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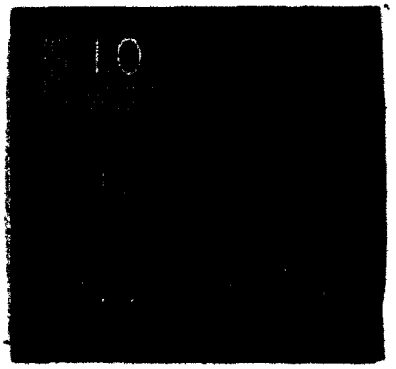

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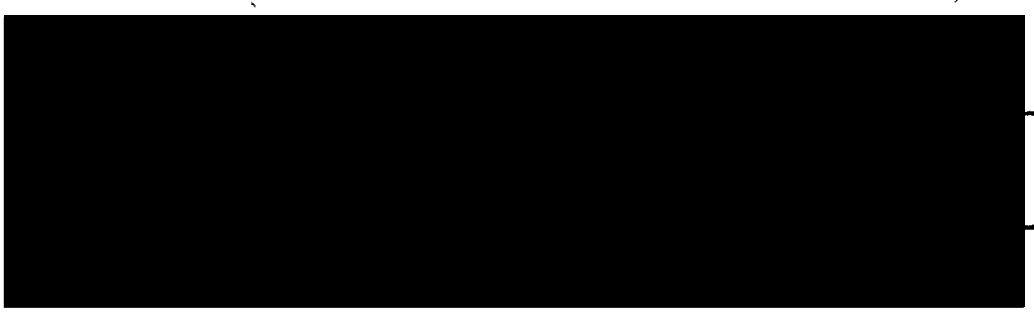
THE FORMATION OF PARTICULATE ORGANIC MATTER IN SEAWATER

BY

Jonathan H. Sharp

Submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Oceanography at Dalhousie University, January, 1972.

Approved by:



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Department or School Oceanography

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CONTENTS

ABSTRACT

The formation of particulate organic matter in seawater is investigated by examination of the pool of organic matter occurring in the sea, by experiments on artificial production of particulate matter in filtered seawater, and by experiments on production of organic matter by marine phytoplankton.

A new method is described for analysis of total organic carbon in seawater in which samples were oxidized by high temperature combustion and the carbon dioxide in the combustion product was measured with a non-dispersive infrared analyzer. This method was compared directly to the now standard persulfate oxidation one and it was shown that the latter method missed a significant portion of the organic matter that is present in seawater. Total, particulate, and colloidal organic matter were measured in the central western North Atlantic Ocean. Examination of these data and of recent literature indicates that organic matter in the sea is neither as conservative nor as predictable a function of depth as had been previously concluded.

Experiments are described in which attempts were made to form particulate organic matter in filtered seawater. These experiments were run under carefully standardized conditions with replicate samples for carbon analyses and microscopic observations. They showed that particle formation occurred which could be attributed neither to experimental artifacts nor to contamination. Experiments with different water samples indicated that there is a natural variability in the potential for particle formation. In the experiments of short duration that were performed, sufficient organic carbon was always present in colloidal form to account for the particulate carbon that was formed in the filtrates.

Preliminary experiments are reported on extracellular organic carbon production by marine phytoplankton. The role of marine phytoplankton as contributors to the filter-passing organic carbon in the sea is discussed.

ACKNOWLEDGEMENTS

I thank Dr. Gordon A. Riley for his unimposing, yet influential, guidance as thesis supervisor. I wish also to thank Drs. Peter J. Wangersky, James S. Craigie, and Robert C. Cooke for serving ably on my thesis committee. Especially, I thank Dr. Wangersky for luring me, with promises of cruises to exotic places, into trying to measure total organic carbon in seawater. I thank Dr. Donald C. Gordon, Jr. for serving effectively and constructively as external examiner of the dissertation. I am grateful to Mrs. Shirley M. Conover for aid and advice with the algal culture work. I have very much appreciated and benefitted from the invigorating educational atmosphere of the Dalhousie University Department of Oceanography which is brought about by an informal association of exceptional students, faculty members, and associates. I acknowledge the Dalhousie Faculty of Graduate Studies for continual financial support throughout my studies. The use of laboratory facilities, granted to Dr. Wangersky, at the Atlantic Regional Laboratory of the National Research Council was appreciated. The ships C.S.S. Hudson, C.S.S. Dawson, and C.F.A.V. Sackville and the boats Sigma-T and Whip-the-Wind were used through Bedford Institute of Dartmouth, Nova Scotia. I thank Dr. William Ford, Director of Bedford Institute, through whose cooperation with Dalhousie it was possible to use these ships. The assistance by the other scientists, the crews, and the officers of these ships is much appreciated as is their companionship for the many weeks in which we traveled the oceans in pursuit of water.

La Nature est un temple où de vivants piliers
Laissent parfois sortir de confuses paroles;
L'homme y passe à travers des forêts de symboles
Qui l'observent avec des regards familiers.

Charles Baudelaire, from "Correspondences"

" O Timbaloo! how happy we are
When we live in a sieve and crockery-jar!
And all night long, in the moonlight pale,
We sail away with a pea green sail
In the shade of the mountains brown."

Edward Lear, from "The Jumblies"

INTRODUCTION

Most of the organic matter in the sea is not alive. In the past, many efforts were made to identify and quantify the small portion that was living while the rest was usually relegated to the role of detritus and was ignored. Recently, the study of non-living organic matter in the sea has become an area of considerable interest.

Organic matter in the sea has been divided by arbitrary definition into particulate and dissolved fractions. Thus, that matter retained by a membrane filter was termed particulate while everything passing the filter was called dissolved. This division initially seemed satisfactory because it apparently separated living matter from non-living. However, the recent realization that some bacteria can pass through the filters and that most of the organic matter retained on the filters is not living make the separation seem less reasonable. Because of analytical convenience, the arbitrary division is usually still employed.

A dissolved organic molecule in an aqueous medium is one in which minimum free energy is attained by hydration of all potentially hydrophilic sites on its structure. Any "loose" association between molecules (as opposed to the "tight" association through chemical bonding that leads to new molecules) that allows for loss of water of hydration and formation of a less hydrophilic species tends to remove the resulting molecular-associate from solution. Clearly then, all that passes a membrane filter is not necessarily in solution. In this dissertation, the terms "particulate" organic matter and "particle" will be used conventionally and they are defined here specifically as that class retained on a membrane filter with a nominal average pore size of eight tenths of a micrometer (0.8 μm). Organic matter

passing this filter will be called filter-passing. That portion of the filter-passing matter that is retained by filters or membranes with smaller nominal pore sizes will be called "colloidal". No attempt is made to measure dissolved organic matter and the only reason for mentioning this class is that much of the importance that is usually assigned to the so-called dissolved class may actually belong to the colloidal class. Although organic matter is discussed above, actual measurements in this dissertation are of organic carbon and all quantitative listings are in weights of carbon. The usual figure of 50% (Birge and Juday, 1934) for carbon in aquatic organic matter seems a reasonable "ball-park" estimate.

It was shown about a decade ago that particulate organic matter could be formed in membrane-filtered seawater by bubbling the filtrate (Riley, 1963; Sutcliffe, Baylor, and Menzel, 1963). The role of non-living particulate organic matter in seawater and the formation of particles have been reviewed by Riley (1970). Some little doubt remains on whether some of the evidence for particle formation is anomalous. There are many unknowns as to the source of the particles that are formed in filtrates. These two areas are the underlying motivation for this dissertation.

In most previous works, experiments on particle formation were done on samples for which very little was known about organic carbon. In the present work, particle formation is considered in light of the overall organic carbon picture. Three main sections make up the dissertation: grouping and distribution of organic carbon, particle formation experiments, and algal culture experiments. The first two sections include surveys that were done at sea, and laboratory and shipboard experiments. Most of the survey work was done in a fairly long and narrow region extending from 44 to 17° N latitude and between 56 and 64° W longitude which is considered

to represent the central western North Atlantic Ocean. By carefully standardized experiments, a comparative estimate was made of a potential for particle formation. These experiemtns were used in conjunction with analyses for total, particulate, and colloidal organic carbon to explore the source and variability of formed particles. The algal culture experiments serve, in a limited fashion, to assess the role of extracellular production by phytoplankton in the organic carbon pool of the sea.

Most of the sampling was done during cruises aboard the C.S.S. Hudson, C.S.S. Dawson, and C.F.A.V. Sackville and station positions and dates are listed in Table 1. The Hudson stations 9-16 were regular stations on two Pacific Ocean legs of the "Hudson-70" cruise and for them considerable water mass and nutrient chemistry data are available from Bedford Institute and particulate organic carbon data from P.J. Wangersky. For all other cruise stations, little or no oceanographic data are available except those included here. Surface samples were taken by bucket while carefully avoiding the ship's bow wake. All other samples were collected in 5- or 28-liter polyvinyl chloride Niskin bottles. The Niskin bottles were periodically cleaned with detergent and isopropyl alcohol; they were rinsed after use with freshwater, and they were scoured with seawater before use by running open bottles up and down the hydrowire. Water collection in St. Margaret's Bay, Nova Scotia was by bucket from the boats Sigma-T and Whip-the-Wind. Water samples were also collected by bucket from a pier at the mouth of the Northwest Arm (the western boundary of Halifax Peninsula, Nova Scotia). Samples were brought back after being collected in aged and well cleaned 8-, 20-, and 60-liter polyethylene carboys. The subsurface cruise samples are listed for nominal depths (determined by hydrowire meter wheel); with the exception of Sackville stations 29 and 30, the depths are not suspected of being appreciably inaccurate.

Table 1. Identification of stations.

STATION	SHIP	LOCATION	DATE
1	Hudson	40°37'N, 63°28'W	24-IV-69
2	"	35°36'N, 63°22'W	26-IV-69
3	"	32°28'N, 63°06'W	28-IV-69
4	"	37°40'N, 63°32'W	30-IV-69
5	"	44°18'N, 56°44'W	18-IX-69
6	"	44°16'N, 58°04'W	20-IX-69
7	"	42°18'N, 60°15'W	22-IX-69
8	"	42°42'N, 64°18'W	24-IX-69
9	"	24°50'S, 150°01'W	8-V-70
10	"	09°50'S, 150°01'W	18-V-70
11	"	04°59'S, 150°08'W	20-V-70
12	"	00°04'N, 149°52'W	21-V-70
13	"	04°47'N, 150°00'W	23-V-70
14	"	10°05'N, 150°04'W	24-V-70
15	"	49°09'N, 150°00'W	4-VI-70
16	"	54°25'N, 150°05'W	6-VI-70
17	Dawson	38°58'N, 63°09'W	12-I-71
18	"	30°19'N, 64°12'W	14-I-71
19	"	17°18'N, 61°31'W	19-I-71
20	"	17°16'N, 61°35'W	23-I-71
21	"	17°20'N, 61°31'W	25-I-71
22	"	17°21'N, 62°29'W	27-I-71
23	"	17°20'N, 62°28'W	4-II-71
24	"	17°18'N, 61°28'W	10-II-71
25	"	19°45'N, 63°30'W	12-II-71
26	"	31°38'N, 63°28'W	15-II-71
27	"	36°30'N, 63°30'W	16-II-71
28	Sackville	42°05'N, 63°05'W	25-VI-71
29	"	40°04'N, 63°02'W	26-VI-71
30	"	38°00'N, 63°00'W	26-VI-71
31	"	38°40'N, 63°00'W	27-VI-71
32	"	41°59'N, 63°23'W	28-VI-71

THE ORGANIC POOL

PREFACE

Three categories of organic matter, measured as carbon, are considered in this section. Two of them, particulate and colloidal organic matter were defined on pages 1-2. The other category is total organic matter, which is the total organic content of a raw unfiltered seawater sample. As will be explained below, the filter-passing (so called "dissolved") organic carbon is quantitatively almost the same as the total organic carbon in the open sea, so the literature on "dissolved" organic carbon may be discussed with this category.

Since organic carbon occurs in comparatively miniscule amounts in seawater, methods for preparation and analysis of samples are extremely critical. Most of this section is devoted to methods, especially that for analysis of total organic carbon. Carbon determinations are also used in the other two main sections of the dissertation. Field surveys of the three categories of organic carbon allow a composite picture to be drawn for the central western North Atlantic Ocean. With the composite picture and with recent literature, organic carbon distribution in the oceans is discussed.

STATISTICS

For all quantitative work in this dissertation, precisions are listed as plus or minus two times the standard deviation divided by the square root of the number of replicates used in calculating the mean. This is the standard error of the mean ($\pm 2\sigma_{\bar{x}}$) and it defines the 95% confidence

interval. A number not exceeding $2\sigma_{\bar{x}}$ in difference from a second number is not considered significantly different from the second number. For composite error calculations, the relative standard error of the mean is used; it is the standard error of the mean divided by the mean ($\pm 2\sigma_{\bar{x}} / \bar{X}$). To compare two groups of data, t-tests were run on the significance of the difference of their means from zero and the 95% confidence interval was used unless otherwise stated.

In carbon analysis, standardizations were done using known amounts of standards as independent variables and their peak areas (integrator counts) as dependent variables. From the standardizations, linear or parabolic regression formulae were calculated by least squares. Correlation coefficients were calculated for linear regressions (this is not strictly a valid statistic for functional relationships, but it provides a quick relative picture of linearity and it was used only as a relative picture).

PARTICULATE ORGANIC CARBON

A somewhat traditional view of both particulate and total organic carbon distribution in the ocean is of considerable variation in the upper 100 to 200 meters with quite constant levels below this. This view is summarized by Menzel and Ryther (1970). According to them, the near surface variability is due to high biological acitivity in the photic zone and the subsurface constancy is due to no appreciable biological activity taking place below about 200 meters. However, there is a midwater (300 to 900 meters) zone of relatively high biological activity which has been called the transition zone (Riley, 1970). Riley (1951) showed subsurface maxima in nitrate and phosphate in this area that were associated with the oxygen minimum layer

and large populations of microorganisms (Fournier, 1970) and zooplankton (Leavitt, 1938) have also been found in this zone. Riley (1970) pointed out some regional and seasonal variations in particulate organic carbon for depths below 200 meters. Additional evidence for particulate organic carbon variability is now available for both within and below the transition zone (Gordon, 1971b; Nakajima, 1971; Wangersky, in preparation). Seasonality has been shown in populations of pigmented microorganisms in the area of the transition zone by Fournier (1971). The concept of particle transport to deep water from the photic zone by overlapping chains of zooplankton (Riley, 1951) has been re-examined and supplemented with transport by fecal pellets to explain deep water variability (Nakajima, 1971). Evidence has been given for particle transport from the transition zone to deep water by fecal pellets and aggregation (Fournier, 1972). Thus, subsurface variability in particulate organic carbon seems fairly well established as a phenomenon.

