06-11011

NRC FORM 313
(1-84)
10 CFR 30, 32, 33, 34
35 and 40

APPLICATION FOR MATERIAL LICENSE

U.S. NUCLEAR REGULATORY COMMISSION
APPROVED BY OMB
3150-0120
Expires: 5-31-87

INSTRUCTIONS: SEE THE APPROPRIATE LICENSE APPLICATION GUIDE FOR DETAILED INSTRUCTIONS FOR COMPLETING APPLICATION. SEND TWO COPIES OF THE ENTIRE COMPLETED APPLICATION TO THE NRC OFFICE SPECIFIED BELOW.

FEDERAL AGENCIES FILE APPLICATIONS WITH:

U.S. NUCLEAR REGULATORY COMMISSION
DIVISION OF FUEL CYCLE AND MATERIAL SAFETY, NMSS
WASHINGTON, DC 20555

ALL OTHER PERSONS FILE APPLICATIONS AS FOLLOWS, IF YOU ARE LOCATED IN:

CONNECTICUT, DELAWARE, DISTRICT OF COLUMBIA, MAINE, MARYLAND, MASSACHUSETTS, NEW JERSEY, NEW YORK, PENNSYLVANIA, RHODE ISLAND, OR VERMONT, SEND APPLICATIONS TO:

U.S. NUCLEAR REGULATORY COMMISSION, REGION I
NUCLEAR MATERIAL SECTION B
631 PARK AVENUE
KING OF PRUSSIA, PA 19406

ALABAMA, FLORIDA, GEORGIA, KENTUCKY, MISSISSIPPI, NORTH CAROLINA, PUERTO RICO, SOUTH CAROLINA, TENNESSEE, VIRGINIA, VIRGIN ISLANDS, OR WEST VIRGINIA, SEND APPLICATIONS TO:

U.S. NUCLEAR REGULATORY COMMISSION, REGION II
MATERIAL RADIATION PROTECTION SECTION
101 MARIETTA STREET, SUITE 2900
ATLANTA, GA 30323

IF YOU ARE LOCATED IN:

ILLINOIS, INDIANA, IOWA, MICHIGAN, MINNESOTA, MISSOURI, OHIO, OR WISCONSIN, SEND APPLICATIONS TO:

U.S. NUCLEAR REGULATORY COMMISSION, REGION III
MATERIALS LICENSING SECTION
799 ROOSEVELT ROAD
GLEN ELLYN, IL 60137

ARKANSAS, COLORADO, IDAHO, KANSAS, LOUISIANA, MONTANA, NEBRASKA, NEW MEXICO, NORTH DAKOTA, OKLAHOMA, SOUTH DAKOTA, TEXAS, UTAH, OR WYOMING, SEND APPLICATIONS TO:

U.S. NUCLEAR REGULATORY COMMISSION, REGION IV
MATERIAL RADIATION PROTECTION SECTION
611 RYAN PLAZA DRIVE, SUITE 1000
ARLINGTON, TX 76011

ALASKA, ARIZONA, CALIFORNIA, HAWAII, NEVADA, OREGON, WASHINGTON, AND U.S. TERRITORIES AND POSSESSIONS IN THE PACIFIC, SEND APPLICATIONS TO:

U.S. NUCLEAR REGULATORY COMMISSION, REGION V
MATERIAL RADIATION PROTECTION SECTION
1450 MARIA LANE, SUITE 210
WALNUT CREEK, CA 94596

PERSONS LOCATED IN AGREEMENT STATES SEND APPLICATIONS TO THE U.S. NUCLEAR REGULATORY COMMISSION ONLY IF THEY WISH TO POSSESS AND USE LICENSED MATERIAL IN STATES SUBJECT TO U.S. NUCLEAR REGULATORY COMMISSION JURISDICTION.

1. THIS IS AN APPLICATION FOR *(Check appropriate item)*

- [ ] A. NEW LICENSE
- [ ] B. AMENDMENT TO LICENSE NUMBER _____
- [X] C. RENEWAL OF LICENSE NUMBER 09-15728-02

2. NAME AND MAILING ADDRESS OF APPLICANT *(Include Zip Code)*

U.S. Department of Commerce (NOAA/ERL)
Atlantic Oceanographic & Meteorological Lab
4301 Rickenbacker Causeway
Miami, Florida 33149

3. ADDRESS(ES) WHERE LICENSED MATERIAL WILL BE USED OR POSSESSED.

At the applicant's address, at sea in international waters, and anywhere in the United States where the Nuclear Regulatory Commission maintains jurisdiction for regulating the use of licensed material.

4. NAME OF PERSON TO BE CONTACTED ABOUT THIS APPLICATION

Dr. Peter B. Ortner

TELEPHONE NUMBER: (305)361-4384

SUBMIT ITEMS 5 THROUGH 11 ON 8½ x 11" PAPER. THE TYPE AND SCOPE OF INFORMATION TO BE PROVIDED IS DESCRIBED IN THE LICENSE APPLICATION GUIDE.

5. RADIOACTIVE MATERIAL
a. Element and mass number, b. chemical and/or physical form, and c. maximum amount which will be possessed at any one time.

6. PURPOSE(S) FOR WHICH LICENSED MATERIAL WILL BE USED.

7. INDIVIDUAL(S) RESPONSIBLE FOR RADIATION SAFETY PROGRAM AND THEIR TRAINING AND EXPERIENCE.

8. TRAINING FOR INDIVIDUALS WORKING IN OR FREQUENTING RESTRICTED AREAS.

9. FACILITIES AND EQUIPMENT.

10. RADIATION SAFETY PROGRAM.

11. WASTE MANAGEMENT.

12. LICENSEE FEES *(See 10 CFR 170 and Section 170.31)*
FEE CATEGORY: N/A
AMOUNT ENCLOSED: $ N/A

13. CERTIFICATION. *(Must be completed by applicant)* THE APPLICANT UNDERSTANDS THAT ALL STATEMENTS AND REPRESENTATIONS MADE IN THIS APPLICATION ARE BINDING UPON THE APPLICANT.

THE APPLICANT AND ANY OFFICIAL EXECUTING THIS CERTIFICATION ON BEHALF OF THE APPLICANT, NAMED IN ITEM 2, CERTIFY THAT THIS APPLICATION IS PREPARED IN CONFORMITY WITH TITLE 10, CODE OF FEDERAL REGULATIONS, PARTS 30, 32, 33, 34, 35, AND 40 AND THAT ALL INFORMATION CONTAINED HEREIN, IS TRUE AND CORRECT TO THE BEST OF THEIR KNOWLEDGE AND BELIEF.

