor to determine the true activity of the samples collected.

As a sensitivity check, chlorophyll a extractions of the phytoplankton samples were made, and the pigment levels correlated with the activity demonstrated by the ^{14}C technique.

As has been shown, the radioisotopic method of detecting photosynthetic activity offers advantages over other techniques available. However, for proper evaluation and calculation of the data collected, knowledge of four factors is required:

1. Uptake of ^{14}C by phytoplankton

2. Total available isotope

3. Isotope correction factor

4. Total available stable inorganic C

The most suitable formula for determining primary productivity is given by:

$$P = \frac{r}{R} \times C \times f \quad \text{(Saunders, 1962)}$$

where:

P= photosynthesis in mgC/m^3

r= uptake of radioisotope in cpm

R= total available radioisotope in cpm

C= stable inorganic carbon in $milligrams/m^3$

f= isotope correction factor (1.06)

Each aspect is discussed in detail below:

A. The uptake of radioactive carbon

$$r = \text{counts/minutes} \left(\text{for volume filtered} \times \frac{\text{volume of experimental bottle}}{\text{volume filtered}}\right)$$

B. Total available ^{14}C

R=uCi x counter efficiency x correction for filter absorption x dps/uCi=cpm

C. Isotope Correction Factor

To account for the selective incorporation of the stable carbon isotope over the radioactive species, a correction factor must be applied. Best estimates to date consider this to be 1.06. That is,

the $^{12}CO_2$ species is incorporated 1.06 times as readily as the heavier $^{14}CO_2$ molecule.

D. Total available stable inorganic carbon

Total available carbon should include carbon contained as dissolved CO_2, carbonic acid, bicarbonate, and carbonate. Since this will vary depending upon temperature, pH, and total alkalinity based upon $CaCO_3$ content of the sample, Saunders has prepared a table (Table 2 , Appendix A) based upon the following equation:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons H^+ + CO_3^=$$

and therefore two acid equivalents are required for each mole of carbonate (c) and one for each mole of bicarbonate (b). The standard alkalinity titration (Welch, 1948) requires 0.02N H_2SO_4 and a 100 cc sample. Letting t equal cc's of acid used,

$$0.02\ t = 100\ (b + 2c)$$

$$t = 5000\ (b + 2c)$$

In the Welch calculation the total alkalinity expressed as ppm of $CaCO_3$ is determined by multiplying the cc's of acid used by 10.

Thus T = total alkalinity = $10 \times t$

or $T = 50,000\ (b + 2c)$

Letting a = molar concentration of carbonic acid and free CO_2, then the total molar concentration of carbon will be (a+b+c) if each radical contains 1 carbon atom/molecule. Since there are 12,000 mg C/mole

$$C = 12,000\ (a+b+c) \text{ in milligrams C/liter}$$

c. Correction for the Effect of Differences in Phytoplankton Density on Integral Photosynthesis

Samples of photosynthesizing cell materials are collected from several depths and placed under a constant illumination for intervals of one and two hours. The only experimental variable is thus the algal population. Differences in photosynthetic activity are then attributed to differences in the metabolizing population itself. Relative photosynthesis may then be plotted against depth and the resulting curve is an expression of the vertical distribution of the photosynthetically active phytoplankton population, (K_p). Since the effects of K_T and K_p are interrelated, they are multiplicative and, in this fashion, a total correction factor for each depth may be obtained. If this is then plotted against depth, curve K_c (see Fig. 3 above) results and gives a good approximation of the photosynthetic activity in any photic column under investigation. The area (A_1) under a curve so obtained is the relative integral photosynthesis for the water depth being sampled. Assuming a uniform phytoplankton population and no light attenuation by the water, the area (A_1 plus A_2) of the rectangle formed by P equals 1.0 (where the ratio of photosynthetic activity at any depth is equivalent to that at the surface) and the depth D is the theoretical integral photosynthesis of a uniform population. The ratio $\frac{A_1}{A_1 + A_2}$ yields a final correction coefficient (K_f) for integral photosynthesis. If absolute photosynthesis for P equals 1.0 is known, integral photosynthesis may be calculated in absolute units for a water column of known dimensions