For particulate organic carbon determinations, seawater samples were filtered and the organic carbon retained on the filters was measured. In the particle formation experiments, 25-mm diameter, 0.8-μm pore size silver filters (Selas) were used; on the Sackville cruise and in the algal culture experiments, 47-mm diameter filters of the same pore size and material were used. With the larger diameter filters and the smaller ones on the Dawson cruise, the filters were prebaked at 500 C before use; this procedure was not done for the other filters. Analysis was done on a particulate carbon analyzer (built by P.J. Wangersky) using the following procedure: Folded filters were put into quartz tubes (5-mm ID by 7-mm OD by 5-cm length), the tubes were filled with 1% HCl (in ultrapure water-see Appendix I), and were dryed by vacuum. Then the tubes were inserted into the combustion

train, and the carbon on the filters within the tubes was burned at 600-900 C in the presence of several catalysts in a stream of oxygen (Wangersky, in preparation). The evolved CO_2 was analyzed as in the total organic carbon analyzer (see below). On the Sackville cruise, filters were frozen in small plastic Petri dishes and brought back to the laboratory in them. But on all other occasions, the filters were placed directly into the quartz tubes which were stored in specially built ceramic racks over silica gel in vacuum desiccators. Analysis of empty tubes and of tubes containing filter blanks indicated that no gross contamination occurred even with several months of storage. The carbon analyzer was standardized with dextrose and calibrated between standardizations with 190- or 1000-ppm CO_2 gas. Since the silver filters contain some organic carbon, filter blanks must be run and for this, blanks were treated like sample filters. Gordon (1969) pointed out that blank values varied for different batches of filters received from the manufacturer. This is verified with blank values of the 25-mm filters that were used in this work as can be seen in Table 2. The reduction of the blank by prebaking and the observation that the variability of the blank is fairly independent of value are illustrated in Table 2. The average blank error is 1.7 so average analytical precision of particulate organic carbon analysis cannot be lower than 1.7 µg C per filter. The blank values that were subtracted from sample values were those from the specific box used and not a composite average.

Most of the particulate organic carbon results are considered in the particle formation and algal culture experiments sections. The only ones that are included here are done so in conjunction with the total organic carbon. Particulate organic carbon in the ocean is often quoted as 10% of the total organic carbon (Parsons, 1963). This figure can be investigated

Table 2. Filter blanks for particulate organic carbon analysis. Silver filters of 25-mm diameter and 0.8-μm pore size were used; n is the number of filters used from each box; the mean and error are in μg carbon. Only the last three batches of filters were prebaked.

n	MEAN	ERROR
6	13.65	1.72
4	6.84	2.48
8	15.07	1.68
8	14.44	1.16
9	15.24	2.36
5	20.01	1.04
4	6.11	3.36
7	5.31	1.52
4	1.86	0.22
	Average	1.73

using some of the data from this study and some from recent literature. Table 3 gives data from the Sackville cruise (the total organic carbon data is explained below). As can be seen, the percent particulate decreases from surface to deep water (with some irregularities) and it is consistently below 10%.

A general picture can be constructed of regional differences in the percent particulate. The data for the second region in the following list are the above mentioned Sackville data plus data from the Dawson cruise; the data from the fifth groupingare from the section on algal culture experiments.

1-North Central Pacific (Gordon, 1971a)	0.7%
2 -central western North Atlantic	1.5
3-Straits of Georgia (Fulton, et. al., 1967)	13
4-Chucki Sea (Loder, 1971)	24
5-one to twenty-five day-old diatom cultures	72

There are some analytical differences between the data of the various groups in the above list but the differences are not sufficient to alter the general picture. The list illustrates a general trend of increasing percent of particulate matter in going from oligotrophic oceanic to more eutrophic waters. As explanation of regional differences: region 1 is a single sub-tropical oceanic station sampled year-round, region 2 includes Gulf Stream and Caribbean waters as well as open ocean North Atlantic, region 3 is inshore North Pacific Ocean, and region 4 is shallow nearshore Arctic Ocean sampled in the summer.

The often quoted 10% particulate seems very much an overestimate for oceanic waters where 1% would be a more reasonable estimate. At 1%, the particulate organic carbon is quantitatively insignificant when compared to

Table 3. Sackville cruise organic carbon data. Sample column is station number and depth (in meters). Particulate and total columns are in mg C/L. Percent column is particulate divided by total times 100.

SAMPLE	PARTICULATE	TOTAL	PERCENT	SAMPLE	PARTICULATE	TOTAL	PERCENT
28 -50	.028	1.25	2.23	30 -3000	.004	0.95	0.38
-75	.015	1.18	1.30	-3450	.004	0.96	0.38
-100	.016	1.23	1.27	-3950	.011	0.77	1.47
-1500	.008	1.07	0.77	31 -200	.010	1.53	0.63
-2000	.005	1.18	0.41	-300	.009	1.54	0.58
-2500	.025	1.14	2.15	-400	.006	1.35	0.48
29 -5	.061	1.50	4.04	-500	.004	1.53	0.26
-25	.049	1.13	4.36	-600	.005	1.39	0.38
-50	.041	1.27	3.23	-1000	.012	1.34	0.92
-100	.023	0.83	2.78	-1500	.003	1.43	0.18
-500	.011	0.91	1.25	-1600	.003	1.39	0.24
-1000	.009	0.94	0.96	-1700	.003	1.52	0.18
-2000	.005	0.80	0.61	-1800	.002	1.42	0.14
-3000	.004	0.84	0.45	32 -10	.045	1.49	3.01
-4000	.006	1.03	0.60	-50	.024	1.38	1.76
-4500	.004	1.09	0.32	-100	.007	1.22	0.61
30 -10	.047	1.30	3.48	-500	.003	1.16	0.28
-50	.080	1.16	6.87	-1000	.003	1.08	0.26
-500	.010	1.04	0.94	-1200	.003	1.14	0.25
-1000	.005	0.83	0.57	-1400	.002	1.07	0.17
-1500	.006	0.86	0.68	-1600	.004	1.00	0.39
-2000	.016	0.91	1.71	-1800	.005	1.24	0.39
-2500	.002	0.76	0.31	-2000	.004	1.07	0.33

total so it is reasonable to consider the "dissolved" organic carbon of the literature as synonymous with total organic carbon when discussing oceanic waters.

TOTAL ORGANIC CARBON

Background

The total organic carbon in seawater is a small amount compared to the total inorganic carbon; it is roughly 4% as calculated from data of Sverdrup, Johnson, and Fleming (1942). Hence, attempts to assess the organic fraction must be preceded by purging the sample of the inorganic. This is usually accomplished by acidifying the sample to a pH below 4 (where HCO_3^- and $CO_3^=$ are unstable) and removing the resultant CO_2 by purging with inert gas. This process also removes volatile organic carbon compounds, the amount of which is unknown. Duursma (1961) and Skopintsev (1966) have discussed the loss of volatiles and concluded that it is fairly small (about 10%). Whatever the amount of volatiles, it is consistently lost in all methods discussed here and therefore is ignored. After the inorganic carbon removal, all the organic carbon is oxidized to CO_2 and the amount of CO_2 is determined.

Early analytical work was relatively crude and probably insufficiently refined by present standards; it is reviewed by Duursma (1961). The early work consisted of wet chemical oxidation of carbon and volumetric detection of CO_2 (usually by titration after the gas was reacted with baryta). This approach reached its peak of sophistication with Duursma (1961) whose method was quite precise, though very tedious. In his method, oxidation was with a mixture of $K_2Cr_2O_7$ and H_2SO_4 and was followed by coulometric titration. Skopintsev and co-workers in the Soviet Union still use a

titrimetric method which is preceded by high temperature combustion of the dried sea salts (see Skopintsev, 1966). The Soviet workers find levels of organic carbon about 100% higher than those of Duursma.

A quick and simple method for organic carbon analysis is the persulfate oxidation one introduced by Menzel and Vacarro (1964). It has been slightly modified and is now considered a standard analysis in seawater (Strickland and Parsons, 1968). In this method, organic compounds are oxidized to CO_2 with $K_2S_2O_8$ and the resulting gas is quantified by non-dispersive infrared analysis. In another recently described method (Armstrong, Williams, and Strickland, 1966) organic compounds are oxidized by exposure to ultraviolet radiation and the resulting CO_2 is measured manometrically.

Efficacy of oxidation by persulfate has been estimated through comparing the amount of CO_2 measured from known compounds to that hypothetically calculated (Menzel and Vacarro, 1964; Fredericks and Hood, 1965; Strickland and Parsons, 1968). These authors concluded that essentially all the carbon that was present was oxidized. P.J. LeB. Williams (1969) also checked persulfate efficiency but he used a residual C^{14} method to determine the completeness of oxidation. He added amounts of organic compounds more closely approximating natural levels than had the previous authors but he also concluded that oxidation by persulfate was complete. He found oxidation to average more than 95% for the 15 compounds tested (ranging from 78 to 100%). In the above checks, relatively simple (low molecular weight and aliphatic), reagent grade, compounds were used rather than naturally occurring marine organic matter.

P.M. Williams (1969) compared the method of ultraviolet radiation to that of persulfate oxidation. He used 24 natural seawater samples for which the mean value by persulfate oxidation was 90% of that by ultraviolet

radiation. The difference was statistically significant but Williams did not feel that the persulfate method missed an appreciable portion of the total organic carbon that was present.

Van Hall, Safranko, and Stegner (1963) proposed a method using high temperature combustion for analysis of organic carbon in aqueous solutions. In their method, liquid samples are injected into a 950 C furnace and carbon compounds are burned to CO_2 in an oxygen atmosphere. The resulting gas, after removal of water, is measured in an infrared analyzer. Their method was not usable at carbon concentrations below 2 mg C/L nor was it tested extensively with solutions of high salt content. It has not been used for oceanographic work because of these limitations.

P.J. Wangersky began development in this laboratory of an analyzer for use with seawater that was similar to that of Van Hall, Safranko, and Stegner. Starting with this prototype, I have developed a high temperature combustion method for liquid samples which is described below. A direct comparison of this method to persulfate oxidation is made on samples from the Pacific and Atlantic Oceans and from several algal cultures.

The Combustion Method

The Carbon Analyzer

The stations that are mentioned in describing the analyzer are identified in Table 1. The analyzer was originally assembled and used at sea on the Hudson for stations 9-16. At that time, the analyzer had a 25-mm OD straight combustion tube of quartz with the injection site at 1000 C; a 14.2-cm long, 18 gauge stainless steel injection needle; and a cold water condenser. Thereafter, it was extensively modified and used at sea on the Dawson for stations 17-21, 23, and 24. The modified version had a 13-mm OD bent combustion tube of quartz with the injection site at 750 C, a

shorter injection needle of 21 gauge stainless steel, and a cold air condenser. After the Dawson cruise, it was slightly modified and used in the laboratory for the rest of the analyses. The differences between the Hudson data and all the rest of the data are considerable; they are discussed later. The differences between the data obtained at sea on the Dawson and the later data are in precision and ease of analysis only. The following description is for the analyzer in its final form. A number of observations made on the development of the analyzer are discussed in Appendix II together with cautions on erratic results from the analyzer. It must be emphasized that the instrument, as it stands, it not readily usable without extensive experience.

Figure 1 is a schematic diagram of the analyzer. Bottled oxygen, as the carrier gas, is purified by passage over 5% platinized asbestos at 1000 C (25-mm OD quartz tube with 30-cm length exposed to the furnace elements) followed by a column filled with Drierite and Ascarite. The carrier gas then goes through the sample valve into the combustion tube. Next, the combustion products pass through the condenser and $Mg(ClO_4)_2$ drying column and into a Beckman IR-215 non-dispersive infrared analyzer (with CO_2-filled detectors and 34.3-cm long sample and reference cells). A flowmeter on the exit of the infrared analyzer allows comparison to the tank flowmeter and monitoring of injections. The flow rate is 225 ml/minute. The Beckman output signal is integrated by an Infotronics CRS-108 digital integrator and recorded on a Honeywell Electronik-194 recorder. The recorder is connected in series to the integrator and is used only for visual checks on peaks.

Figure 2 shows the sample valve. It is a Chromotronix eight-way valve with a two-position switch. In position one, the gas stream is divided so

Figure 1. High temperature combustion carbon analyzer. A-O_2 supply and regulator. B-needle valve. C-tank flowmeter. D-preburner furnace. E-Drierite and Ascarite column. F-sample valve. G-combustion tube and furnace. H-cold air condenser. I-water collector. J-$Mg(ClO_4)_2$ column. K-infrared analyzer. L-analyzer flowmeter. M-integrator. N-recorder.