WARNING: 18 U.S.C. SECTION 1001 ACT OF JUNE 25, 1948, 62 STAT. 749 MAKES IT A CRIMINAL OFFENSE TO MAKE A WILLFULLY FALSE STATEMENT OR REPRESENTATION TO ANY DEPARTMENT OR AGENCY OF THE UNITED STATES AS TO ANY MATTER WITHIN ITS JURISDICTION.

SIGNATURE–CERTIFYING OFFICER	TYPED/PRINTED NAME	TITLE	DATE
Peter B. Ortner	Dr. Peter B. Ortner	Supervisory Oceanographer	6/27/85

14. VOLUNTARY ECONOMIC DATA

a. ANNUAL RECEIPTS
- [ ] <$250K
- [ ] $250K–500K
- [ ] $500K–750K
- [ ] $750K–1M
- [ ] $1M–3.5M
- [ ] $3.5M–7M
- [ ] $7M–10M
- [ ] >$10M

b. NUMBER OF EMPLOYEES *(Total for entire facility excluding outside contractors)*

c. NUMBER OF BEDS

d. WOULD YOU BE WILLING TO FURNISH COST INFORMATION *(Dollar and/or staff hours)* ON THE ECONOMIC IMPACT OF CURRENT NRC REGULATIONS OR ANY FUTURE PROPOSED NRC REGULATIONS THAT MAY AFFECT YOU? *(NRC regulations permit it to protect confidential commercial or financial–proprietary–information furnished to the agency in confidence)*
- [ ] YES
- [ ] NO

FOR NRC USE ONLY

TYPE OF FEE	FEE LOG	FEE CAT.	$	APPROVED BY
	8509120406 850828 REG2 LIC30 09-15728-02 PDR			19070
AMOUNT RE			FEE EXEMPT	DATE

PRIVACY ACT STATEMENT ON THE REVERSE

PRIVACY ACT STATEMENT

Pursuant to 5 U.S.C. 552a(e)(3), enacted into law by section 3 of the Privacy Act of 1974 (Public Law 93-579), the following statement is furnished to individuals who supply information to the Nuclear Regulatory Commission on NRC Form 313. This information is maintained in a system of records designated as NRC-3 and described at 40 Federal Register 45334 (October 1, 1975).

1. **AUTHORITY:** Sections 81 and 161(b) of the Atomic Energy Act of 1954, as amended (42 U.S.C. 2111 and 2201(b)).

2. **PRINCIPAL PURPOSE(S):** The information is evaluated by the NRC staff pursuant to the criteria set forth in 10 CFR Parts 30, 32, 33, 34, 35 and 40 to determine whether the application meets the requirements of the Atomic Energy Act of 1954, as amended, and the Commission's regulations, for the issuance of a radioactive material license or amendment thereof.

3. **ROUTINE USES:** The information may be (a) provided to State health departments for their information and use; and (b) provided to Federal, State, and local health officials and other persons in the event of incident or exposure, for their information, investigation, and protection of the public health and safety. The information may also be disclosed to appropriate Federal, State, and local agencies in the event that the information indicates a violation or potential violation of law and in the course of an administrative or judicial proceeding. In addition, this information may be transferred to an appropriate Federal, State, or local agency to the extent relevant and necessary for an NRC decision or to an appropriate Federal agency to the extent relevant and necessary for that agency's decision about you.

4. **WHETHER DISCLOSURE IS MANDATORY OR VOLUNTARY AND EFFECT ON INDIVIDUAL OF NOT PROVIDING INFORMATION:** Disclosure of the requested information is voluntary. If the requested information is not furnished, however, the application for radioactive material license, or amendment thereof, will not be processed. A request that information be held from public inspection must be in accordance with the provisions of 10 CFR 2.790. Withholding from public inspection shall not affect the right, if any, of persons properly and directly concerned need to inspect the document.

5. **SYSTEM MANAGER(S) AND ADDRESS:** U.S. Nuclear Regulatory Commission
Director, Division of Fuel Cycle and Material Safety
Office of Nuclear Material Safety and Safeguards
Washington, D.C. 20555

ITEM 5
RADIOACTIVE MATERIAL

Element	Form	Amount in Millicuries
Carbon-14	Any	30
Sulfur-35	Any	500
Hydrogen-3	Any	30
Phosphorous-32	Any	10
Phosphorous-33	Any	10

NOTE: Previous license covered in addition to the above Nickel-63 gas chromatograph detector cells. These are omitted because the equipment has been transferred to the University of Miami and is now included under their license. The types and amounts above are the remaining ones from our current license (Appendix III).

ITEM 6
PURPOSES

1) Carbon-14 will be used in measuring marine primary productivity. Basic experimental and precautionary protocol is attached (Appendix I). The amount is necessary because individual experiments now require about 1 millicurie, one experiment per day has been the rule, and cruise durations have been extended to 30 days.

2) Sulfur-35 and Phosphorous-32 will be used in marine microbiological uptake experiments just like Carbon-14. One principal difference is that the dilute aqueous solutions of labeled carbonate will be acidified and the CO_2 collected on Ascarite while sulfate will be precipitated and collected as the Barium salt. The second major difference is that considerably higher activities are necessary because sulfur is much less abundant in individual living cells than is carbon but far more abundant in seawater. Therefore individual experiments require as much as 15 millicuries. Attached are sections of our research plan (Appendix II). Safety precautions given in Appendix I are applicable as well to sulfur as sulfate. As noted in our application all radioactivity is strictly confined to the mobile laboratory both at sea and at the home laboratory. Hydrogen-3 uptake experiments are essentially similar as are requisite safety precautions except that residual activity is concentrated on activated charcoal. Activities per experiment are 0.5 to 1.0 millicuries.

3) At sea all the isotopes in the application are used for precisely the same purposes and in the same form as on shore and with exactly the same safety and experimental procedures as noted above. The very same mobile laboratory is used at the home base and aboard N.O.A.A. or U.N.O.L.S. research vessels. Cruises are taken both within and without US territorial waters but we feel the NRC retains jurisdiction because we are on US flag ships. In foreign waters appropriate clearances are secured for us by the State Department.

ITEM 7
RESPONSIBILITY

Dr. Ortner will continue to conduct all aspects of our radioactive substances research, safety and disposal. He has had six years of experience at the Woods Hole Oceanographic Institute and eight years at the Atlantic Oceanographic and Meteorological Laboratory in the principles and practice of radiation protection, measurement, monitoring and biological effects. He has been routinely employing the listed isotopes in uptake experiments at AOML under NRC 09-15728-02.

ITEM 8
TRAINING

See item 7.

ITEM 9
FACILITIES AND EQUIPMENT

Our laboratories are located on the second floor of the AOML laboratories on Virginia Key, Miami, Florida. The Ocean Chemistry Division Labs have fume hoods as well as sinks. ^{14}C, ^{35}S and ^{3}H work is conducted in a mobile laboratory equipped with a fume hood and sink. Stock solutions are prepared and kept in this facility which is located at the Virginia Key address when not aboard ship. All filtration is conducted in the mobile laboratory.