Because the solubility and this availability of soluble carbonate for metabolic purposes is affected by pH, this factor must also be considered. Total alkalinity can be related to carbon content as follows:

$$C/T = \frac{12,000\ (a+b+c)}{50,000\ (b + 2\ c)} \quad \text{or} \quad C = 0.24T\ \frac{a+b+c}{b + 2c}$$

a, b and c are related by the equibrium constants K_1 and K_2:

$$K_1 = \frac{b\ (H)}{a}\ ; \text{ or } a = \frac{b\ (H)}{K_1}$$

Similarly,

$$K_2 = \frac{c\ (H)}{b}\ ; \text{ or } c = \frac{b\ K_2}{(H)}$$

where: (H) = hydrogen ion concentration.

$$C = 0.24T\ \frac{\frac{b(H)}{K_1} + b + \frac{bK_2}{(H)}}{\frac{2b\ K_2}{(H)} + b}$$

and

$$C = T\ \frac{0.24(H)^2 + 0.24K_1(H) + 0.24\ (K_1\ K_2)}{0.24K_1\ (H) + 0.48K_1\ K_2}$$

K_1 and K_2 values of Hained and Bonner, (1945) and Hained and Scholes (1941) respectively were used to evaluate the expression above, and provide the values shown in Table 2 , Appendix A.

In order to correlate the uptake of carbon-14 with the fixation of inorganic carbon, it is essential, as in any isotopic tracer study, to correct for isotope dilution by the unlabeled inorganic carbon present in the seawater sample itself. This problem is well understood and

established standard procedures for dealing with it are clearly defined. In manual determinations of primary productivity, the total inorganic carbon is determined by measuring several interrelated parameters. Theoretically, the total alkalinity, chlorinity or salinity, temperature, and pH all must be determined in order to evaluate the available carbon content of the medium. For these determinations rather elaborate laboratory procedures are required. By the use of established tables of conversion factors, the raw data may be manipulated to provide a determination of the total carbon content.

Fortunately, however, most marine environments are fairly stable; changes in pH, alkalinity, and other factors with time in a given location are very slight. Since much difficulty and expense would be involved in instrumenting an automated system to make these ancillary determinations at the time of measuring ^{14}C incorporation, it is possible that these characteristics could be manually determined by infrequent periodic measurements when the equipment is being maintained and serviced. On the other hand, it may be necessary to automate the determination as part of the instrument.

Another less favorable but also feasible procedure, based upon the assumption that the alkalinity and pH remain relatively constant at each sampling site, is the measurement of relative photosynthetic activity as a function of time. By simply observing changes in the rate of photosynthetic activity at each site the difference in primary

productivity can be determined. Thus, an indication of photosynthetic activity can be obtained in the absence of knowledge of the total inorganic carbon present. Since this will make correlation between activity at different sites difficult, where the inorganic carbon might differ this approach leaves much to be desired.

However, in practice one is interested only in the dilution of the radioisotope by unlabeled inorganic carbonates. Rather than measuring the alkalinity or salinity of the environment for the purpose of an independent calculation of total inorganic carbon from which the dilution may be calculated, a more direct approach seems promising. It may be possible to substitute periodically a different experimental program. If during incubation period in which photosynthetic uptake occurs, a small known quantity of barium hydroxide is introduced followed by immediate filtration, the activity recovered as a result of the formation of a precisely known quantity of precipitate might serve as an indication of the dilution of label. In the absence of any unlabeled carbonate, all of the ^{14}C sample will be recovered in the form of barium carbonate and may be measured accordingly. However, should significant quantities of unlabeled carbonate be present in the seawater sample, then the barium carbonate which is precipitated out will show significantly lower activity because of the inclusion of the carrier. By carefully defining the amount of barium hydroxide provided, and knowing the specific activities of both the label provided and the barium carbonate recovered, one can readily obtain a measure of the dilution. Selective precipitation of the

^{14}C over the ^{12}C isotope may be determined and a necessary correction factor provided. Determinations should be run to establish the optimum concentration and quantity of barium hydroxide to be used for this purpose.