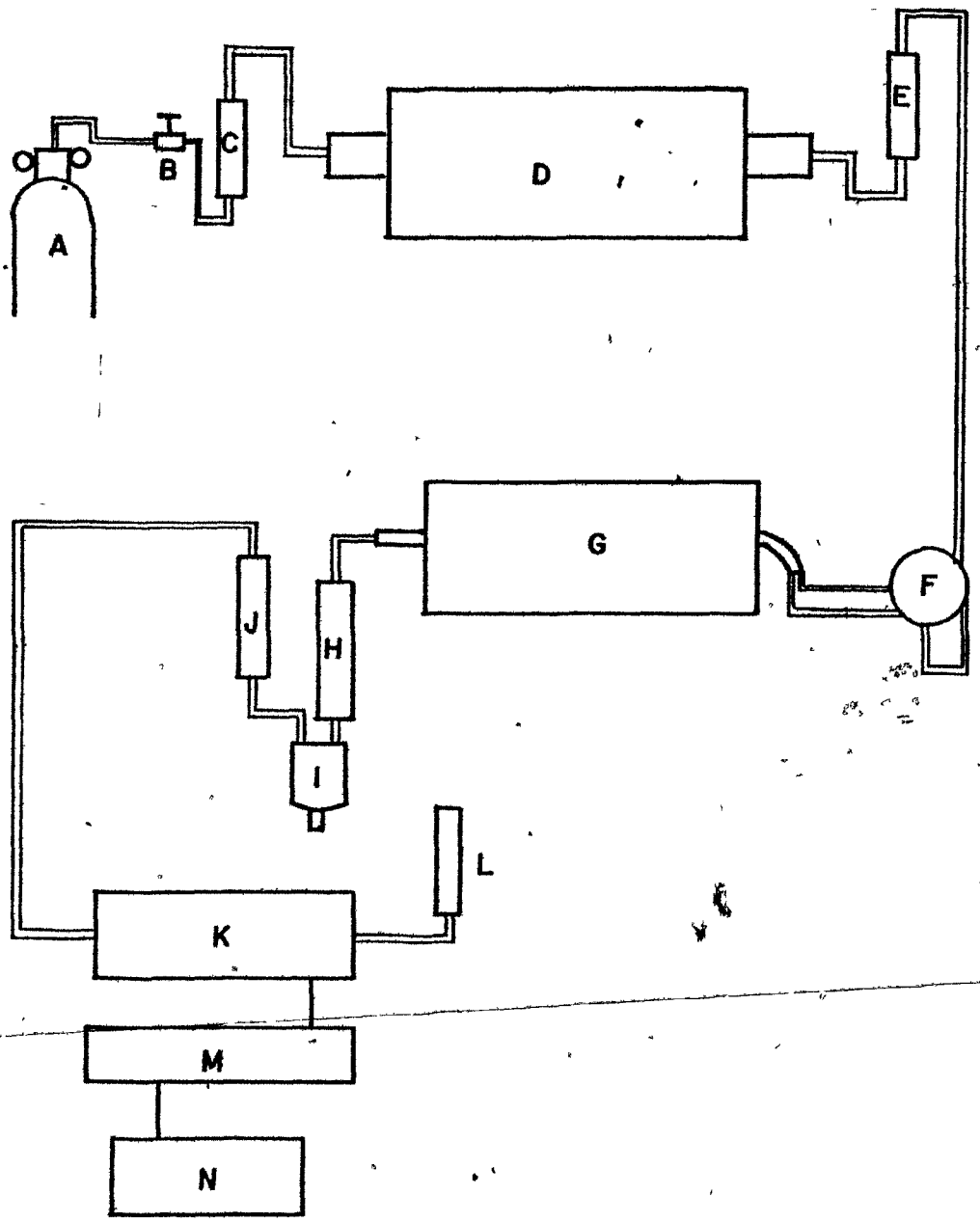
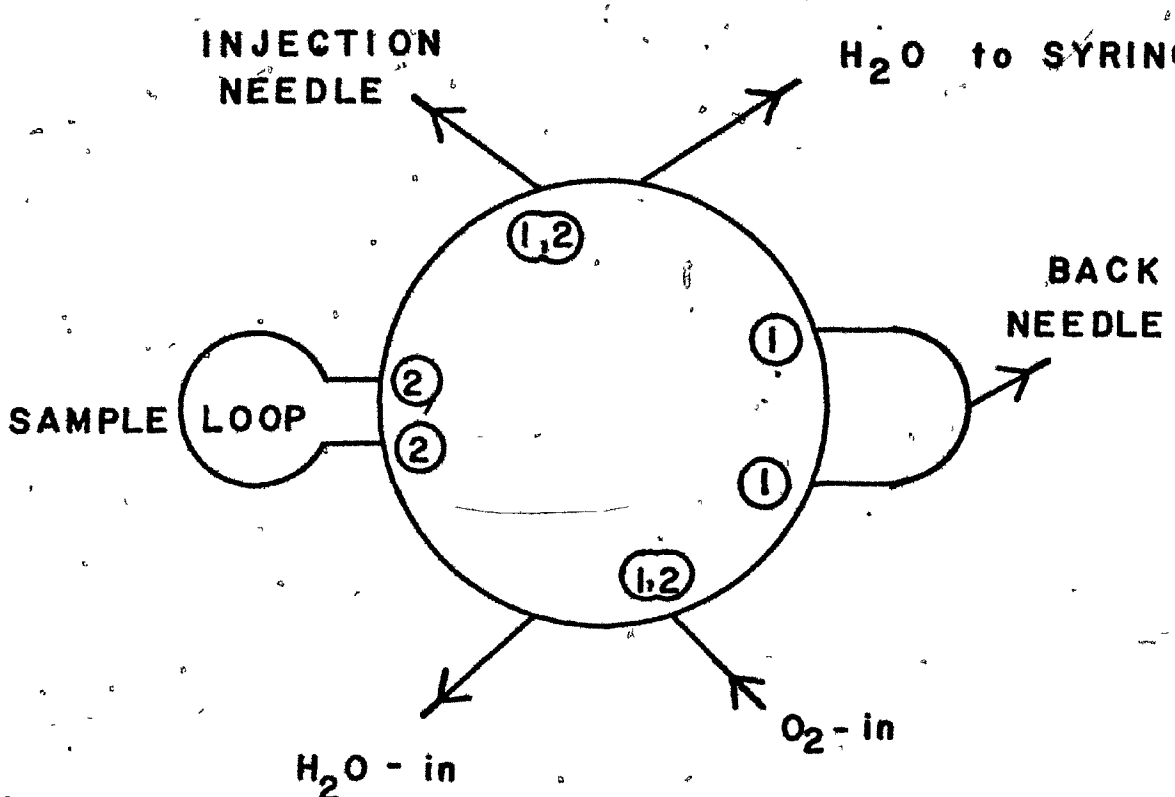

A
B
C
D
E
F
G
H
I
J
K
L
M
N

Figure 2. Sample valve. In position one, the oxygen flows through the ports indicated by "1"; here, the sample loop is open to the "H_2O in" and "H_2O to syringe" ports. In position two, the oxygen flows through the ports indicated by "2".


INJECTION NEEDLE
H2O to SYRINGE
1,2
BACK NEEDLE
1
SAMPLE LOOP
2
2
1
1,2
H2O - in
O2 - in

that it enters the furnace through the injection and backflow needles; it bypasses the sample loop which is open for rinsing and filling (filled by drawing liquid from "H_2O-in" port through the loop with a 2-ml hypodermic syringe at "H_2O-to syringe" port). In position two all the gas flows through the sample loop (thus injecting the sample) and out the injection needle.

The combustion tube is entered by the injection and backflow needles from the sample valve. It is a 13-mm OD X 11-mm ID fused quartz tube with a 30° bend beginning just inside the furnace block. To retard sample flow and to aid in oxidation, the tube is packed with quartz wool and 5% platinized asbestos. The entrance to the tube is fitted with a number 00 silicone rubber stopper carrying the two needles. At the other end, the tube is connected by a 12.7-mm Swagelok union, with teflon ferrules, to the condenser. The tube is placed in a 1000-watt Electro-Applications furnace which was designed so that the 30-cm long heating elements have only a 6.3-mm insulating sheet separating them from the outside at the entrance end and a 19-mm sheet at the exit. The position of the tube in the furnace allows the tip of the injection needle to be in a region of about 750 C while the center of the tube is heated to about 950 C. The temperature of the furnace is controlled by an 8-amp Variac. With the combustion tube thus placed, the silicone stopper is only 15 mm from the heating elements and a thermal buffer was needed to prevent it from burning. The entrance port of the furnace is packed with quartz wool and is shielded with an aluminum foil sheet. Another aluminum shield is placed around the combustion tube at the juncture with the silicone stopper and this one is cooled with a light flow of compressed air. This arrangement allows the tube to be maintained at about 150 C at the stopper while at the tip of the injection needle, it

is at 750 C. The exit of the combustion tube protrudes 7 cm beyond the heating elements so that room air cooling keeps it around 1000 C. The entrance and exit of the combustion tube are kept near 100 C to prevent buildup of water, but are at temperatures low enough to prevent thermal damage to the silicone stopper and teflon ferrules (see Figure 3).

The needle assembly is made up and replaced as a unit. The backflow needle is a 32-mm long, 20 gauge, stainless steel hypodermic needle. The injection needle is made of quartz and has a bore of approximately 21 gauge. To make an injection needle, a quartz capillary is drawn out to the correct bore, is then placed in a length of 2-mm OD X 1-mm ID quartz tubing so that both ends just protrude, and the cold end is secured with epoxy cement. The injection needle is placed through the stopper so that the tip extends 41 mm from it while the backflow needle barely protrudes through the stopper. Figure 4 shows the needle assembly with connections to the sample valve. The injection needle is embedded within the combustion tube in a plug of quartz wool to prevent excessive backflow of liquid. The plug is made up and burned clean before insertion; it sits in the combustion tube so that it is 10 mm from the silicone stopper and has the injection needle extending just beyond its distal end.

At its exit, the combustion tube is connected to the glass tubing of the condenser by a 12.7-mm to 6.3-mm Swagelok reducing union. The condenser tubing has a right angle bend directly past the union from which it goes through a 9.5-mm Swagelok Union-T fitted with a 6.3-mm reducer at the upper end and with 6.5 cm of open-ended 9.5-mm OD stainless steel tubing at the lower end. The side arm of the T is connected to the cold end of a number 106 Vortex tube (with 4L bushing) so that the T becomes a cold air condenser jacket. The Vortex tube is a device that channels incoming air into cyclonic

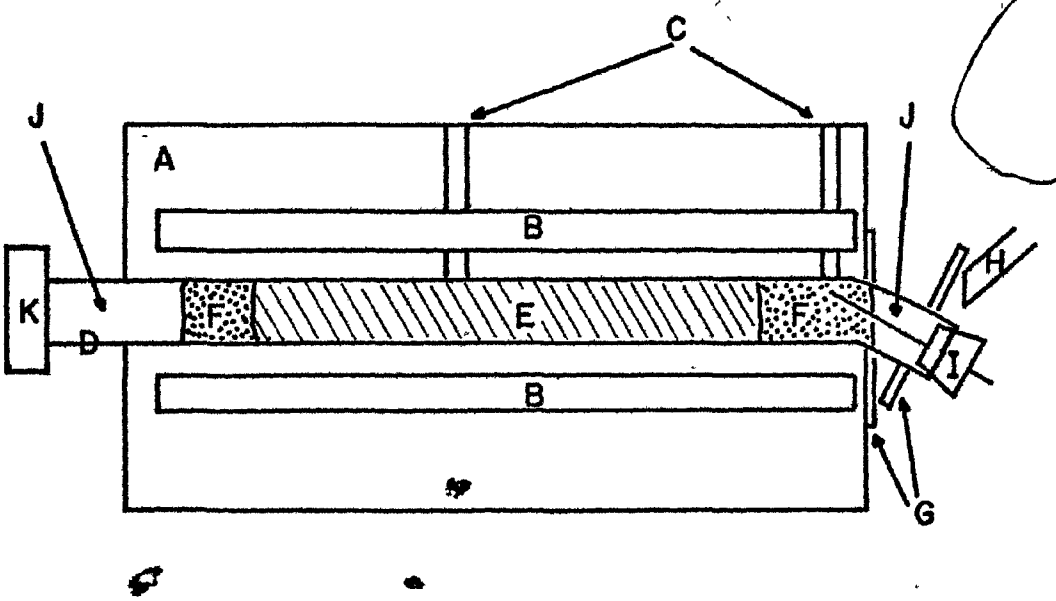


Figure 3. Combustion tube in furnace. A-furnace block, B-heating elements. C-thermocouple ports. D-quartz combustion tube. E-platinized asbestos. F-quartz wool. G-aluminum shields. H-tube for compressed air. I-silicone stopper with injection needle. J-air spaces. K-Swagelok connector with teflon ferrules.

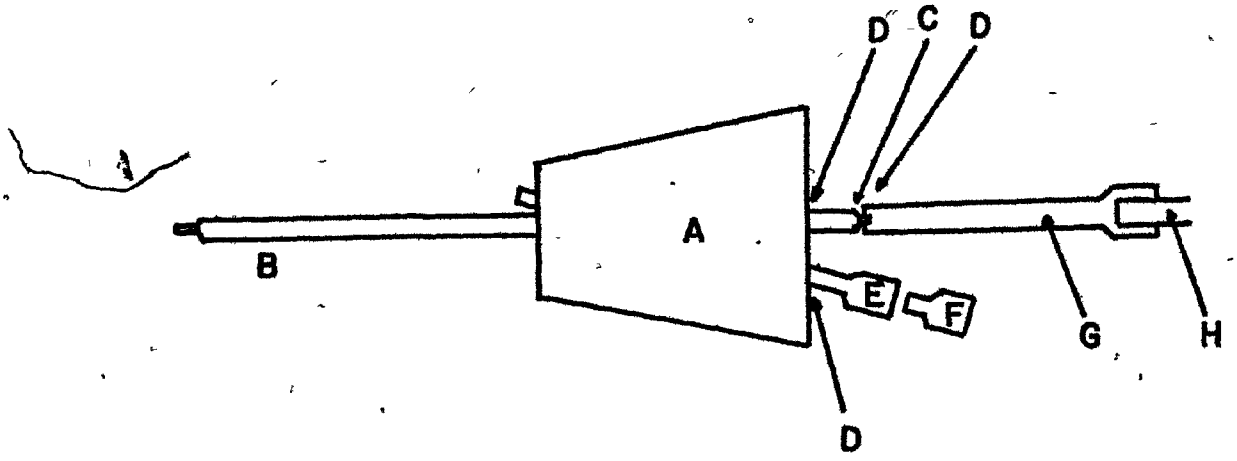


Figure 4. Needle assembly. A-silicone stopper. B-injection needle (2-mm quartz tubing with quartz capillary held within by epoxy at C). D-silicone sealant at these junctures. E-backflow needle with female Luer connection to receive male Luer connection (F) from sample valve. G-1.5-mm OD by 0.5-mm ID tygon tubing connected to teflon tubing (H) from sample valve.

and anticyclonic cones at very high speeds (up to 10^6 cycles per second) which produces hot and cold air outflows. Depending upon pressure and volume of input, outflowing air can be raised or lowered by several hundred degrees centigrade. As used here, with laboratory compressed air, it lowered the temperature at the cold end to about 12 C. Beyond the condenser jacket, the glass tubing is connected to a water-collector which consists of a 15-ml coarse-frit glass funnel that is closed below the frit by a pinch clamp on a tygon tubing extension (tygon secured to the funnel with silicone sealant). The top of the water-collector is an inverted cut-off bottom of a 20-ml beaker with two holes drilled in it for the glass tubing (the collector top and glass tubing are secured with epoxy cement). The condenser and water-collector are illustrated in Figure 5.

Procedure

For each run on the carbon analyzer, the $Mg(ClO_4)_2$ in the drying column was changed. If 10-20 samples had been previously done, the salt was removed from the combustion tube. This was accomplished by removing the quartz wool plug with forceps, scraping out the combination of disintegrated quartz wool and salt that built up in frontof the plug, putting in fresh quartz wool and reinserting or replacing the quartz wool plug. An hour or two was required to burn off organic contaminants from the new quartz wool packing. Usually 5-10 injections of a standard or sample were required to stabilize the analyzer at the beginning of the run. Samples were run in random order with standards staggered among them.