ITEM 10
SAFETY

Our radiation protection program involves the use of film badges as well as spot checks of lab backround activity by filter wipes of benches and occasional urine sample checks. The activity of the last two is then measured by liquid scintillation counting. For phospherous experiments Guardway Film badges are obtained from Landauer, Inc. through the University of Miami and are collected monthly. Standard laboratory rediotracer procedures are followed for uptake experiments and all radioactivity is strictly confined to the mobile laboratory with the exception of biological incubations: e.g., sugers or amino acids. The scintillation counter is also located in a mobile laboratory.

ITEM 11
WASTE

Dilute aqueous solutions of bicarbonate, sulfate and phosphate and of labelled organic constitute the bulk of our waste. Bicarbonate solutions are acidified and the CO_2 collected on Ascarite. Sulfate is precipitated and organics are scavenged by activated charcoal. These concentrated wastes, particulates collected on filters, and contaminated disposable plastic and paperwares are transferred to our collaborator, Dr. Russell Cuhel, for disposal through the University of Miami Radiation Control.

APPENDIX I

V.3. UPTAKE OF RADIOACTIVE CARBON

INTRODUCTION

The following method is given for workers who wish to use the radioactive carbon technique as a research tool or who have other reasons for carrying out the method entirely without external assistance. For others there already exists a service for providing 4-μc carbon-14 sources, filtration equipment, holders, etc., with facilities for counting and standardizing the radioactive plankton samples obtained as a result of a photosynthetic rate experiment. Details and prices can be obtained from the Danish Institute for Fisheries and Marine Research, Charlottenlund Castle, Denmark. Working Group 20 of the UNESCO Scientific Committee on Oceanic Research has recently issued a report of recommendations for the radiocarbon estimation of primary production which should be consulted.

METHOD

A. CAPABILITIES

Range: 0.05–100 mg C/m³ per hr

The range of this method depends very largely on the amount of radioactive carbon added and the precision of the radiochemical part of the procedure. There is virtually no upper limit and the lower limit could probably be reduced to 0.01 mg C/m³ per hr by using great care. An absolute lower value will exist, however, depending upon the reproducibility of "dark blanks" (*see later*). The above range is a realistic one under routine conditions.

1. PRECISION AT THE 25 MG C/M³ PER HR LEVEL

The correct value lies in the range:

Mean of n determinations $\pm 3/n^{\frac{1}{2}}$ mg C/m³ per hr (5 hr incubation, 1 μc added).

2. PRECISION AT THE 1.5 MG C/M³ PER HR LEVEL

The correct value lies in the range:

Mean of n determinations $\pm 0.15/n^{\frac{1}{2}}$ mg C/m³ per hr (7 hr incubation, 5 μc added).

B. OUTLINE OF METHOD

A known amount of radioactive carbonate, $^{14}CO_3^{2-}$, is added to sea water of known total carbonate content. (The addition of inactive carbonate with the "labelled" material is negligible.) After photosynthesis by the endemic phytoplankton population has continued for a suitable period the cells are filtered onto a membrane filter, washed and dried, and the radioactivity from the carbon in the plants is measured with a suitable Geiger counter. This uptake of radioactive carbonate, as a fraction of the whole, is assumed to measure the uptake of total carbonate, as a fraction of the whole, and hence the rate of photosynthesis may be evaluated.

C. Special Apparatus

BOD bottles (clear and opaque) cleaned, etc. as described in Part V.2, Section C.

Small sacks of black cloth and lighttight boxes as described in Part V.2, Section C.

The 25-mm diam Millipore filtration apparatus should be fitted with a funnel to hold more than 300 ml of liquid. A manostat device (there are several cheap commercial laboratory models based on the Cartesian diver) set to regulate the suction to the filtration unit to prevent the vacuum becoming greater than 150–200 mm of mercury.

A Geiger counter and decade scaler. It is strongly recommended that an ultra-thin end-window flow-counter be used rather than a windowless model. There is only a slight decrease in sensitivity with the former and the reliability and ease of use is greatly improved. In many sea areas it is difficult to filter the entire contents of a 300-ml BOD bottle through 25-mm filters, as described below. Although aliquots can be taken it is preferable to filter the entire contents of the bottle. If a counter accepting 50-mm planchettes is available it should be used as then samples can be filtered on 47-mm HA Millipore filters which are counted after mounting on a suitable copper or aluminum planchette. A pair of metal forceps for handling planchettes, and suitable tongs for placing holders in and out of the counter are required.

A 2-ml capacity "insulin" syringe with a supply of hypodermic needles, 2 and 6 inches long.

Cylindrical cardboard pill boxes with one or two small perforations in the lid, for storing copper planchettes and filters.

A desiccator containing small sacks of silica gel and soda lime to ensure a dry carbon dioxide-free atmosphere.

D. Sampling Procedure and Sample Storage

The general sampling methods and precautions specified in Part V.2 apply equally well to the present method. However, less sample is required (ideally two clear and one dark BOD bottle per sample) and it is unnecessary to ensure that there is no oxygen supersaturation. Subsurface samples should never be exposed to full sunlight during the filling operation or else "sun damage" of the phytoplankton cells may occur.

E. Special Reagents

1. Neutral formaldehyde solution

Take good quality 40% formaldehyde solution and decant the clear liquid from any precipitate. Store the solution in a well-stoppered dark glass bottle to which have been added a few small calcium carbonate (marble) chips.

2. Filtered sea water

As a wash liquid use sea water taken at the same time as the samples and filtered through an AA Millipore filter. Do not use synthetic sea water or a sodium chloride solution.

3. NITRIC ACID WASH SOLUTION

Prepare approximately 30% v/v solution of the concentrated acid. Add about 1 g of sulphamic acid or urea to each liter.

4. RUBBER CEMENT

Use a good quality *thin* rubber cement of the type prepared commercially for office use.

5. RADIOACTIVE CARBONATE SOLUTION

a. *Sodium chloride dilution solution*

Prepare 5% w/v sodium chloride solution from the analytical quality salt and distilled water. Add 0.3 g of anhydrous sodium carbonate, Na_2CO_3, and 1 pellet (*ca.* 0.2 g) of sodium hydroxide to each liter of this solution. Many liters may be required.

b. *Stock solution of active carbonate*

Obtain a "carrier free" solution of $Na_2{}^{14}CO_3$ (generally in the presence of a trace of sodium hydroxide) with an activity of about 0.5–1 mc/ml. The activity, correct to about 10%, should be stated by the manufacturer.

c. *Working solutions*

Prepare suitable quantities (at least 2 liters at a time is recommended) of dilute radiocarbon solutions by diluting the stock solution of active carbonate with the sodium chloride dilution solution. These working solutions should be prepared *immediately* before ampules are filled (*see below*) and care must be taken when handling the stock radioactive carbonate solution to see that none of it touches the hands or mouth or contaminates the benches of the laboratory in which subsequent work with radioactive carbon is to be carried out. Work over a large enamel tray covered with paper towels to catch and absorb any drips. Ordinary volumetric glassware may be used in this dilution operation but should be rinsed very thoroughly with acid and with distilled water after use. Use some form of automatic sucking device with pipettes.