5. Frequency, Duration, and Timing of Carbon-14 Primary Productivity Measurements

No in situ primary productivity data is available from which ideal geographical or temporal sampling frequencies can be selected. The expense and difficulty involved in obtaining sufficiently detailed information by manual determinations makes extrapolating from laboratory work the only way to estimate the sampling frequency required for this study phase.

Laboratory studies on mixed phytoplankton populations (Jorgensen, 1964; Goldman, 1966) have shown that relatively slight changes in light intensity, temperature, nutrient concentration, etc. have profound effects upon primary productivity. Since tides and currents will have the greatest effect upon the in situ environment of phytoplankton, a non-uniform bouy distribution, with the greatest number of bouys distributed in the vicinity of estuaries, continental shelves, and heavily worked fishing grounds should provide the best description of in situ conditions.

The length of time that a sample of water should be retained in the instrument for the determination of primary productivity is limited by the power and storage capacity of the instrument on one hand, and by the desire to maintain in situ conditions on the other. If a short

incubation period is utilized, many samples must be taken during a daylight period to achieve an adequate representation of fluctuations in photosynthetic activity that normally occur as the angle of incident sunlight changes (Fogg, 1966). But frequent sampling will increase the usage rate of the isotope filters, titrant, and power supply and is difficult even with manual determinations (Saunders, 1962). If samples are retained in the incubation chamber for too long, the exhaustion of growth factors and the excretion of toxic by-products into the chamber will change the in situ condition. Therefore, most authors agree that a two to six hour incubation period is best.

In order to obtain primary productivity data which are most representative of the in situ condition, changes in the angle of incident sunlight (directly related to penetration depth and thus depth of the photic column) must be considered (Jorgensen et al, 1964) in timing the measurements. Measurements should be taken at least under morning, noon, and late afternoon sunlight to obtain an accurate picture of primary productivity.

In order to establish appropriate in situ locations for adequate sampling by an automated device numerous ecological factors must be considered. Several of the salient features are discussed below:

A. Sampling Depths for In Situ Measurements

Investigation of photosynthetic activity in the East Indian Ocean has shown that the maximum photosynthetic activity in northern regions occurs at a depth of 25 meters. In more southern locales

maximum activity was found at 50 meters and, in one case, at 75 meters. The greatest depth at which photosynthesis has been reported is 300 meters (Raymont, 1962). Therefore, these is no reason to sample beneath this depth. Differences observed in the determination of primary productivity in situ versus bringing the samples to the surface for testing indicate that changes in metabolic activity occur when the organisms are transferred to the new environment. Samples taken aboard ship for photosynthesis studies consistently show greater activity than sample for the same depth studied in situ (National Working Group on Photosynthesis, 1966). Findenegg (1966) has reported that maximum photosynthetic activity occurs in several European Lakes at an average of 10 meters below the surface with virtually all activity occurring in the top 20 meters. Fogg (1965) reported maximum activity three meters below the surface. Surface activity is sub-maximal due to light inhibition (Saunders, 1962). It must be concluded that the measurement of primary productivity by an automated buoy system will depend upon the specific environment in which it is placed. Therefore, it is recommended that the detecting device be designed to operate from the surface down to depths no greater than 300 meters and probably no greater than 50 meters.

B. Optimal Sample Volume for Measuring ^{14}C Incorporation

In studying the competition by bacteria and algae for organic

substrate, Hobbie and Wright (1966) have shown that 50 ml samples of seawater are sufficient for the determination of ^{14}C incorporation. Saunders has done extensive work with 100 ml samples (1962). Sorokin (1956) conducted numerous isotope studies with samples of under 100 ml. Since the APPI as presently conceived should easily be capable of handling samples of 100 to 200 ml (Levin, 1967), the size of the sample for an adequate test should pose no problem to the instrument.