The sample loop, in position one, was rinsed by pulling through enough sample to remove air bubbles; thus filled, it was injected by pushing the switch to position two. The switch was left in position two until the sample

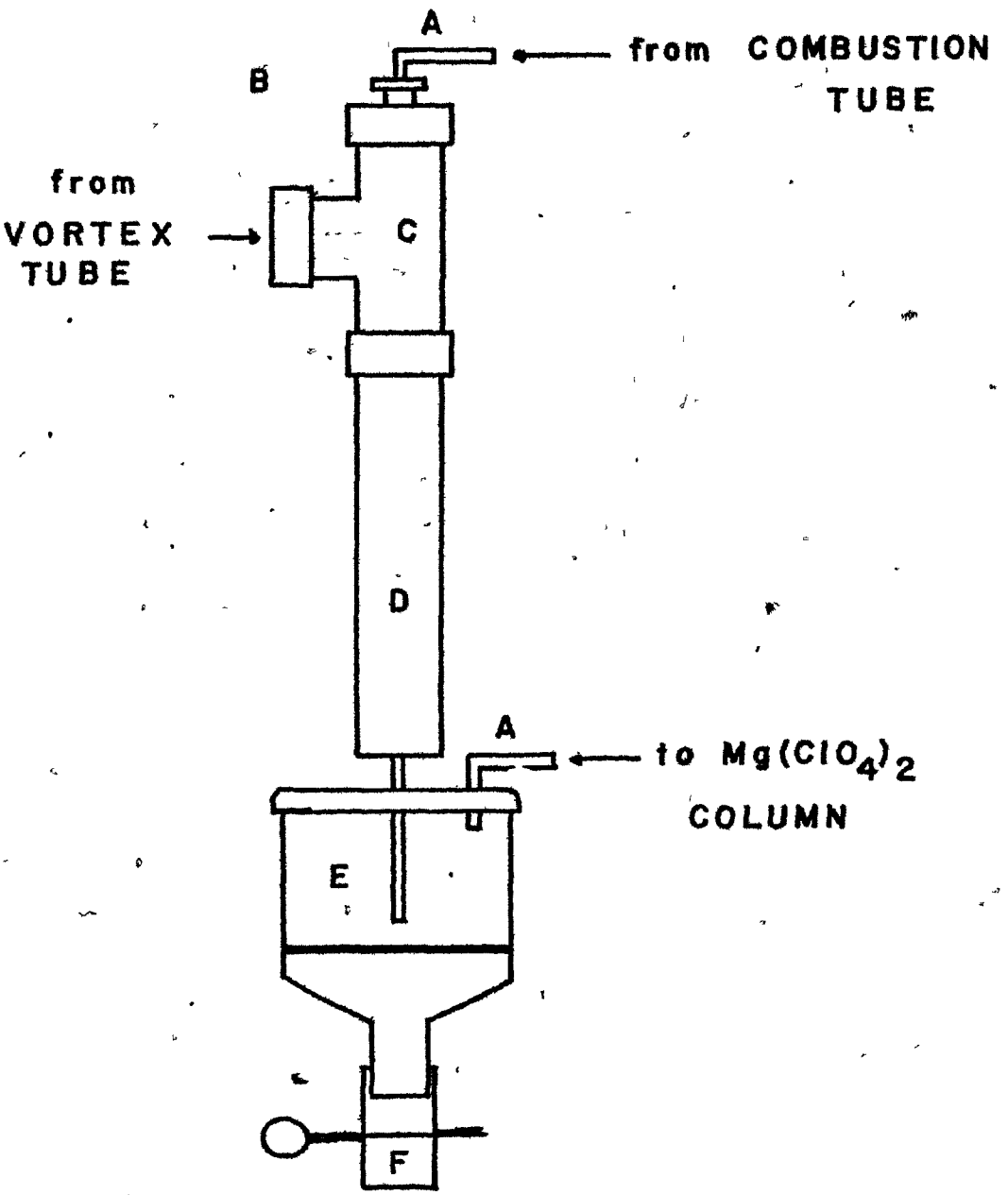


Figure 5. Condenser. A-6-mm pyrex tubing. B-Swagelok reducer. C-Swagelok Union-T. D-9.5-mm stainless steel tubing. E-water-collector. F-tygon tubing with pinch clamp for draining.

had visibly left the tygon tubing and the analyzer flowmeter began to return to normal. Upon injection, this flowmeter showed a sudden large rise, followed by a slow drop to zero and then a slow recovery to its original reading. The sample was injected within 10-20 seconds and the switch was then returned to position one. If the switch were left in position two, it allowed CO_2 accumulation behind the injection needle and a long tailing of the peak occurred. When the sample hit the hot quartz wool, back pressure caused some water to condense on the combustion tube between the quartz plug and the silicone stopper; this water re-evaporated when the switch was returned to position one. The CO_2 was recorded as a Gaussian peak in the period from about 45 to 150 seconds after the start of the injection. As soon as the recorder pen returned to the baseline, the next injection was done. In this way four injections could be run in about 10 minutes. At the end of running the replicates of one sample, the water-collector was flushed by opening the pinch clamp, the syringe was flushed through the sample loop, and the next sample was drawn into the syringe and started.

The Persulfate Oxidation Method

The procedure used was that of Strickland and Parsons (1968) with the following modifications: The $K_2S_2O_8$ was not recrystallized. Seawater samples of 30 ml were acidified with conc H_3PO_4 in the sample bottles and were bubbled there for 5 minutes (see sample preparation) before 5-ml aliquots were measured out into the prepared ampoules; the ampoules were purged for about 30 seconds before sealing (this is instead of 5-ml samples samples being acidified with 0.25 ml of 3% acid and bubbled in the ampoules in the presence of the $K_2S_2O_8$ for 5 minutes). The sample bottles were prebaked (applied to glassware, this term means heated to 550° C to burn off organic contaminants) directly before use, as were the ampoules. The $K_2S_2O_8$ was put in the ampoules before the seawater was added. Immediately

after seawater addition, the ampoules were briefly purged and then were sealed with a propane torch. The sealed ampoules were autoclaved for one hour within a few hours of sealing. In CO_2 analysis, oxygen was used as the carrier gas; the silicone tubing was connected directly to the tygon tubing; and no KI gas scrubber was used as Cl_2 removal was not necessary for infrared analysis nor for protection of the sample cell. The analyzer exhaust was vented outside the laboratory. The same infrared analyzer, integrator, and recorder as used for the combustion method were used for the persulfate oxidation method.

Strickland and Parsons indicate a number of precautions necessary for using the persulfate oxidation method; a few additional notes are added here. The cannula used in the CO_2 analysis must be replaced after 150-200 ampoules are run since it develops holes from persulfate corrosion and begins to leak. The silicone and tygon tubing must be replaced after about 50 ampoules due to leaks from glass cuts. Carrier gas leaks result in decreased flow rate and increased peak areas and they are easily detected because of the pronounceddifferences in peaks and the odor of Cl_2. It appears that some loss of organic matter can be caused by extended bubbling of the sample in the presence of the $K_2S_2O_8$, so acidifying and bubbling of seawater before addition of the persulfate seems advisable (this is discussed further below).

Comparison of the Two Methods

Sample Preparation

Information about sample collection is found on page 3 while that about preparation of colloidal fractions is on page 46 and the explanation of the algal culture samples is on page 80. In all cases, a single sample was

used for analyses by the two methods and replicate determinations of each sample were made by both methods. Thus the comparison is direct and can be well defined in terms of statistical errors.

The Hudson samples were unfiltered; the Dawson ones were either filtered of unfiltered as indicated in Appendix IV; The Sackville and algal culture samples were filtered through 0.8-μm pore silver filters. For analysis, a prebaked sample bottle was filled with about 35 ml of sample. The sample bottles were 30-ml reagent bottles with inverted T ground glass caps for which the caps had 5-mm diameter holes drilled near the top so that the bottles were sealed when the caps were on tightly but had openings when the caps were slightly lifted. A sample was immediately acidified with 0.05 ml of H_3PO_4 and was bubbled to remove CO_2. For the bubbling, a bent 21 gauge cannula was inserted through the hole in the cap down to the bottom of the bottle so that the bottle remained capped during the bubbling. Bubbling was for 5 minutes with a flow of 100-200 ml/minute of nitrogen purified by passage through charcoal, Ascarite, and a 14-μm Millipore filter. From the acidified and bubbled sample, three 5-ml aliquots were measured out as replicates for persulfate oxidation and combustion analysis was done directly on some of the remainder from the sample bottle, or a 5-ml aliquot was transferred to an ampoule and frozen for later analysis. For the latter, 5-ml aliquots were put in prebaked ampoules, purged for about 30 seconds, sealed, and immediately frozen.

Standards were treated in exactly the same way as samples both in the laboratory and at sea. Hence, aliquots of individual samples were standardized against aliquots of individual standards by the two methods.

The Hudson samples and the Dawson stations 17-21, 23, and 24 samples had combustion determinations done immediately at sea; the Dawson stations 22,

25, and 26 samples and all the Sackville samples had combustion determinations done in the laboratory on frozen aliquots. The combustion determinations of the algal culture samples were done immediately upon collection. The persulfate oxidation aliquots for Hudson samples were run in several groups at sea; all others were run as unit groups (all Dawson, all Sackville, and all algal culture) in the laboratory at the end of their respective collection periods.

Standardization of the two methods is very critical if accurate results are to be obtained and this subject is thoroughly discussed in Appendix III.

Results and Discussion

All the data on total organic carbon are listed in a single table as Appendix IV. For the combustion determinations, four replicates of each sample were run to obtain the mean values. Three aliquots were taken from each sample for the persulfate oxidation determinations, but occasionally one aliquot was lost in preparation and often, in analysis, one or two appeared to be anomalous. This method differs from the combustion one in that it is not possible to repeat a lost or anomalous aliquot. So an arrangement was devised for discarding disparate values: The error of the three replicates was calculated and if it exceeded 10%, the most divergent value was rejected and a new error was calculated for the two; if this were still in excess of 10%, the median value of the three was used and an error of 10% assigned to it. This arbitrary arrangement is necessitated by the fact that extreme values often occur from contamination to or leakage from the ampoules. In the analyses of 173 samples; 2 were lost completely; 18 were based upon only one aliquot; 52, upon two aliquots; and the remainder, 101, upon all three aliquots. Barber (1967) used median values for all

his data instead of means; as the data here indicate, that is rather extreme data rendering.

Since the method, at that time, was not very satisfactory and since the values appear to be high, the Hudson combustion data are viewed as questionable. They are listed for possible future interest, but are not included in the interpretation of the two methods. The algal culture data are not from samples of natural seawater since the culture media are artifically enriched, so they are also excluded from the discussion that follows immediately. The Dawson and Sackville data allow 102 direct comparisons of the two methods for samples of natural seawater. The Dawson data are especially good for assessing the methods, for the filtrates must be equal to or lower than the original unfiltered samples. Values of filtrates that are higher (outside statistical limits) than the raw values indicate that something is amiss. Three of the 64 combustion samples and five of the 63 persulfate oxidation ones can thus be considered as too high (these are indicated in Appendix IV). Of the high values by persulfate oxidation, 4 of the 5 are from samples with fewer than three replicates and might be considered as inadequately replicated. All three of the high values by combustion analysis are from frozen aliquots and might be suspected of having excessive handling. These observations emphasize the advantage of three or more replicates and the necessity for limiting handling of the samples. Having no reason nor means to suspect any of the Sackville data, all are kept, but it is reasonable to expect occasional undetected erroneous values.

Strickland and Parsons (1968) give a precision for the persulfate oxidation method of $\pm 0.06/\sqrt{n}$ mg C/L which is based upon a number of carefully run replicates of a single sample and really represents the

the potential precision of the method rather than the actual precision of running samples. The average error for the determinations by the persulfate oxidation method of the Dawson and Sackville samples is ±5.5%. By the calculation of Strickland and Parsons, the error of these data would be ±3.9% (based upon 102 averages in which n=2.54 and $\bar{X}$=0.96 mg C/L). It can be seen that the actual precision of samples when three replicates were run is not as good as the potential precision of the method. However, it is close enough to indicate that the method can approximate the potential when run carefully.

Table 4 lists averaged analytical precisions for the two methods; the Dawson samples for the combustion data are divided because the analyzer was improved for the second group of stations. The combustion method (excluding that first group of Dawson stations) is slightly more precise than the persulfate oxidation one. Also the combustion method becomes even more precise with the higher carbon contents encountered in the algal culture work and this improvement seems not as marked a feature of the persulfate oxidation method.

Table 5 lists differences in total organic carbon by the two methods. The differences between the two methods for all depths, for shallow, and for deep are significant at the 99.9% confidence level thus establishing that the differences between combustion and persulfate oxidation determinations are real. The combustion method should measure all the organic carbon that is present, so the persulfate oxidation method must fail to measure all of it. The differences seem to indicate that there is organic matter in seawater that is resistant to persulfate oxidation. From Table 5, the difference between the two methods appears greater in deep water than in shallow, but it is not significant.

Table 4. Analytical precision. Percent relative standard error of the mean averaged for blocks of analyses by the two methods. Number in parentheses is n for that block.

SAMPLES		PERSULFATE	COMBUSTION
Dawson	(Stations 17-21, 23, and 24)	5.42 (63)	7.83 (39)
	(Stations 22, 25 and 26)		3.75 (25)
Sackville	(Stations 28-32)	5.77 (48)	4.34 (48)
Algal culture	(<10 mg C/L)	4.19 (18)	3.13 (18)
	(>10 mg C/L)	4.36 (23)	1.18 (23)

Table 5. Comparison of organic carbon determinations. Total organic carbon from the Central Western North Atlantic Ocean by both methods. Percent is persulfate value divided by combustion value times 100. All differences are significant at the 99.9% confidence level.

DEPTH	n	mg C/L			PERCENT
		PERSULFATE	COMBUSTION	DIFFERENCE	
All depths	68	0.94	1.21	0.27	78
0-100 meters	27	1.16	1.36	0.21	85
>100 meters	41	0.80	1.11	0.30	72

Figure 6 a and b are composite depth plots for the central western North Atlantic Ocean of the total organic carbon as measured respectively by persulfate oxidation and by combustion (aliquots of the same samples were used for the two methods). Though these figures show the combustion values to be more variable than the persulfate oxidation ones in deep water samples, no clear trend is seen. To get a picture of the depth trend, composite depth curves are constructed. For the curves, all values from the same depths are averaged, for depths must be combined to get large enough groups for statistical analysis. This procedure of averaging samples in depth groups is based upon the premise that horizontal variability is minor compared to vertical variability. It is not necessarily a correct premise and the averaging is used only to look for a trend and not to establish verities in depth profiles. Table 6 gives the total organic carbon data in the depth groups; Figure 7 shows the depth curves. From this grouping, it can be seen that the difference between the two methods of carbon analysis is most apparent in a region below the surface layer and above the very deep water. Further discussion of the depth curves follows later and it is sufficient here to note that some of the organic matter resistant to persulfate oxidation can be explained as being from subsurface waters (and, hence, fairly old).