Prepare one or more working solutions having the following *nominal* strengths (assuming the activity assay of the initial carbonate solution by the supplying agency is approximately correct):

2 ml ≡ 1 μc (microcurie) of ^{14}C
2 ml ≡ 5 μc (microcuries) of ^{14}C
2 ml ≡ 25 μc (microcuries) of ^{14}C

Generally speaking only one or two of these stock working solutions will be required by any one establishment (*see* Section F).

d. *Stock ampules*

Use 2-ml soft glass ampules. Fill each ampule with exactly 2.00 ml of radioactive working solution, using an "insulin" syringe with a 2-inch hypodermic needle or some suitable semiautomatic filling device. Ensure that no drops of solution collect at the mouth of the ampules and seal each one in a low-temperature blowpipe. Autoclave the ampules, submerged in a strongly coloured aqueous solution of

methylene blue, at 15 psi of steam for at least 20 min. Cool and remove the ampules, discarding any which are defective, as indicated by blue solution's having entered through the glass.

The preparation of dilute working solutions (c *above*), the filling and sealing of ampules, and the autoclave sterilization operation should be carried out on the same day. Working solutions are then stable almost indefinitely. They are standardized as to their exact content of radioactivity by the method to be described in Section H.

F. Experimental

Procedure

1. The sample for analysis is poured into a clear BOD bottle (preferably in duplicate) leaving an air space of about 3–5 ml at the top of the bottle (Note *a*).

2. Take an ampule containing 2.00 ml of suitable strength radiocarbon solution in 5% sodium chloride (Note *b*), score the tip with a sharp glass file and break it off. Carefully suck out the entire contents of the ampule by an "insulin" syringe fitted with a 6-inch hypodermic needle, transfer the needle to the *bottom* of the BOD bottle, and empty the contents of the syringe *slowly* into the sample. Raise the needle to the top of the BOD bottle, remove about 2 ml of sample and use this to rinse the ampule. Finally suck the ampule dry again and squirt the rinsing back into the BOD bottle. Add a little saline wash solution (if necessary) to fill the bottle, and close it with its ground glass stopper which should be secured by wire (Note *c*). The operations described in this paragraph should be carried out on a large paper-covered tray remote from the part of the building or laboratory in which the subsequent counting of radioactive plankton is to be carried out (*see later*).

3. Mix the contents of the BOD bottle thoroughly by shaking, cover it with a black sack, and put it into a lighttight box. When all samples have been "inoculated" expose them to illumination for a suitable time for photosynthesis to occur (Note *d*).

4. At the end of the experiment remove the stopper from the BOD bottle and add 1 ml of neutral formaldehyde solution from a small hypodermic syringe kept specifically for this purpose (Note *e*).

5. Assemble the filtration apparatus with a plain white 25-mm HA Millipore membrane filter in place. Filter the entire contents of the BOD bottle through the membrane, sucking it dry (Note *f*). Rinse the sample bottle twice with about 10-ml portions of filtered sea water, scrubbing the sides and bottom of the bottle with a rubber policeman, suck each washing through the membrane and finally wash the filter funnel and membrane with two or three more small rinses (2–4 ml) of filtered sea water. Suck the membrane free from all excess liquid, remove the source of vacuum, and unclamp the funnel (Note *g*).

6. Using clean uncontaminated forceps remove the membrane disc holding the radioactive plant cells and roll it onto a copper planchette previously smeared with rubber cement (Note *h*). Place the planchette holding the sample into a numbered pillbox and store the pillbox in the desiccator. Samples should be allowed to dry and lose their rubber cement solvent for at least 2 hr before being counted and may be stored for as long as several weeks if necessary (Note *i*).

7. Place the planchette and sample into a suitable Geiger counter assembly and measure the radioactivity. Details of this operation are not given as they depend largely on the equipment used but at least 5000, preferably 10,000, counts should be recorded for each sample. (Notes *b* and *j*). "Normalize" all counting rates to the rate found with a standard source, checked at least once a day or more frequently according to the stability of the counter system (Section H.2 (*i*)). Let R_s be the normalized counting rate (in counts per minute) of the sample planchette (Notes *k* and *l*), let R_b be the normalized counting rate of a blank determined as described in Section G, and let R be the normalized counting rate to be expected from the entire activity of the ampule. R varies with the microcurie level in the ampules and is determined as described in Section H.

8. Determine the total carbonate contents of the water by Part I.4. In nearly all sea locations, except in areas of excessive land drainage, it is only necessary to know the chlorinity and *p*H. The assumption of a specific alkalinity of about 0.125 and a density of 1.025 is made for calculation of the total alkalinity (*see* Part I.4.I.2). The *p*H of the sea water should be known in order to make the correction in Table VIII but the assumption of *p*H 8.0 is probably adequate in most cases. If A is the total *carbonate* alkalinity in milliequivalents per liter, calculate the weight W of carbonate carbon present in the water in mg C/m³ by the relation:

$$W = 12{,}000 \times A \times F_T$$

where F_T is read from Table IX and may generally be approximated to 0.95.

9. If N is the number of hours during which the sample was exposed to light, then the rate of photosynthesis is given by

$$\text{Radiocarbon-measured photosynthesis (mg C/m}^3\text{ per hr)} = \frac{(R_s - R_b) \times W \times 1.05}{R \times N}$$

The factor 1.05 is put in to allow for the fact that the carbon-14 isotope behaves rather differently from the carbon-12 isotope found in nature. This correction is somewhat uncertain.

NOTES

(*a*) A small air space should be left so that the contents of the bottle do not overflow when the radioactive solution is added. This precaution lessens the chances of contamination of the laboratory and personnel with radioactive solution.

(*b*) The amount of radioactivity to add depends upon the photosynthetic potential of the water, time of incubation, etc. If E is the percentage efficiency of the counting assembly used (generally 25–50), U the anticipated uptake of carbon in mg C/m³ per hr (say a tenth of the daily photosynthetic production rate), and R_s is the desired number of counts per minute from the radioactive plankton after an illumination period of N hr, then:

$$\mu\text{c (microcuries) carbon-14 to be added} = \frac{R_s}{E \times U \times N}$$

As R_s should be at least 1000 count/min and N is generally about 5 hr it will be seen that 25-μc ampules should be used for most open ocean work, 5-μc for moderately productive inshore waters, and 1-μc ampules for work in coastal areas during a phytoplankton bloom.