C. Optimal Light Intensity for Photosynthetic Activity

Considerable disagreement exists in the literature concerning the optimal light requirement for maximum primary productivity in the marine environment (Strickland, 1958; Jorgensen, 1964; Ryther, 1956). Because of the varying requirement for illumination in the photosynthetic activity of different marine algal forms and the known inhibitory effects of light on the metabolic activity of a number of organisms, consideration must be given to the selection of the nature and method of illumination required in ^{14}C fixation studies. Several points must be kept in mind:

(1) At similar or equal light intensities, temperature, and algal densities, the photosynthetic activity of an ecosystem can vary over greater than one order of magnitude (Elster, 1965).

(2) A survey of the experimental literature indicates that only broad limits to the upper and lower sensitivity intensities may be determined.

(3) In the majority of studies, inhibition of activity is achieved at 20,000 lux (although some species grow quite well at this level).

(4) Growth is also detected with light intensities as low as several hundred lux. A better approach to the determination of optimal light intensity will be achieved by designing the automated system so that each detecting instrument will have the capability of finding several levels of photosynthetic activity.

In the light of this discussion, the value of determining the compensation point (that level of illumination where photosynthetic processes are exactly counterbalanced by respiratory processes for the cell population under study) becomes academic. To establish a meaningful contour map of the primary productivity over a wide area, it will be necessary to provide instruments at the point of maximal net photosynthetic activity. This by definition will be above the compensation point.

6. Distribution of Measurement Stations

The question of determining a scheme for the adequate sampling of large bodies of water has never been fully considered. Because of the great difficulty involved in manual determinations of primary productivity - including extensive effort and equipment as well as the full-time utilization of a seagoing vessel - no truly adequate sampling procedure has yet been devised (Saunders, 1962). Researchers have used as few as three sampling stations in a three-mile long lake (Wetzel), and many studies have been done at a single sampling station from which the data obtained were extrapolated to measure photosynthetic activity

for a entire region (Findenegg, 1966). Limited efforts have been done on the determination of a means for adequately determining the activity throughout the photic column at each site. However, essentially all workers today use one of two techniques.

Described briefly in the technical proposal for the development of the APPI, one method of assaying photosynthetic activity over a vertical column has been to take measurements at 100%, 10%, and 1% of the surface ambient light intensity. Differences in photosynthetic activity with level are shown in this manner, and a mean value of activity throughout a photic column may be determined. More routinely, however, investigators tend to select samples at arbitrary depths, frequently five-meter intervals, down to the level at which net photosynthetic activity ceases. This is quite uniformly reported to be at a point where the illumination does not exceed 300 lux.

To determine the photosynthetic activity in mg/carbon meter^3 per day in the photic column, the data obtained by such vertical sampling are plotted as a graph of depth versus photosynthetic activity, either absolute photosynthetic activity or again more commonly in percent of surface activity. By integrating the area under the curve thus obtained, one has a farily accurate measure of the photosynthetic activity in the photic column. With various nuances of this basic sampling procedure which allow for corrections of differing phytoplankton populations and levels of illumination, this procedure is universally utilized. The application of in situ determinations of photosynthesis will alleviate

the corrections for differing populations, etc., that are required, but a standardized sampling procedure will have to be determined. As long as several samples are taken between the surface and the depth at which net photosynthetic activity ceases, sufficient data will be available for the calculation of a meaningful curve.

The question of the temporal deployment of the sampling devices is also still open to discussion. Various workers in the field have utilized sampling and incubation times for the $^{14}CO_2$ primary productivity experiment ranging from one hour to the full daylight period (Steeman-Nielsen, 1952, Ryther et al, 1954). The standard values for primary productivity are always given in grams carbon per meter^3 of seawater per either hour or day, where "day" represents the hours of sunlight. While lengthened sampling periods may tend to have an averaging effect upon fluctuations in the photosynthetic activity that occur as a result of weather conditions, the greatest value will be obtained from data that has been collected over several conditions of daylight illumination.