As explained below, two classes of colloidal organic matter can be calculated for the Dawson data and they are shown in Table 7. Both colloidal classes are significant by combustion analysis at the 95 % level; neither is significant by persulfate oxidation; however, the 0.003-μm class would be significant at the 90% level. Thus some of the persulfate resistant matter seems to be in colloidal form.

Figure 6. Total organic carbon determinations. Composite depth profiles of all samples from the central western North Atlantic Ocean. Bars in the upper left-hand corners represent average analytical errors ($2\sigma_{\bar{x}}$) for the determinations.

Figure 6a. Persulfate oxidation analyses.

Figure 6b. Combustion analyses.

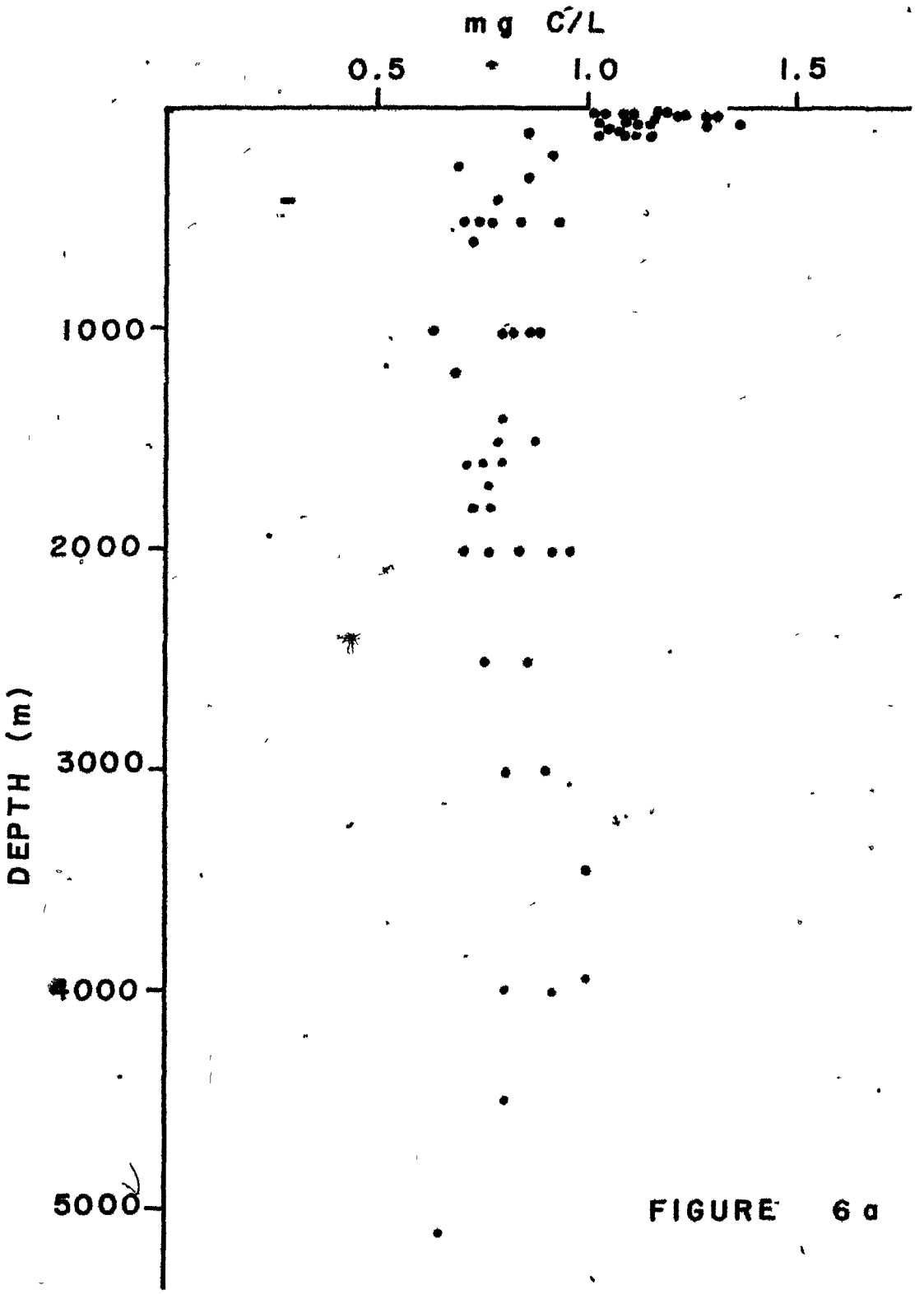


FIGURE 6a

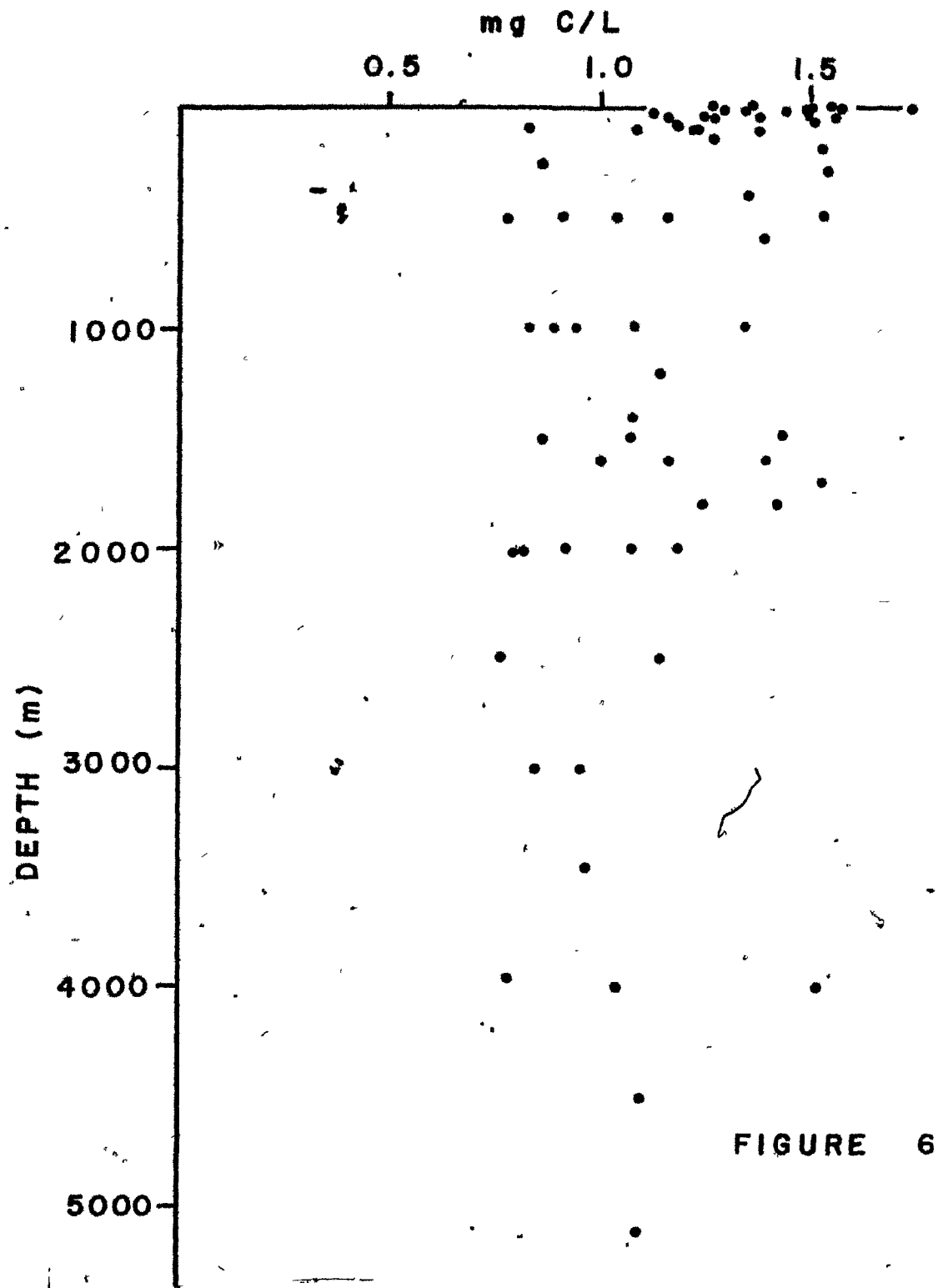


FIGURE 6b

Table 6. Total organic carbon grouped by depth. The data are the same as used for Table 5, as is the table format. Depths are in meters. Standard deviations ($\sigma_{\bar{x}}$) are listed for the combustion data for statistical analysis.

			mg C/L			
DEPTH (Average)		n	PERSULFATE	COMBUSTION	$\sigma_{\bar{x}}$	DIFFERENCE
0-25	(10)	13	1.17	1.46	0.05	0.29
50-100	(75)	12	1.10	1.26	0.06	0.16
150-400	(260)	5	0.81	1.31	0.11	0.50
500-600	(510)	6	0.78	1.14	0.10	0.36
1000-1200	(1010)	6	0.77	1.04	0.06	0.27
1400-1800	(1600)	10	0.77	1.22	0.07	0.45
2000-2500	(2140)	7	0.82	0.95	0.07	0.13
3000-5100	(3880)	8	0.85	1.03	0.08	0.18
75 & 510 groups		18		1.22	0.05	
1010 & 2140 groups		13		0.99	0.05	

Figure 7. Total organic carbon in the central Western North Atlantic Ocean. Depth curves are from averaged values shown in Figures 6 a and b. Open circles are from persulfate oxidation determinations; closed circles are from combustion determinations.

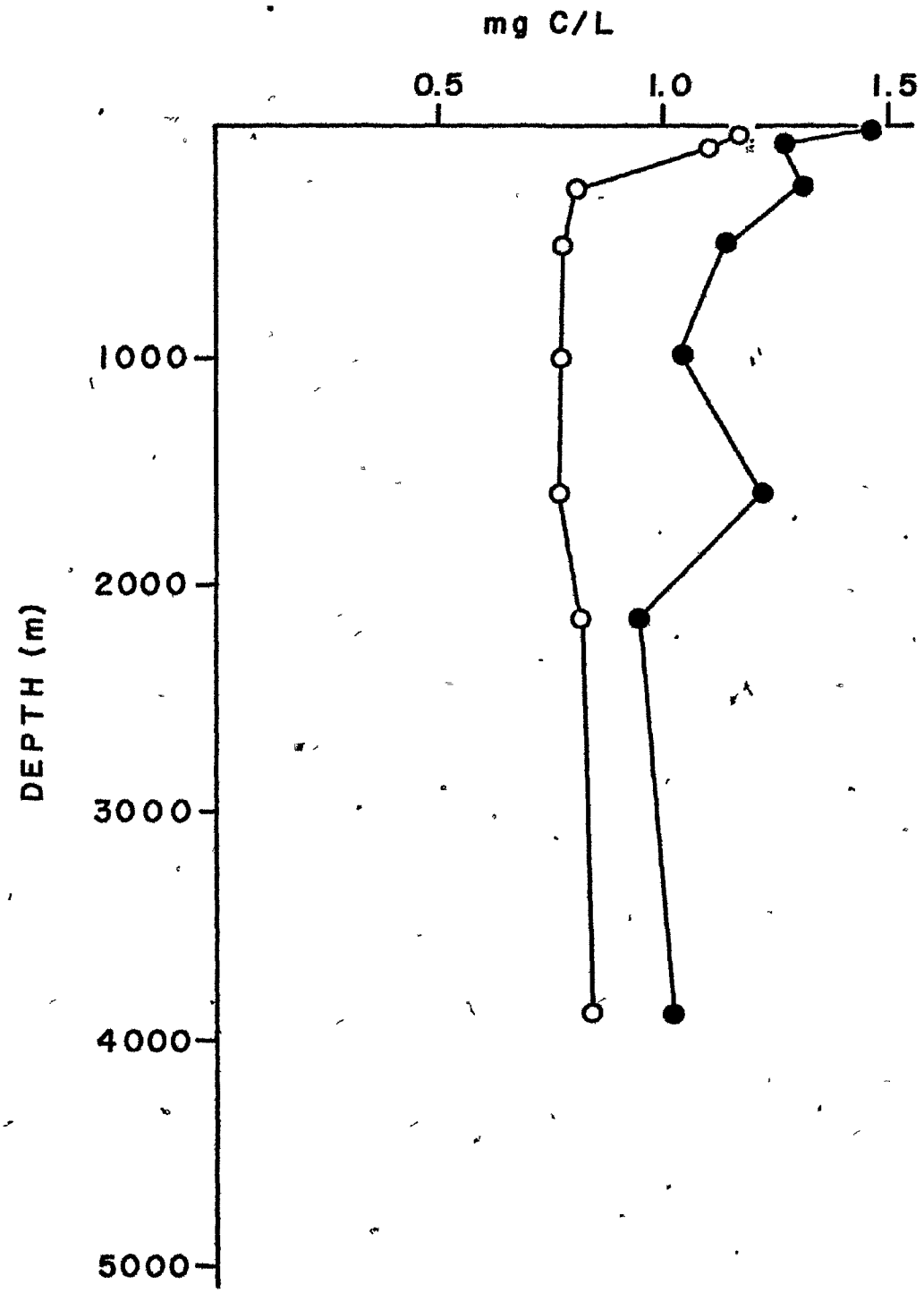

mg C/L
0.5
1.0
1.5
1000
2000
3000
4000
5000
DEPTH (m)

Table 7. Colloidal classes by the two methods for organic carbon analysis. Samples are Dawson cruise stations and depths (in meters). See text for explanation of collidal classes. Values are listed as mg C/L. t-tests of the differences of the mean from zero are shown by probabilities (p).

SAMPLE	GREATER THAN 0.025 μm		GREATER THAN 0.003 μm	
	Combustion	Persulfate	Combustion	Persulfate
17-Sfc	.07	.15	.44	---
20-Sfc	.11	-.06	.20	.03
26-Sfc	.02	-.05	.19	.03
19-5	.04	-.02	0	.07
22-5	---	.07	.17	.04
23-5	.08	---	-.03	---
25-10	.23	---	.17	-.04
24-15	.09	-.08	-.03	.04
23-25	.12	---	.28	.11
23-50	.08	---	.14	.05
23-75	.05	.01	.25	-.02
24-100	.13	---	.25	-.03
25-100	.21	---	---	---
19-150	.24	---	.29	---
22-250	-.04	-.01	-.01	-.02
24-500	.21	-.02	.02	-.01
22-1000	---	0	.21	---
25-1600	.15	-.01	.20	.13
21-2000	.12	-.03	.17	-.05
18-4000	.10	-.05	.25	.02
25-5100	.13	0	---	.03
MEAN	.11	-.01	.17	.02
p	<.001	.7>p>.5	<.001	.1>p>.05

As mentioned above, the algal cultures do not represent natural seawater for the carbon levels are considerably higher and the organic compounds probably are quite different from those ofnatural seawater. Comparing the two methods for samples from old cultures (carbon levels exceeding 30 mg/L), the combustion method measures 17% less than persulfate oxidation and this is a significant difference. It appears that the combustion method is inadequate for such high carbon levels and probably the inadequacy is due to incomplete conversion of the carbon to CO_2 (from CO) rather than being due to resistant organic compounds. Considering only the data under 30 mg C/L, the combustion method still measures significantly less thanpersulfate oxidation but now only 6%. If only samples with less than 5 mg C/l are considered, there is no significant difference between the two methods. Hence, maybe all the algal culture combustion data show inadequate estimates of the carbon present due to an oxidation period that is too short for the high carbon levels encountered. In the section on algal culture experiments, the data for the samples of greater than 30 mg C/L are not used; the combustion data for the other samples are used and they are not enough different from the persulfate oxidation data to give a misleading general picture of the extracellular production.