Making up the radiocarbon solution in 5% sodium chloride ensures that it is denser than all

seawater samples and sinks to the bottom of the BOD bottle when it is added. The final electrolyte strength and composition of the sample are not materially affected.

(*c*) The bottle should be filled completely to minimize leakages and exchange when the BOD bottle is placed in a light incubator or, more particularly, at depth in the sea. The introduction of a little filtered sea water does no harm. As long as a known amount of activity is added to a bottle of sea water and *all* the water is filtered the present method is *not* affected by the volume of the water initially in the bottle.

(*d*) The illumination period should not exceed 10 hr and should preferably be between 2 and 6 hr.

(*e*) Organisms are killed and photosynthesis is stopped by this addition. From then on samples may be exposed to light whilst they are filtered, etc., without introducing error, but filtration should not be delayed for more than a few hours. If filtration can be carried out in subdued light immediately after a sample has been incubated and if the filtration time does not exceed a few minutes the formaldehyde addition should be omitted. Even in the small concentrations used here formaldehyde may affect the excretion or loss of organic matter from the more delicate algae and should not be used for the most precise work. However, when a large batch of samples has to be filtered the errors brought about by not killing the algae will probably be greater than those introduced by this treatment, especially when bottles cannot be conveniently stored in total darkness or the filtration cannot be carried out in very subdued light.

(*f*) Filtration should be rapid and the time should not exceed about 10 min. However, with samples having large population densities or containing clay particles, filters will clog rapidly and nothing is to be gained by greatly increasing filtration times. Either take an aliquot from the BOD bottle (check that the volume is 300 ml) or filter the water through a 47-mm diam filter. In the latter case count the whole filter or cut a small disc from the stained portion with a sharp cork borer and multiply the resulting count by a factor which is the ratio of the area of the stained part of the filter to the area of the cut disc. Occasionally with heavy coastal blooms the plankton on the filter from 300 ml will not be "weightless" (less than about 0.1 mg/cm^2). If the chlorophyll *a* content of the water exceeds about 10 mg/m^3 use a 47-mm filter or take only a 100-ml aliquot from bottles. It has been shown that not all the radioactivity in a seawater sample is retained even by an HA porosity filter (*ca.* 0.5-μ pore-size) if samples are filtered with full suction from a water pump. Losses depend on populations, etc., but are greatly reduced or eliminated if the suction is regulated so that it never exceeds about $\frac{1}{4}$–$\frac{1}{3}$ atm.

(*g*) Washing with isotonic sodium chloride solution (*ca.* 3–4%), as previously recommended, has been shown to give a loss of radioactivity. Use only filtered sea water from the location of the samples. If filters are sucked dry only a very small washing is necessary. Various treatments of the filters by acid have been recommended to remove the inorganic labelled carbon taken up as carbonate. The amount of this uptake is still in question but can only be significant if the phytoplankton contains an appreciable proportion of coccolithophores. For most coastal samples no acid treatment is necessary. If coccolithophores are suspected hold the filter with bone-tipped forceps in the mouth of a flask containing concentrated hydrochloric acid and bathe the filter in the moist hydrogen chloride vapour for about 0.5–1 min before attaching it to the planchette. A washing with dilute acid solution is *not* recommended.

(*h*) The analyst should not touch planchettes or filter membranes by hand and should give his hands a thorough wash in water at this stage for safety, in case small amounts of radioactive sample solution contaminate the planchette.

(*i*) Planchettes may be stored for several weeks before counting, if adequately protected from physical damage, but we are not certain of the upper limit of storage time. If the pillboxes are stored in a desiccator having both a dry and carbon dioxide-free atmosphere there seems little reason to suppose that counting could not be delayed for many months.

(*j*) A suitable activity for the radioactive carbonate solution has been discussed in Note *b*. The standard deviation of any radiochemical determination is ultimately limited by the properties of radioactive atoms, as the coefficient of variation can not be less than $100/(R_s \times t)^{\frac{1}{2}}$ where R_s is the number of counts per minute and t the time of counting. For the precision to be $\pm 2\%$ or better from counting statistics alone the total number of counts must therefore exceed

10,000. As t should not have to exceed 5–10 min for practical reasons R_a should be at least 1000 count/min.

(*k*) The natural background count of the Geiger assembly should not exceed about 20–40 count/min and should be reproducible from day to day. In most circumstances this count is allowed for in the blank determination R_b obtained as described in Section G, but if there is reason to suppose that the natural count will change much in between measuring R_a and R_b *each* rate should be corrected separately for natural background before they are used as in paragraph 9 of Section F. Care must be taken to see that "coincidence corrections" are made if the counting rate of a sample becomes too great for the Geiger assembly. With most equipment this critical rate lies between 5,000 and 10,000 count/min and the analyst is referred to textbooks on radiochemistry for particulars.

(*l*) One should be on guard that the self absorption of the radioactive material on the planchette does not become sufficiently great to cause errors. This will be rarely the case in open ocean waters. In coastal areas, especially in the presence of suspended mineral matter, the amount of material on the filter may exceed 0.05 mg/cm^2 if too much sample is filtered and thus cause an error. Even with "weightless" sources, there is evidence that the efficiency of counting depends on the plankton species that have been filtered (C. Goldman, *J. Conseil, Conseil Perm. Intern. Exploration Mer.* In Press at the time of writing). This source of variability is not generally appreciated. The errors introduced by assuming a constant efficiency will generally not exceed 10% and can be accepted for most ecological work but for the specialized studies, where a high precision and accuracy is sought, end-window geiger counting should be replaced by the use of a scintillation counter.

G. Determination of Blank

Even in total darkness there is some uptake of radioactive carbon by plants, animals, and bacteria, largely by "exchange," although there may be a little true fixation. The amount depends mainly on time and is generally only 1–2% of the photosynthesis over a comparable period but in some ocean samples, especially in the tropics, the fraction may be 10% or greater. In any case the determination is worth making and acts as an overall safeguard against serious errors being undetected.

Fill a blackened BOD bottle with the sample and treat it exactly as described in Section F, paragraphs 1–7, placing the bottle near to the sample bottle (hence at the same temperature) during the illumination period. The final activity, R_b, may only be a few counts per minute greater than the natural background and a total count of 5,000–10,000 is clearly *not* warranted. The most suitable counting period is left to the discretion of the analyst.