Automated in the fashion proposed, the ultimate APPI could be housed in a submerged buoy and be programmed to conduct measurements of primary productivity at desired times and depths. The instrument would be autocycling and would store the data obtained. The device could operate for considerable lengths of time as determined by periodic resupply of isotope, filters, and power. The data could be removed upon maintenance visits or could be retrieved by interrogation from

ships or satellites. A grid pattern of automated stations in the sea could provide the degree of information required to predict forthcoming fish crops, critical times and locations. Subsequently, the primary productivity measurements could be augmented and integrated with other important measurements, such as those of nutrient concentration, light penetration, temperature, pH, and the like. If successful, it is possible to visualize hundreds of such buoys on permanent locations in the principal fishing grounds of the oceans.

7. Recommended Approach to Instrumentation

Based upon the literature search, the following recommendations for the design of an automated instrument for the radioisotope determination of primary productivity are proposed:

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 one 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 walls of the sample chamber.

8. A conference of investigators actively at work in the appropriate fields should be organized by Biospherics Research, Incorporated.

GLOSSARY

APPI - Automated Primary Productivity Instrument

Autotrophic - refers to those phytoplankton which require no organic material for normal growth and synthesize carbohydrates directly from carbon dioxide.

Auxotrophic - refers to organisms which require a specific, limited number of organic molecules for normal growth and reproduction.

Day - generally refers to the hours of daylight during which photosynthetic activity occurs on the ocean surface. In keeping with the convention of Strickland (1964), the term "day_{24}" is used when a full 24-hour period is implied.

Eutrophic - is used to describe marine condition of extreme planktonic abundance.

Facultative - refers to organisms which metabolize organic material in the dark but may behave as autotrophs in the light.

Gross photosynthetic activity - is defined as the total amount of carbon fixed in the photosynthetic process.

Heterotrophic - refers to all organisms that require an organic carbon source to satisfy either the carbon or energy requirement for growth.

Microplankton - range from 50 to 500 microns across the largest dimension.

Monomictic - refers to a plankton population which consists essentially of a single species (90% or more).

Nannoplankton - range from 10 to 50 microns across the largest dimension.

Net photosynthetic activity - is a measure of the organic matter remaining after catabolic losses which occur simultaneously with photosynthesis. It is equal to net carbon assimilation by photosynthetic organisms.

Oligotrophic - is used to describe marine conditions of extreme planktonic paucity.

Pantomictic - refers to a plankton population in which no single species predominates.

Polymictic - refers to a plankton population consisting of several species in approximately equal abundance.

Photosynthetic quotient - is defined as the ratio of molecular oxygen liberated during photosynthesis to molecular carbon dioxide assimilated (O_2/CO_2).

Photosynthetic rate - is defined in several ways, depending upon the measurement chosen:

1. The dry weight increase of phytoplankton per unit time resulting from photosynthetic activity may be considered the photosynthetic rate. Since many marine algae are heterotrophic for a significant portion of their survival time and occur in mixed populations, this is a rather imprecise measurement of photosynthesis.

2. The rate of photosynthesis may be measured by the amount of oxygen released to the extracellular environment as a result of photosynthetic activity. However, a significant fraction of the oxygen liberated in photosynthesis is utilized in respiratory processes. This utilization varies widely with environmental conditions.

3. Probably the most accurate physical determination of the photosynthetic rate is achieved by measurement of the rate of carbon dioxide uptake in the photosynthetic organism with the use of $^{14}CO_2$. This rate of uptake is an excellent measure of the rate of carbohydrate formation and the use of the radioisotope allows measurements that are both highly precise and accurate.

Primary productivity - is defined as the increase in organic matter as a direct result of the fixation of carbon dioxide without correcting for respiratory or excretory processes.

Productivity index - is the primary productivity per unit of primary standing crop at a given light intensity and is a direct measurement of the fertility of a specific area.

Respiratory quotient - is defined as the ratio of molecular carbon dioxide liberated during respiration to molecular oxygen consumed in the process (CO_2/O_2).

Ultraplankton - range from 0.5 to 10 microns across the largest dimension.

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