The conclusion from comparing the two methods for analysis of total organic carbon in seawater is that the persulfate oxidation method underestimates the value (by an average of 22% in the central western North Atlantic Ocean). The underestimation is due to incomplete oxidation of resistant organic matter, some of which seems to be in old subsurface water and in colloidal form. In retrospect, the works of both P.J. LeB. Williams (1969) and P.M. Williams (1969) lend support to evidence of incomplete oxidation by persulfate oxidation.

Looking now at the distribution of total organic carbon in the sea, reference is made again to Figures 6 and 7. The traditional view of carbon distribution (Menzel and Ryther, 1970) shows total organic carbon below the 100-200 m surface level to be a fairly straight vertical line. This view is based upon averaged data and the averaged curve for persulfate oxidation data from the present work is also a fairly straight line (Figure 7). However, Figure 6a illustrates how the averaging hides the variability of the data and Figure 6b shows that the same samples by combustion analysis are even more variable. Indeed, by combustion analysis, the deep water samples are no less variable than are the near surface ones. Hence, it is not reasonable to consider total organic carbon as a constant for deep water. The averaged curve for combustion analysis in Figure 7 shows a general trend of gradual decrease all the way down to 2000 meters. Superimposed upon this general trend are two pronounced peaks. The significance of these peaks can be tested by contrasting them with combined averages of groups immediately above and below them (see Table 6). Thus the peak at 260 m is not significant while that at 1600 m is significant at the 95% confidence level. Excessive stratification of the central western North Atlantic Ocean by water mass intrusion is not seen from other chemical observations (Riley, 1951 and 1970), so that vertical transport and biological activity would seem to be involved. The gradual decrease with depth suggests that biological activity is occurring and that all the water below 100 meters is not a biological wasteland. The peak at 1600 m is somewhat puzzling; it is below thearea in the transition zone of relatively high biological activity. Possibly increases in total organic carbon occur below areas of high biological activity. More data are needed before an explanation can be assigned to this deep water peak because not enough is known about deep water

horizontal variability to assess the ecological significance of the peak.

Although the averaged depth curve by persulfate oxidation is [redacted] in shape to those reported in the literature, it is higher in value than averaged curves from the same area (Menzel, 1967). Menzel's curves for the Sargasso Sea and Caribbean have deep water averages of 0.5 to 0.6 mg C/L while that reported here has an average of about 0.8 mg C/L. Although a direct comparison cannot be made on the data, an explanation is proposed for the apparent difference in the curves. The usual procedure in persulfate oxidation analysis is to add the $K_2S_2O_8$ and H_3PO_4 to the sample in the ampoule and then purge with N_2 for about 5 minutes. In the present work, the sample was acidified and bubbled before the $K_2S_2O_8$ was added, and then it was flushed quickly (for only about 20-30 seconds) with N_2 before sealing. When the sealed ampoule is autoclaved, the heat enhances solution of $K_2S_2O_8$ and its oxidation of organic compounds. However, both solution and oxidation will proceed at room temperature and bubbling the sample for 5 minutes in the presence of the persulfate probably causes some of the organic carbon to be oxidized and lost as CO_2 before the ampoule is sealed. For samples below 100 m, direct comparison here shows that persulfate oxidation (as run here) missed an average of 22% of the total organic carbon. By indirect comparison of samples from the same area, persulfate oxidation (by the usual procedure, Menzel, 1967) apparently missed 50% of the total organic carbon. An experimental check should be made to test the persulfate oxidation method for this possible procedural error and comparisons of the two methods for other waters should be made. However, it does appear that that previous estimates of the total organic carbon in the sea are too low and that there is anywhere from one and one-half to two times as much there as is presently thought.

COLLOIDAL ORGANIC CARBON

Colloidal organic carbon was defined in the Introduction as that passing an 0.8-μm filter but retained by a filter of a smaller pore size. Colloidal carbon was determined on the Dawson cruise by using 25-nm Millipore filters and 50,000 MW Diaflo membranes. Actually, the Diaflo membrane does not act as a sieve as the Millipore filter does, but is a molecular diffusion barrier that can be assigned an effective pore size. Both these filtration methods retain matter that is not particulate (in excess of that retained by an0.8-μm filter) but that is probably not in solution. The 47-mm diameter Millipore filter was pretreated by boiling breifly in doubly distilled water, similar to the treatment used by Nakajima and Nishizawa (1968). Then it was placed in a Sterifil filter holder, rinsed with 100 ml doubly distilled water, similarly rinsed with 100 ml of sample, and then the sample was collected in a sample bottle (placed within the Sterifil holder so that the bottle was filled directly). The rinsing and collecting was done using vacuum filtration with a gauge pressure of -0.5 kg/cm^2. The Diaflo sample cell, with 76-mm diameter membrane in place, had 100 ml of doubly distilled water rinsed through, followed by 100 ml of sample, and then the sample was collected directly in a sample bottle. The rinsing and collecting was done using forced dialysis with a compressed nitrogen gauge pressure of 5 kg/cm^2.

The Dawson cruise data allow calculation of several size classes of organic carbon. Nominally the 25-nm filter should retain matter larger than 0.025 μm and the 50,000-MW membrane should retain that larger than 0.003 μm. If the retention behaviors are similar to those of the filters measured by Sheldon and Sutcliffe (1969), the nominal pore sizes are

reasonably close estimates of the actual average pore sizes. The two classes of colloidal matter can be calculated by subtracting the values for the filtrates from those of raw seawater and then subtracting the values for particulate carbon from these. Table 7 listed values for these two classes (without subtracting the particulate). Table 8 lists the three classes of retention (particulate and two colloidal) as cumulative percentage groups. The dashed spaces represent lost values (eliminated in Appendix IV) or calculated values that were negligible. The classes of retained matter were separately determined, but should cumulatively increase, going from 0.8 to 0.003-μm. In only one case is there failure for the three to show the cumulative increase (sample 25-10). The averages for all depths and for shallow and deep grouped depths are shown at the bottom of the table. As was previously illustrated, the amounts of total and particulate organic carbon are lower in deep water than in shallow. From the table, the two classes of colloidal carbon are shown to be higher in deep water than in shallow. Due to the limited data and appreciable scatter, none of these depth differences can be shown to be statistically significant, but they are suggestive. The increase with depth of colloidal carbon seems somewhat surprising at first, but is confirmed by observations from the total organic carbon section. It was found that the two classes of colloidal matter were not as prominent when measured by persulfate oxidation as by combustion and that the curve by combustion analysis of total organic carbon did not decrease in mid-water as precipitously as did the one by persulfate oxidation.

A second observation from Table 8 is that an average of 82% of the total organic carbon is smaller than 0.003 μm and 90% is smaller than 0.025 μm. Ogura (1970) gave data that was somewhat complementary to this, though

Table 8. Classes of retention of organic carbon. Samples are Dawson cruise stations and depths (in meters). Particulate organic carbon (0.8-μm retained material) and colloidal organic carbon (0.025-μm and 0.003-μm filtrates subtracted from the total organic carbon) classes are listed as percentage of the total organic carbon.

SAMPLE	mg C/L Total	PERCENT OF TOTAL		
		0.8-µm	0.025-µm	0.003-µm
17-Sfc	1.55	2.39	4.52	28.39
20-Sfc	1.27	2.36	8.66	15.75
26-Sfc	1.36	2.06	----	13.97
19-5	1.29	2.33	3.11	----
22-5	1.57	2.80	----	10.83
23-5	1.74	1.95	4.69	----
25-10	1.74	2.36	13.22	9.77
24-15	1.44	2.43	6.25	---
23-25	1.49	3.09	8.06	18.79
23-50	1.56	2.37	5.13	8.97
23-75	1.51	2.19	3.32	16.56
24-100	1.09	1.93	11.93	22.94
25-100	1.38	2.25	15.22	---
19-150	1.27	2.20	18.89	22.83
22-250	0.86	1.63	---	---
24-500	0.78	2.82	26.92	---
22-1000	0.89	1.01	---	23.60
25-1600	1.16	1.64	12.93	17.24
21-2000	0.81	1.73	14.82	20.99
18-4000	1.51	0.33	6.62	16.56
25-5100	1.08	1.20	12.03	---
AVERAGE	1.30	2.05	10.37	17.66
0-100 m	1.46	2.35	7.65	16.22
>100 m	1.04	1.57	15.37	20.24

his was based upon analysis by persulfate oxidation. He found 7% difference between 0.45 and 0.10-μm retention of organic carbon. A similar number from the present data would be 8% difference between 0.8- and 0.025-μm. Looking at retention of C^{14} labeled organic compounds, Jeffrey and Hood (1958) found about 6% difference between 0.45 and 0.01-μm. These two works support the finding here that some quantity, and a variable one, of organic carbon exists that can be classified as colloidal. As is discussed below, there is no reason to postulate that the filters used for retention of particulate matter, or the filters used above, adsorb dissolved organic matter. This is also partially confirmed by the fact that smaller effective pore sizes retain more organic carbon (as shown by observations here and by Ogura's data). Hence, the retention is probably of tiny colloidal particles. Although only about one percent of the total organic carbon in oceanic seawater was shown to be particulate, it is seen here that a comparatively large portion of the rest of it is not necessarily dissolved.

EXPERIMENTS ON PARTICLE FORMATION

I think that we shall always see
Organic particles from the sea
Formed without provocation
Almost like spontaneous generation.
"Ode to CRAP" - Sharp, unpbl.

PREFACE

Having described methods of carbon analysis and discussed distributions of total, particulate, and colloidal organic carbon in the central western North Atlantic Ocean, experiments on particle formation are now considered.

In most of the experiments that have been published, filtered seawater samples were bubbled with compressed air for relatively short periods and were then refiltered to collect the particulate matter that was formed. Wangersky (1965); Sheldon, Evelyn, and Parsons (1967); and Riley (1970) also noted particle formation in filtrates that had not been bubbled. In the bubbling experiments, particle formation has been attributed to coalescence of organic matter on the surfaces of bubbles; MacIntyre (1965) has examined this mechanism. Particle formation in undisturbed filtrates is at least partially due to bacterial activity. The existence of "filter-passing" bacteria has been known for some time (Anderson and Heffernan, 1965). Riley (1970) and Sheldon (personal communication), in their experiments, have noted that bacteria in the filtrates were larger than the nominal pore size of the filters that they employed. Sheldon and Sutcliffe (1969) have found that very little material will pass through Millipore or Selas filters that is larger than the nominal pore size of

the filters. Thus, bacteria in the filtrates probably get through the filters in some smaller form and then attain larger size. Weibull (1965) has reported bacillus L-forms that were on the order of 0.1 μm (smaller than the nominal pore sizes employed by the above workers). These L-forms were viable and were able to grow into cell-wall bearing bacilli. It is not known whether there are L-forms or some other pleomorphic forms in seawater that could pass the filters. However, it does appear that in the essentially abiotic environment of the filtrates, a few bacteria could grow and multiply rapidly. Probably these "filter-passing" bacteria are responsible for part of the particle yield in long term experiments on particle formation. Although it is difficult to separate biological and physical contributions in these experiments, it would seem that in relatively short bubbling periods, physical phenomena would be the main mechanisms for partiele formation.

EXPERIMENTAL METHODS

In an attempt to get maximum consistency and minimum contamination in experiments on particle formation, a routine procedure was established. This procedure was followed for experiments done in the laboratory and at sea. Figure 8 (parts A-D) illustrates the apparatus and the procedure which is described below. Six identical setups were used, each consisting of two 1500-ml Florence flasks which were connected together and were closed off from the outside by filters. The flasks were joined together and the filter holders were connected to them with 6.4-mm OD silicone rubber tubing. The flask stoppers were also of silicone rubber; they were fitted with three lengths of pyrex tubing as entry and exit ports to the flasks. Swinnex-25

Figure 8. Apparatus for experiments on particle formation.

Figure 8A. Vending and receiving flasks assembled in preparation for the initial filtration. A-filter holder for filtration of the second sample, closed with rubber cap and pinch clamp. B-silicone rubber tubing connecting the flasks. C-air vent with rubber cap. D-filter holder for filtration of the sample (initial). E and F- air vents with 0.45-μm Millipore filters. See text.

Figure 8B. Schematic representation of the arrangement of filter holders for: A-initial filtration. B-shaking period. C-second filtration. See text.

Figure 8C. Six filtering setups on the Dawson.

Figure 8D. Flasks being shaken on the Hudson.