As only a few per cent of the total activity in a BOD bottle will be taken up by plants the contamination of planchettes, etc., by only a trace of radioactive seawater sample will cause serious errors. The "inoculation" of BOD bottles from ampules and the filtration of samples should preferably be carried out in a part of the laboratory away from where planchettes are mounted and counted. BOD bottles and filtration equipment must be rinsed thoroughly with distilled water after use. From time to time all glassware and planchette holders should be rinsed in nitric acid wash solution and then water. Copper planchettes, with adhering membrane filters, should be discarded when counting is completed but pillboxes may be used again.

H. Calibration

Note: The calibration of this method is the most critical step. The following "classical" procedure has been repeated from earlier editions for the use of laboratories where scintillation

equipment, etc., is not available but we do not recommend its use. We believe the only satisfactory method is to determine the absolute disintegration rate of the source using a scintillation counter and the efficiency of the end-window counter by counting membranes holding radioactive phytoplankton and subsequently finding their absolute disintegration rates (*but see* Section F, Note *l*). The counting rate of an ampule, R, will then be the efficiency of the counter (20–50%) multiplied by the disintegration rate of the material in an ampule and should be "tied" to a standard source as described in H.2.*(i)* below. Eventually the entire radiocarbon method will be done by scintillation counting when this equipment is generally available. For details see Wolfe and Schelske (*J. Conseil, Conseil Perm. Intern. Exploration Mer*, 31: 31, 1967).

1. SOLUTIONS REQUIRED

a. *Radioactive working solutions*

Take two or more ampules *at random* from the stock prepared as described in Section E.5. If several strengths of solution are to be prepared and analysed it is often convenient to prepare the weaker activity solutions by exact volumetric dilution of the strongest solution used. In this way direct calibration is necessary only for the most strongly active solution and the other values may be obtained by simple arithmetic.

b. *Carbonate solution*

If A is the area (in cm^2) covered by the solid filtered onto a membrane filter by the equipment used, dissolve $0.54A$ g of anhydrous sodium carbonate, Na_2CO_3, in 1000 ml of distilled water.

$$1 \text{ ml} \equiv 1 \text{ mg } BaCO_3/cm^2$$

c. *Barium hydroxide solution*

Boil 1 liter of distilled water for a few minutes to remove dissolved carbon dioxide and then add 10 g of analytical reagent quality barium hydroxide, $Ba(OH)_2 \cdot 8H_2O$. Allow the solution to cool in an aspirator or similar vessel with a carbon dioxide trap. Any fine precipitate will settle out in a day or so and the clear supernatant solution should be used. This reagent must be kept out of contact with atmospheric carbon dioxide until immediately before use.

d. *Ammonium chloride solution*

Dissolve 5.0 g analytical reagent quality ammonium chloride in 500 ml of distilled water.

e. *Wetting agent*

Prepare a 0.2% solution of a stable neutral detergent.

2. STANDARDIZATION (DETERMINATION OF R)

(*a*) Remove the active solution from an ampule with an "insulin" syringe and transfer it to a volumetric flask containing one or two pellets (0.2–0.5 g) of sodium hydroxide. Rinse the ampule and hypodermic syringe once or twice with a little water and make the solution to a suitable volume such that 1 ml of the resulting solution contains about 10^{-2} μc of activity. Secondary dilutions may be necessary; e.g., 2 ml of 25-μc solution is made to 100 ml and 10 ml of the resulting solution is rediluted to 250 ml, whence 1 ml contains 10^{-2} μc. It is well to prepare exactly similar replicate dilutions from a further one or two ampules. These dilute solutions

should be used within a few hours of preparation. The initial flask and other flasks used to make secondary dilutions must contain a little sodium hydroxide to prevent the exchange of labelled carbon dioxide and atmospheric carbon dioxide above the solutions. This can be very serious with large solution–air interfaces unless solutions are appreciably alkaline (as could be the case if only distilled water were used for these dilutions). Flasks must be filled *immediately* to the neck after adding the radioactivity.

(*b*) Add to one or more 100-ml beakers (fitted with rods and coverglasses) 2.00-ml aliquots of the diluted radioactive standards, using an "insulin" syringe. Add about 5 ml of ammonium chloride solution and pipette in 20 ml of sodium carbonate solution. Make the volume to about 50 ml with recently boiled-out distilled water and add 20 ml of barium hydroxide solution slowly, stirring solution as this reagent is added. Add about 5 drops of wetting agent (the exact amount must be found by trial and will depend upon the detergent used) and allow the precipitate to settle for about 15 min, with the coverglass in place. The detergent will prevent excessive "creep" of the precipitate.

(*c*) Place an HA filter and copper planchette on a holder (do *not* cement the filter) and dry the assembly for about 1 hr in a desiccator. Remove and weigh the assembly to the nearest $\frac{1}{10}$ mg.

(*d*) Assemble the filtration apparatus with the weighed HA filter in place and filter the suspension of barium carbonate. Remove all the carbonate from the beaker with the aid of a policeman, rinsing with a little distilled water. Suck the precipitate dry on the filter and wash it once or twice with *small* volumes of distilled water. Remove the HA filter holding the precipitate and place it onto the weighed copper planchette. Clamp the planchette and filter into the weighed holder but do *not* cement or lose any of the membrane filter in the process. The filtration assembly, beakers, etc., should be rinsed with nitric acid wash solution and water before re-use.

(*e*) Allow the assembled holder, planchette, filter, and precipitate to dry for at least 2 hr in a desiccator (check for constant weight) and finally reweigh to the nearest $\frac{1}{10}$ mg. Find the weight, in milligrams, of barium carbonate precipitate (containing all the added radioactive carbon) as the difference between this weight and the initial weight (paragraph (*c*) above). Measure the area of the precipitate in cm^2.

(*f*) Find the radioactive count of the sample, using precisely the same assembly as that used for phytoplankton samples. Evaluate the counting rate in counts per minute, having counted a total of at least 10,000 — preferably 50,000 — counts. Correct this rate for any "coincidence correction" and for the natural background rate if this is significant.

(*g*) From the weight of precipitate per unit area and a self-absorption correction graph (*see* 3 *below*) find the standard count at *zero* thickness of precipitate. This count should be obtained (as a mean) from duplicate experiments on one dilute solution. Two or more dilute solutions are prepared from separate ampules. R is obtained by correcting these observed counts for self absorption and for the dilution factor necessary to relate counts back to the rate expected from the entire content of an ampule.

(*h*) Take a *standard source* of radiocarbon, having a counting rate of between about 2,000 and 10,000 count/min (such sources are obtainable commercially and their true counting rate, i.e. disintegrations per minute, are assumed not to change with time), and measure the exact rate on the same Geiger assembly as used above, counting at least 50,000 counts. Let this counting rate be R_c.