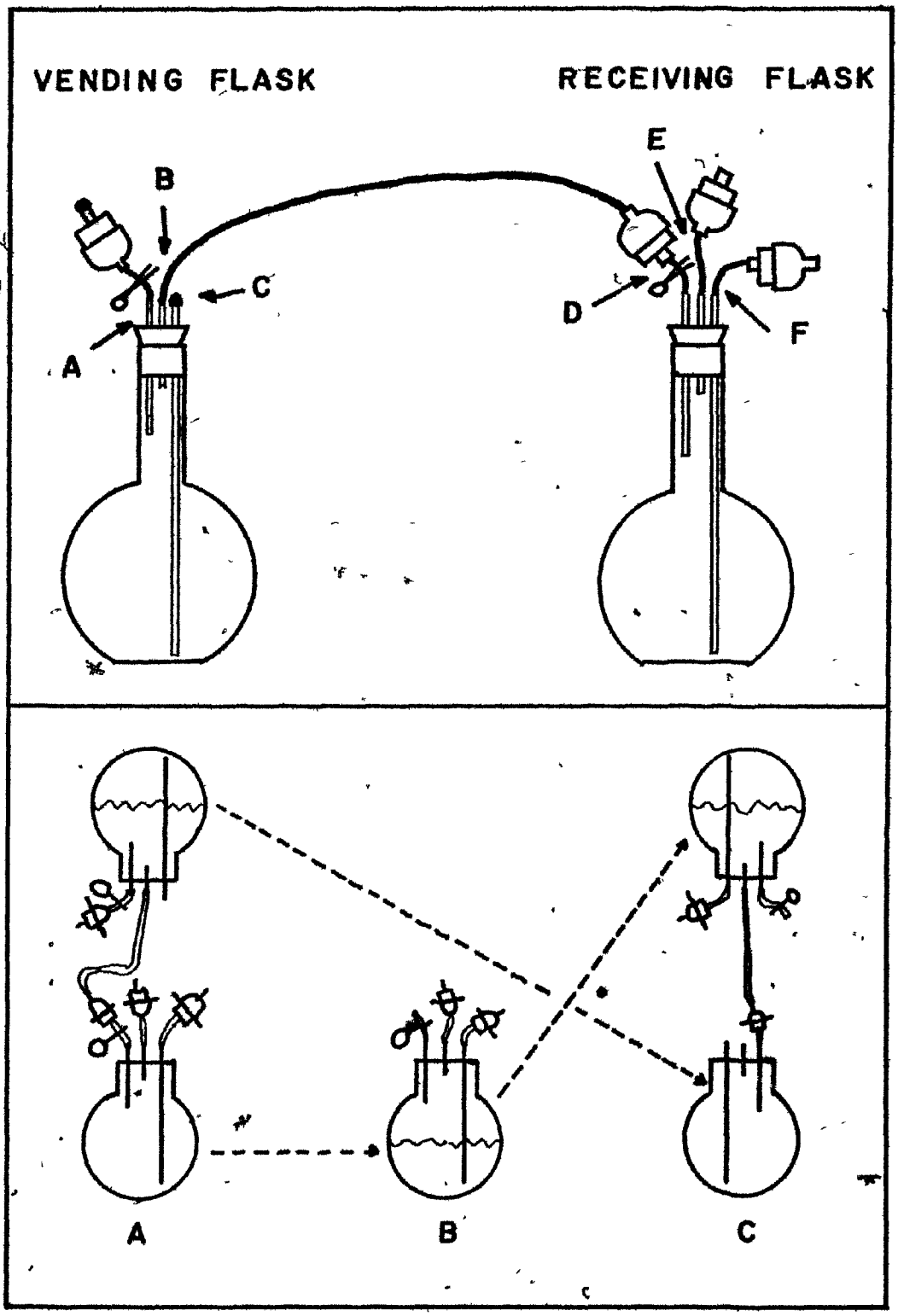


Figure 8 A (upper) and B (lower).

C

D

Figure 8. Continued.

filter holders were adapted for in-line use by placing the tops of Swinnex-13 holders over the outlets of the larger ones so that both inlets and outlets subsequently had female Luer connectors. Two of the filter holders had sample collection filters in them and the other two had air-venting filters. Filter holders were sealed between use by capping with gum rubber caps and pinching the silicone tubing connections with spring clamps.

Both the initial filtration of raw seawater and the second filtration were done by gravity in order to minimize distortion of material on the filters by pressure. One-liter samples were measured out and poured into the vending flasks. Then flasks were inverted, air bubbles were squeezed from the silicone tubes, and the initial samples were collected by filtration. Filtration took anywhere from 15 minutes to several hours, depending upon the samples. After filtration, the receiving flasks were sealed by pinching off the tubing, and the particle formation was done. Bubbling was used for particle formation, usually through shaking the flasks. Shaking was done for three hours on a reciprocating shaker with a speed of 2 excursions (of about 10 cms) per second. After the particle formation period, the flasks were inverted and the water was filtered a second time to collect the particulate matter that was formed in the filtrates.

About one-third of the flask volume was the air headspace above the sample. Shaking the filtrates caused vigorous production of bubbles of a fairly uniform size in the water. There was little possibility of contamination by the air introduced for bubbling since the headspace air was recirculated. Also, it was easy to run six samples at once under the same conditions. To insure that this was not an inferior method for particle formation, shaking was compared to gas-stream bubbling. This was done in

preliminary experiments by shaking and bubbling replicate filtrates. Bubbling was done with tank compressed gases that were purified by passage through a charcoal column, a water bath, and a Millipore filter. No appreciable difference was found between gas-stream bubbling and shaking.

In preparation for experiments, the flasks were washed with chromic acid cleaning solution; the flask stoppers, filter holders, and silicone rubber tubing were soaked in 0.5 N HCl. All equipment was rinsed with scalding hot water followed by two rinses with doubly distilled water (see Appendix I). The final rinses and assembly of setups were done wearing disposable plastic gloves. The silicone connecting tubes were cleaned before the second filtration also. The setups, with filters in holders, were assembled before sample collection so experiments could be started immediately. In a routine experiment, a single water sample was used with three setups for particulate organic carbon replicates and three for microscopy replicates. In other experiments, setups were used for separate samples with fewer or no replicates.

Microscopy was used for qualitative confirmation of the measurements of particulate organic carbon and for chemical interpretation of results. Appendix V describes staining and microscopy; only passing reference is made to this subject within the text.

Preliminary experiments were done on the length of the shaking period. For these, replicate samples were filtered initially and their filtrates were subsequently refiltered after varying periods of shaking. Three experiments are shown in Table 9. In experiments 1 and 2, immediate refiltration was done by harvesting one of the replicate filtrates that had no shaking period. In experiment 3, all samples were immediately refiltered after the initial filtration and the particle formation was done

Table 9. Shaking time of filtrates. See text for explanation of the experiments. Carbon yields are in μg C/L; standard error of the mean and the number upon which it is based are in parentheses ($\pm 2\sigma_{\bar{x}}$ / n).

Experiment 1	YIELD
Initial content	35.1 (8.1/5)
Immediate refiltration	12.9
Shaken for 5 hours	22.3
" 9 "	20.4
" 20 "	29.2
" 48 "	24.6
" 72 "	50.4
Experiment 2	
Initial content	20.0 (6.2/6)
Immediate refiltration	7.4
Shaken for 5 hours	11.6
" 11 "	5.2
" 50 "	14.4
" 75 "	>150
Experiment 3	
Initial content	59.3 (31.6/3)
Immediate refiltration	9.0 (1.8/3)
Shaken for 3 hours	31.6
" 19 "	10.8
" 48 "	15.7
" 67 "	48.3

on doubly filtered samples. The immediate refiltrations in experiment 3 were done by placing a second filter directly below the first one in the filter holder. The two filters were separated by a coarse grid nylon separator. Water for the experiments was surface water, several weeks old, from the Sargasso Sea (1 and 3) and from the Nova Scotia slope area (2). It can be seen in these experiments that afew hours' shaking gave yields comparable to an extended period of up to two days; after two days, there was an increase in the yield (possibly mediated biologically).

Riley (1970) has emphasized the importance of using controls for experiments on particle formation. One kind of control is to compare an unbubbled filtrate to one that was bubbled. The immediate refiltration yields of Table 9 served as unbubbled controls. Clearly, the results are equivocal. Immediate refiltration controls were used in experiments done at Hudson stations 1-4. In these experiments, three replicates were used of each sample (two replicates for microscopy were also used). Samples were initially filtered and immediately refiltered. Immediate refiltration, in this case, was done by placing a second filter holder tandem to the first one. All filtrates (doubly filtered) were shaken for about 18 hours and then filtered for yields of particles that were formed. Results are shown in Table 10. Examining the results it appears that bubbling enhances particle yields; but a variable yield seems recoverable even without bubbling.

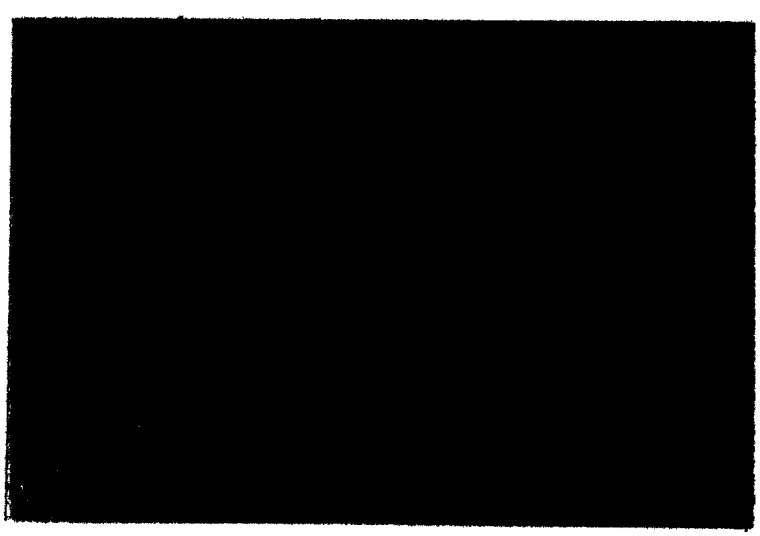
Menzel (1966) has suggested that yields from unbubbled filtrates are due to adsorption of dissolved organic matter onto filters. Some explanation is needed for the varied yields from the immediate refiltrations, but possibly adsorption is not the proper term for the phenomenon. Microscopy can be employed for information on the immediate refiltration yields.

Table 10. Immediate refiltration controls in particle formation. Experiments were done on freshly collected samples at Hudson stations 1-4. Three replicates of each sample were filtered for initial content and were immediately refiltered; doubly filtered samples were shaken for about 18 hours and then filtered for filtrate-formed yields. Yields are in μg C/L; Standard errors of the mean for the three replicates are in parentheses.

	YIELD
Station 1 - Surface Gulf Stream	
Initial content	90.0 (11.1)
Immediate refiltration	13.9 (6.5)
Filtrate-formed	13.8 (3.7)
Station 2 - Surface Sargasso Sea	
Initial content	45.4 (1.6)
Immediate refiltration	6.4 (3.3)
Filtrate-formed	21.6 (13.3)
Station 3 - Surface Sargasso Sea	
Initial content	32.3 (4.2)
Immediate refiltration	12.0 (4.7)
Filtrate-formed	12.1 (2.2)
Station 4 - 1000 m Sargasso Sea	
Initial content	6.3 (1.7)
Immediate refiltration	-0.3 (1.2)
Filtrate-formed	45.8 (20.0)

With the Nomarski optics, the Millipore filters can be focused upon. They appear as pure white membraneous pads with pores that are sinuous lacunae (easily seen by focusing down into the filters). Most of the initial filtration slides had "blotches" of indistinctly stained areas on them. Under highest magnification, these blotches were found to be composed of tiny spots that were near the limits of resolution of the microscope (about 0.4 μm); they often could be found within the pores of the filters. Slides of organic matter from filtrates (those from the previously mentioned experiments and those from the following section) rarely showed these indistinct blotches. With little exception, only quite distinct particles were found on these filters. The presence or absence of these blotches can be easily seen on the filters stained with periodic acid-Schiff's Reagent and, to a lesser extent, on those stained with bromophenol blue and osmic acid. Figure 9 illustrates the difference between indistinct blotches in naturally occurring particulate matter and distinct particles formed in a filtrate. With immediate refiltration, microscopy again revealed only distinct particles. In the filtering of seawater, extensive bubbling occurs at the underside of the filter and there is considerable splashing as the filtered water falls into the collection flask. Hence, filtrates have been exposed to bubbling even before the shaking period is begun. The difference between samples that were immediately refiltered and those that were shaken would hence seem to be one of extent of bubbling (the inevitable bubbling due to filtering alone as compared to that bubbling plus the additional vigorous bubbling from the shaking). The variable results from immediate refiltration probably represent the variability in the potential to form particles and slight differences in the bubble action inherent in the initial filtration. Surely, some adsorption of dissolved matter, adherence

A



B

Figure 9. Material on filters stained with periodic acid Schiff's Reagent. Bars represent 20 μm. A-natural particles and indistinct blotches. B-filtrate-formed particle.

of colloidal matter, and absorption (entrapment within the pores) of both dissolved and colloidal could occur. But there is no evidence that these processes do occur to an appreciable extent from samples after the initial filtration (some of the indistinct blotches encountered on initial filtration slides might be due to retention of organic matter that could otherwise pass the filters). There is good evidence that distinct particles are retained from samples after initial filtration.

The phenomenon of particle formation has been well illustrated by Riley (1970). That the phenomenon is not due to experimental artifacts can be further verified with the present experiments. The purpose of the ensuing section is to examine the variability and source of formed particulate matter. The method that was employed for particle formation was not designed to imitate nature nor to give maximum yields. It was used because it was carefully standardized and should not be extensively vitiated by contamination or experimental variability. Thus it is an examination of the potential for particle formation.

POTENTIAL FOR PARTICLE FORMATION

The organic matter in the sea originates in the surface water or, if its origin was exogenous, at least passes through the surface. In transport to deep water, organic matter probably has a varied and complex history. The processes that organic matter in deep water have undergone include settling by gravity of particulate matter and degradation by bacteria of particulate and filter-passing matter. By aging seawater samples in the laboratory, similar, but not analogous, settling and degradation can be investigated.

Seawater samples of large volume were collected, experiments on particle formation were performed with the fresh samples, then the water was stored in carboys for subsequent experiments on the aged samples. Polyethylene carboys were used and they were stored in the laboratory (at about 20 C) with no attempt to pressurize or darken them. The aging was similar to that in deep water in that the organic input was stopped (plankton growth halted fairly rapidly in the closed carboys, probably because of exhausted nutrients). Also, particle settling was allowed to occur since the carboys were left undisturbed and sampling was done by siphon. The results of four aging series are given in Table 11. Water for series 1 was collected at Hudson station 3; for series 2 and 3 from the surface of St. Margaret's Bay (on 8 September and 15 October, 1969, respectively); for series 4 at Hudson station 5. The seven week old sample from series 1 was taken after the carboy was shaken (it can be seen in Table 11 to be anomalous), but in all other cases, the carboys were undisturbed. Aging profiles for series 1 and 2 are given by Figure 10. In both cases, the curves for filtrate-formed yields are parallel to and of lower value than those for initial yields. In both series, the initial particle content dropped to about 20 μg C/L after a few weeks and remained fairly constant at that level. Correspondingly, the filtrate-formed particle content dropped to zero and remained fairly constant. Similar trends are shown by the data from series 3, which was of surface water, as were series 1 and 2. Series 4, a deep water sample, shows a somewhat different general picture. It is too limited and isolated for much interpretation, but it probably does represent a somewhat different aging process (in this case, nutrient-enriched deep water may have slowly sustained phytoplankton growth in an otherwise phytoplankton poor sample).

Table 11. Particle formation in aged seawater samples. Initial and filtrate-formed particulate organic carbon yields are in μg C/L. Standard errors of the mean and the number of replicates upon which they are based are in parentheses ($\pm 2\sigma_{\bar{x}}$ / n).