(*i*) The standard count R is then "tied" to the counting rate of the standard source R_c *obtained on the same day the calibration is made*. R need not be redetermined until a fresh batch of ampules is prepared as long as the standard source is unchanged and all sample counts obtained in the photosynthesis experiments (*see* Sect. F, paragraph 7) are "normalized" to the count R'_c. This is done by counting the standard source each day and finding a counting rate R'_c (which should be generally within a few per cent of R_c). Sample counts are multiplied by a factor R_c/R'_c before being used in calculations (Sect. F, paragraph 9), i.e.

$$R_s \text{ (Sect. F.7)} = \text{Rate found} \times R_c/R'_c$$

For counting equipment with 2π geometry R should be approximately 10^6 for each microcurie in the radioactive working solution in the ampules. R values are determined for each batch of ampules and refer to the count from the entire content of the ampule.

3. DETERMINATION OF A SELF-ABSORPTION CORRECTIVE CURVE

a. *Introduction*

The "soft" beta rays from ^{14}C are readily absorbed by matter. The absorption is largely a function of the "thickness" of the absorber (defined in radiochemical work as a mass per unit area, generally mg/cm^2) and is nearly independent of the type of matter comprising the absorbing material. Because of a near-cancellation of several effects the final absorption pattern of the beta rays is reminiscent of the attenuation of light through a uniform medium. It is evident, therefore, that a precipitate such as the one used above will partly absorb radiation from the ^{14}C atoms in the precipitate itself, giving rise to the phenomenon of "self absorption." The counting rate for a given amount of activity in a precipitate 20 mg/cm^2 "thickness," used for standardization, will be only about 10% of the count that would have been recorded if the same amount of radioactivity had been present on a filter with only a negligible amount of solid material on it, as is the case with phytoplankton filtered from a few hundred milliliters of sea water. To find the relationship between the counting rate determined on standards and the counting rate at "zero thickness" we must construct a self-absorption curve showing the *relative* counting rate of a fixed amount of radioactivity plotted (on the ordinate) against the thickness of the precipitate containing the radioactivity (on the abscissa). The shape of this curve is approximately (but by no means exactly) exponential and depends upon the radiochemical equipment and counting equipment used. The curve need only be determined *once* and is not required for the entire range of possible weights. One needs to be concerned only with the change of relative rate with weight of barium carbonate in the range 10–30 mg/cm^2, measured as a fraction of the counting rate at zero thickness. The latter is obtained by *extrapolation*, using successively smaller weights of barium carbonate.

b. *High-weight portion of graph*

Carry out the procedure described in Section H.2(*b*)–(*f*), finding the normalized corrected counts of precipitates containing the same amount of radioactivity (2.00 ml of active solution) with thicknesses of approximately 10, 15, 20, 25, and 30 mg/cm². These are obtained by adding 10, 15, 20, 25, and 30 ml of sodium carbonate solution to the beakers. It is desirable to carry out each determination in duplicate.

Count at least 10,000 counts for each sample. Construct a curve showing the count of each sample relative to the zero thickness count (*see* c *below*) against the thickness of each precipitate in mg/cm². Whenever standardization is carried out (as described in H.2 *above*) the count R is calculated from the relationship shown on this graph and a knowledge of the thickness (mg/cm²) of the standard (Sect. H.2(*g*)).

c. *Determination of zero-thickness count*

Prepare a set of twelve planchettes, holders, and HA filters; dry and weigh *two* as described in Section H.2(*c*). To a 250-ml beaker add 50.0 ml of the same dilute radioactive solution as was used in the "high-weight" experiments above, and about 25 ml of ammonium chloride solution. Pipette in 50 ml of sodium carbonate solution. Make the volume to about 150 ml with recently boiled-out distilled water and add 50 ml of barium hydroxide solution, stirring the solution as the reagent is added. Cover the beaker and allow the precipitate to settle for about 1 hr and then decant off most of the supernatant liquid *without* appreciable loss of precipitate. Add about 100 ml of distilled water, stir up the precipitate vigorously and again allow it to settle, and decant off the clear supernatant liquid. Finally transfer the solid quantitatively to a 250-ml volumetric flask and make to a volume of exactly 250 ml with distilled water. Shake the flask vigorously to suspend the solid uniformly and immediately pipette a 50-ml aliquot. Filter this aliquot of precipitate onto one of the weighed filter assemblies, dry in a desiccator, and reweigh. Repeat with a further aliquot from the thoroughly mixed suspension.

Place a small covered magnetic stirring bar in the flask of precipitate and whilst stirring vigorously pipette duplicate portions of 10, 5, 2, 1, and 0.5 ml of slurry directly onto membrane filters in the Millipore filtration equipment. A little water should be put in the filter funnel first and the aliquot of slurry added (rinse the pipette into the funnel) before applying suction. This ensures a more uniform dispersion of precipitate on the membrane filter. Dry the ten separate planchettes and precipitates thus obtained. Count each precipitate, "normalize" each count to the radioactive standard, and correct for "coincidence error," natural background, etc. The counts found for the 10-, 5-, 2-, 1-, and 0.5-ml aliquots are multiplied by 1, 2, 5, 10, and 20, respectively, to make them comparable to the activity of the precipitates used in the "high-weight" experiments (b *above*). If W is the mean weight of the precipitate per unit area found from the 50-ml aliquot experiments (the activity of these precipitates does not have to be measured) then the weights per unit area corresponding to 10-, 5-, 2-, 1-, and 0.5-ml aliquots are 0.2 W, 0.1 W, 0.04 W, 0.02 W, and 0.01 W, respectively. Plot the corrected counts on a logarithmic scale against these thicknesses on an arithmetic scale. Extrapolate to zero

thickness by the best line, which should be approximately linear at thicknesses below about 1 mg/cm^2. The count at zero thickness (about 10 times the count for the same amount of radioactivity in a precipitate of 20 mg/cm^2 thickness) is called unity and is used to fix the curve plotted in the high-weight experiment (b *above*), showing the self-absorption effect of samples having thicknesses between 10 and 30 mg/cm^2.

ating bacterial and algal protein synthesis in mixed populations. The plant sulfolipid is an integral component of the chloroplast thylakoid membrane, and its absence results in seriously decreased ability to perform the Hill reaction of photosynthesis (Sinensky, 1977). Because it is an essential constituent of the chloroplast, sulfolipid may be synthesized at a rate strictly related to the growth rate of the chloroplast itself. The growth rate of the chloroplast, in turn, should be relatively tightly coupled to the cellular growth rate, owing to its central importance in the biosynthesis and energy metabolism of the cell.

Although the role of the sulfolipid in chloroplast development and function is not yet clear, certain key points encourage the investigation of relationships between sulfolipid synthesis and growth. Sulfolipid is covalently bound to protein in the chloroplast membrane, but is unrelated to the chlorophyll-protein complex (Gregory Schmidt, personal communication). This suggests a relationship between sulfolipid and components of the photosynthetic electron transport chain. The absence of a physical interaction between sulfolipid and the light harvesting pigments is a positive feature. Sulfolipid is less likely to be subject to the fluctuating levels of chlorophyll often encountered as a result of light stress (c.f. Beardall and Morris, 1976) and hence may be a more conservative measure of growth.

PROPOSED RESEARCH

A. Specific Objectives:

The proposed research is designed to determine the relationships among protein synthesis, sulfur and carbon metabolism, and growth of marine phytoplankton with respect to possible application to natural population rate measurements. Initially, pure culture studies with representative phytoplankton species will be conducted to provide the following background data:

(1) ratios of carbon to sulfur in whole cells and principal biochemical components (i.e. protein, sulfolipid, soluble materials, etc.) during balanced, exponential growth,

(2) ratios of central metabolic processes (e.g. protein synthesis, chlorophyll a synthesis, photosynthetic carbon fixation, sulfolipid synthesis, sulfur incorporation into protein, etc.) during balanced growth,

(3) relative rates of central metabolic processes during growth under a light/dark regime to determine which processes are most strongly affected by daily rhythms and at what time during the cycle a given measurement is most meaningful, and

(4) variations in the ratios of elements and rates as functions of representative applied stresses.

When the relationships between sulfur metabolism, protein synthesis, and growth are sufficiently well understood, mixed pure cultures of algae and bacteria will be used to attempt the quantitative separation of the rates of protein synthesis attributable to each.

Finally, rates of carbon and sulfur incorporation will be measured with natural populations. Local populations will be used to optimize incubation and sample processing conditions, after which natural rates will be measured in locally available areas of varying productivity (e.g. inshore waters off Florida, within and outside of the Gulf Stream, and in deep chlorophyll maxima) in order to determine the limit of sensitivity of the proposed method.

B. Analytical Methods:

Pure culture studies will be carried out in a sulfur-free artificial seawater modified from the formulation of Lyman and Fleming (1940) or in natural seawater. Nutrient and vitamin additions will be made as necessary. Cultures will be grown at constant temperature both with or without a light/dark cycle. Bulk analyses (direct counts, chlorophyll a, protein, carbohydrate, particulate organic carbon and nitrogen, ATP, etc.) will be performed by commonly accepted techniques.

Data on the distribution of radioisotopes (^{35}S and ^{14}C) will be obtained by the same procedure as described in NSF Grant OCE77-12172. The method is the serial extraction procedure of Roberts et al. (1963) in which the cells are chemically fractionated as follows:

(1) extraction with cold 5% trichloroacetic acid (TCA); 5000xg supernatant containing low molecular weight organic compounds;

(2) residue extraction with warm 80% EtOH and EtOH-ether; supernatant containing lipids and alcohol-soluble protein (phases separated with water and lipids extracted with ether);

(3) residue extraction with boiling 5% TCA; supernatant containing hydrolyzed nucleic acids and polysaccharides;

(4) residue containing protein.

In some cases, RNA and DNA will be separated by warm KOH hydrolysis of RNA after the alcohol-ether steps (Neidhardt and Magasanik, 1960).

Investigators using whole cell sulfate uptake as a potential measure of microbial growth have experienced some difficulty with the sensitivity and interpretation of uptake data (Jordan and Peterson, 1978; Jordan et al., 1978; Monheimer, 1978). Large and variable radioisotope blanks were encountered as a result of the high activity of ^{35}S which must be added to detect uptake by natural populations of microorganisms, even in freshwater environments. This problem is even more pronounced in marine systems where the sulfate concentration is 25mM. A filter-excision technique was adopted by Jordan et al. (1978) which alleviated this problem substantially. Nonetheless, Monheimer (1978) felt that sulfate uptake would not be a useful technique because there appeared to be no predictable relationship between sulfate uptake and microbial carbon assimilation. Our results substantiate this finding: the last line of Table 1 demonstrates that the carbon to sulfur ratio in whole cells of a single microorganism in pure culture spans the entire range of reportedly constant C:S ratios of 50:1 (Jassby, 1975) to 500:1 (Stuiver, 1967) when measured under a variety of nutritional states. However, both of these problems have been largely resolved by the use of the fractionation procedure described above in conjunction with a specially constructed punch funnel serving the function described by Jordan et al. (1978). In addition to providing a predictable relationship between sulfur assimilation and growth (Figure 2), the initial extractions in cold TCA effectively wash out contaminating sulfate. Reproducible blanks of

under 1000 DPM are obtained after passage of a 100ml sample containing 50uCi $^{35}SO_4^{=}$/ml (1.1×10^{10} DPM). If twice the blank value is required to be sure of incorporation, the sensitivity of the measurement is slightly greater than 5ug C assimilated per liter during the incubation period. Production rates of this magnitude frequently occur in one hour for inshore waters. The method will not be sensitive enough to detect growth in highly oligotrophic areas such as the Sargasso Sea in late summer, but under these conditions even carbon dioxide fixation is difficult to detect. When purchased in bulk, the cost of processing a sample under these constraints is about $7, similar to that of ^{14}C.

C. Experimental Protocol:

Initial pure culture studies with representative axenic phytoplankton species will be carried out in one-liter syringes made from chromatography columns. The concentrations of bulk parameters (e.g. protein, chlorophyll *a*, cell counts, etc.) and the distribution of ^{14}C and ^{35}S will be determined from parallel incubations. During the mid-exponential phase of growth, aliquots will be removed to smaller syringes and the desired perturbations applied. Similar incubation methods will be used in both pure culture and natural population studies; subsampling from a single sample to minimize heterogeneity.

A regular sampling schedule using laboratory simulated in situ incubations can be carried out in the nearby Gulf Stream. Details of specific activity of radioisotopes, incubation time, sample volume, etc. can be optimized easily in this fashion. In addition, the site provides the benefits of nearby mesotrophic and oligotrophic water masses as well as a deep chlorophyll maximum. This allows the sampling of a "trophy gradient" for comparing metabolic features of populations under widely differing physical and nutrient regimes. Field experiments will be directed towards the measurement of total microbial protein synthesis, its relationship to heterotrophic

and autotrophic carbon metabolism, and delineation of the relative contribution of phytoplankton and bacteria to total biomass production.

SIGNIFICANCE OF THE PROPOSED RESEARCH

Development of a quantitative measurement of the rate of microbial protein synthesis will be useful in determination of the nutritional state of natural populations. Comparison of phytoplankton protein synthesis and total carbon metabolism can reveal regions of nutrient limitation and physico-chemical stress which may affect the interpretation of routine CO_2 fixation studies. The estimation of de novo protein synthesis will be a valuable contribution to trophodynamic studies, and may elucidate the currently unknown state of bacterial growth in natural aquatic environments.