	INITIAL	FILTRATE-FORMED
Series 1		
Freshly collected	32.3 (4.2/3)	24.1 (4.7/3)
2 weeks old	35.1 (8.1/5)	22.3
7 " "	59.3 (31.6/3)	31.6
10 " "	19.4 (4.3/4)	5.2 (3.6/4)
12 " "	18.6 (0.9/4)	2.9 (3.4/3)
19 " "	20.9 (4.5/3)	4.5 (5.1/3)
Series 2		
Freshly collected	92.0 (18.8/3)	24.0 (8.5/3)
4 days old	25.4 (4.5/6)	14.5 (3.3/2)
4 weeks old	15.7	2.9 (7.6/2)
8 " "	33.2 (22.8/3)	6.3 (3.1/3)
26 " "	18.2 (4.1/3)	3.1 (0.7/2)
Series 3		
Freshly collected	78.0	28.7
4 weeks old	23.1 (2.8/3)	11.4 (3.3/3)
Series 4		
Freshly collected	5.6 (2.5/2)	-0.8 (3.6/3)
26 weeks old	12.0 (2.8/2)	15.7 (8.1/2)

Figure 10. Aging profiles from experiments on particle formation. Closed circles represent initial (naturally-occurring) particulate organic matter; open circles represent particulate organic matter formed in filtrates from the same samples. Bars on open circles are the errors for the individual values.

Figure 10 a. From series 1. Water for the 7 week sample was drawn off after the carboy was shaken.

Figure 10 b. From series 2.

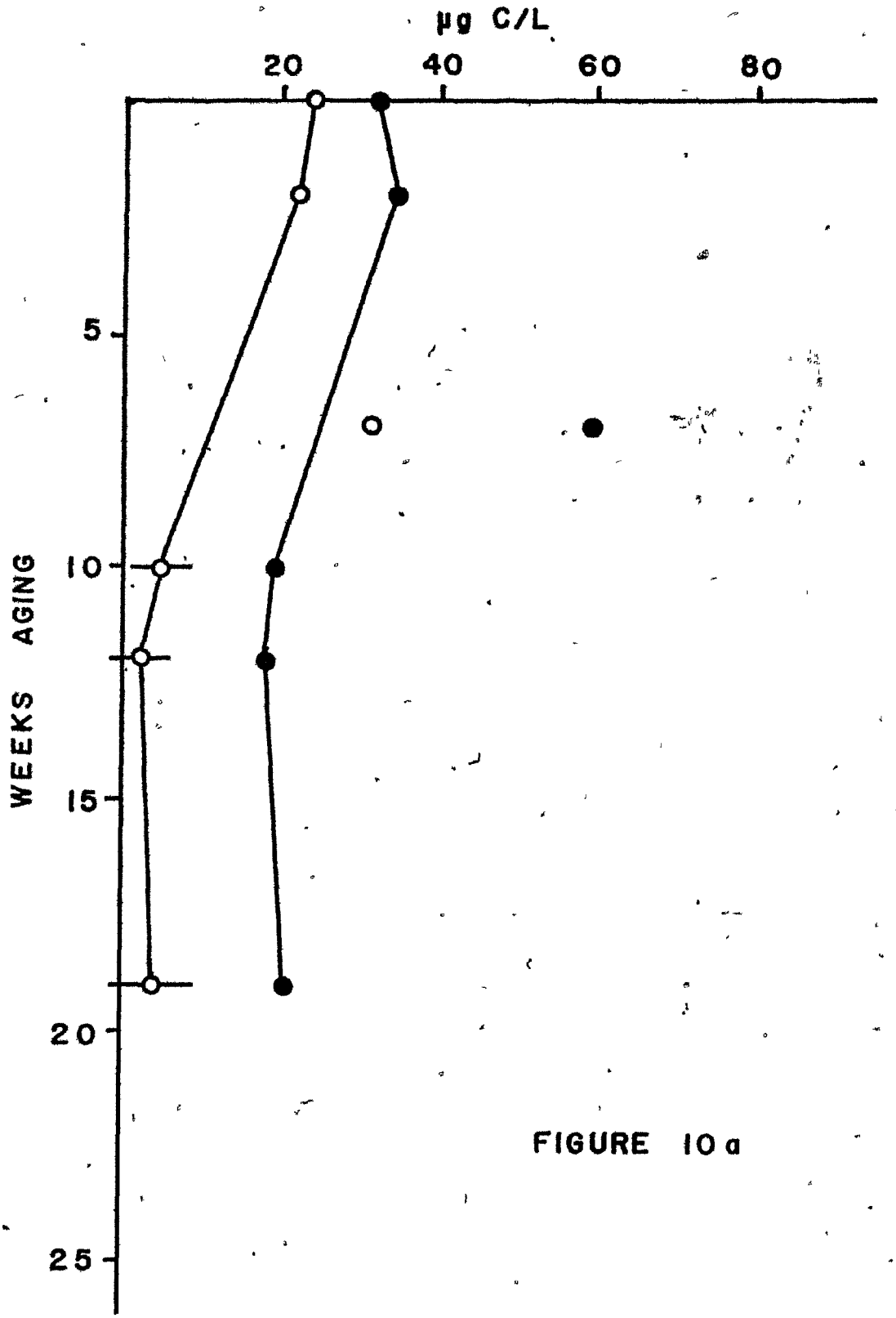


FIGURE 10 a

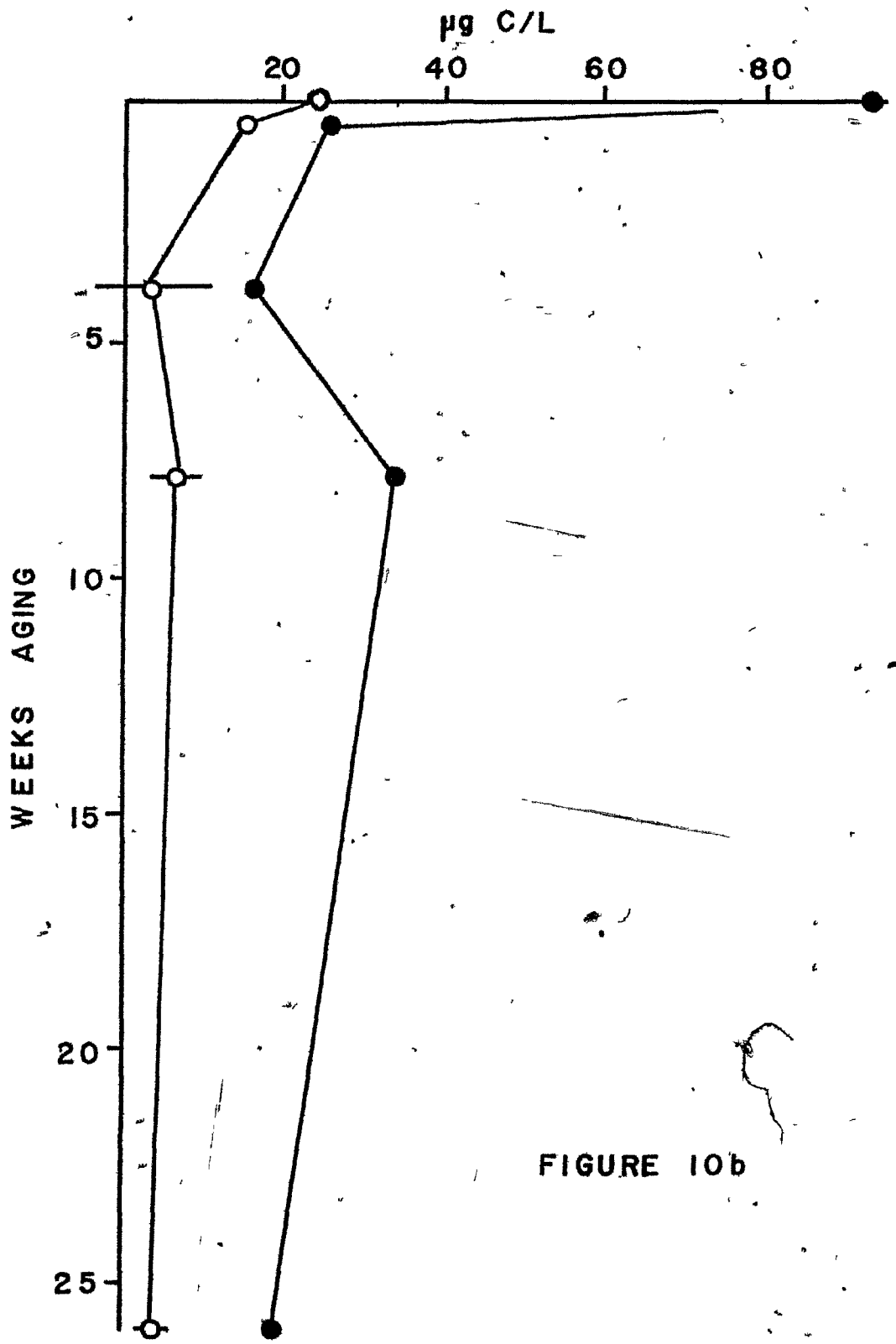


FIGURE 10b

Riley, VanHemert, and Wangersky (1965) did experiments on particle formation with some deep water samples. They found somewhat lower yields in deep water than in the surface waters. To look more thoroughly at this, the potential for particle formation was examined in water samples from various depths in the central western North Atlantic Ocean and in an area which is considered "eutrophic" in comparison to the oligotrophic oceanic waters. All the data are listed in Appendix VI and are plotted as composite depth plots in Figure 11. As in the experiments on laboratory aging, the filtrate-formed particulate carbon usually paralleled, at a lower value, the naturally-occurring carbon. Decreases with depth in both initial and filtrate-formed carbon were similar to the decreases with age. A difference between aging and natural depth trends is that the initial values were lower in deep water than they were in aged water and that the filtrate-formed values were higher in deep water than they were in aged water.

In the aging series, microscopic observations showed that much of the naturally-occurring particulate matter in the old samples was in the form of indistinct blotches. There was no sign of organisms other than bacteria in these samples. In the deep water samples, blotches were rare while organisms were comparatively abundant (coccoid cells similar to those described by Fournier (1970) and remnants of diatoms and coccolithophorids were often found). The particulate matter formed in filtrates did not appear by visual examination to be appreciably different between aged and deep samples. From the aging and depth sequences, a few chemical characterizations can be made. Lipids were not very abundant except in living matter and they were found only as minor inclusions in naturally-occurring non-living particles while they were almost never in particles formed in filtrates. Both protein and carbohydrates were found on all

Figure 11. Depth profiles from experiments on particle formation done at sea. Closed circles represent initial (naturally-occurring) particulate organic matter; open circles represent particulate organic matter formed in filtrates of the same samples. Bars in upper right-hand portions of graphs represent averaged experimental errors.

Figure 11 a. From "eutrophic" waters (St. Margaret's Bay, Nova Scotia Shelf and Continental Slope regions).

Figure 11 b. From oceanic waters (Gulf Stream, Sargasso Sea, and the Caribbean).

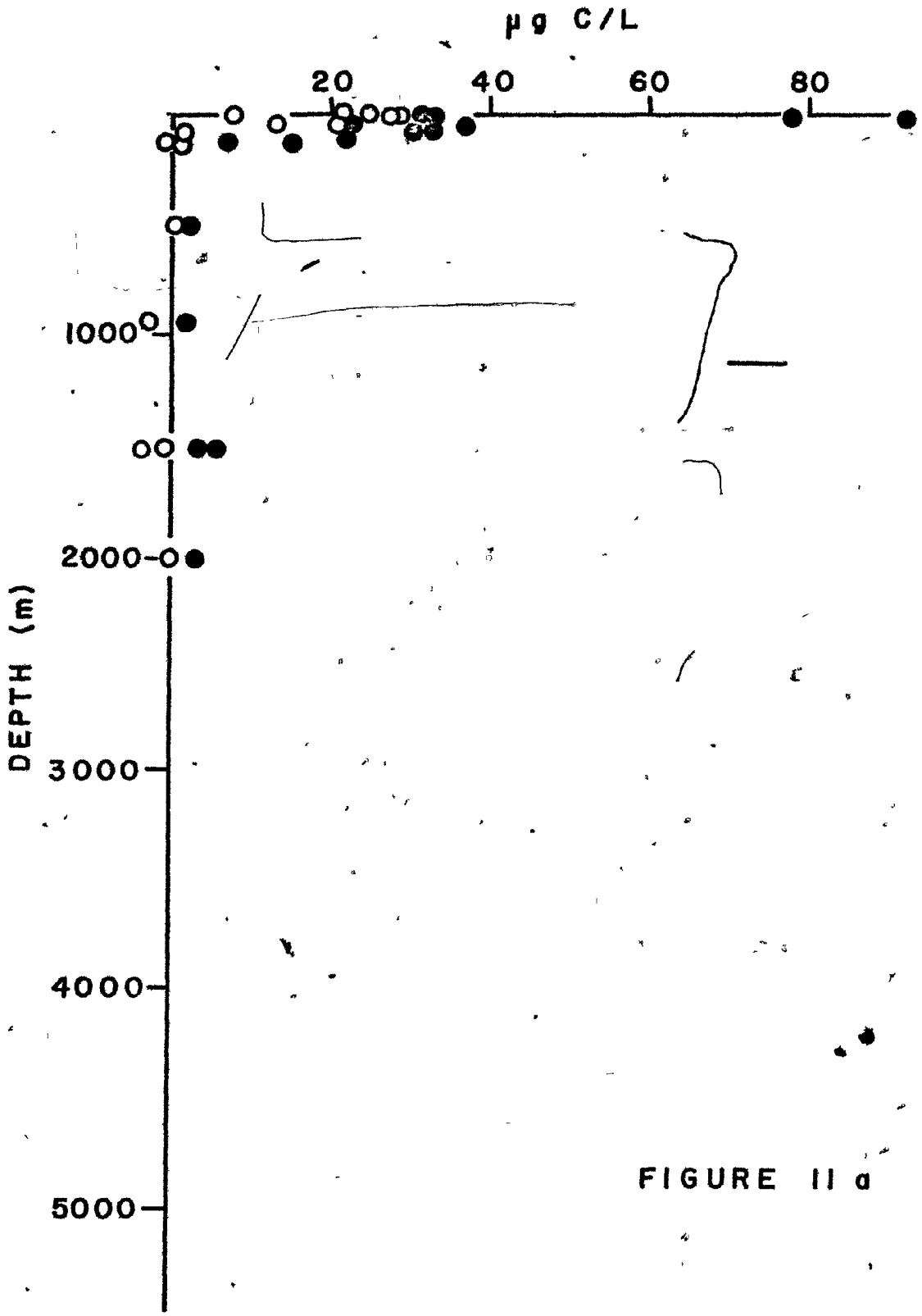


FIGURE II a

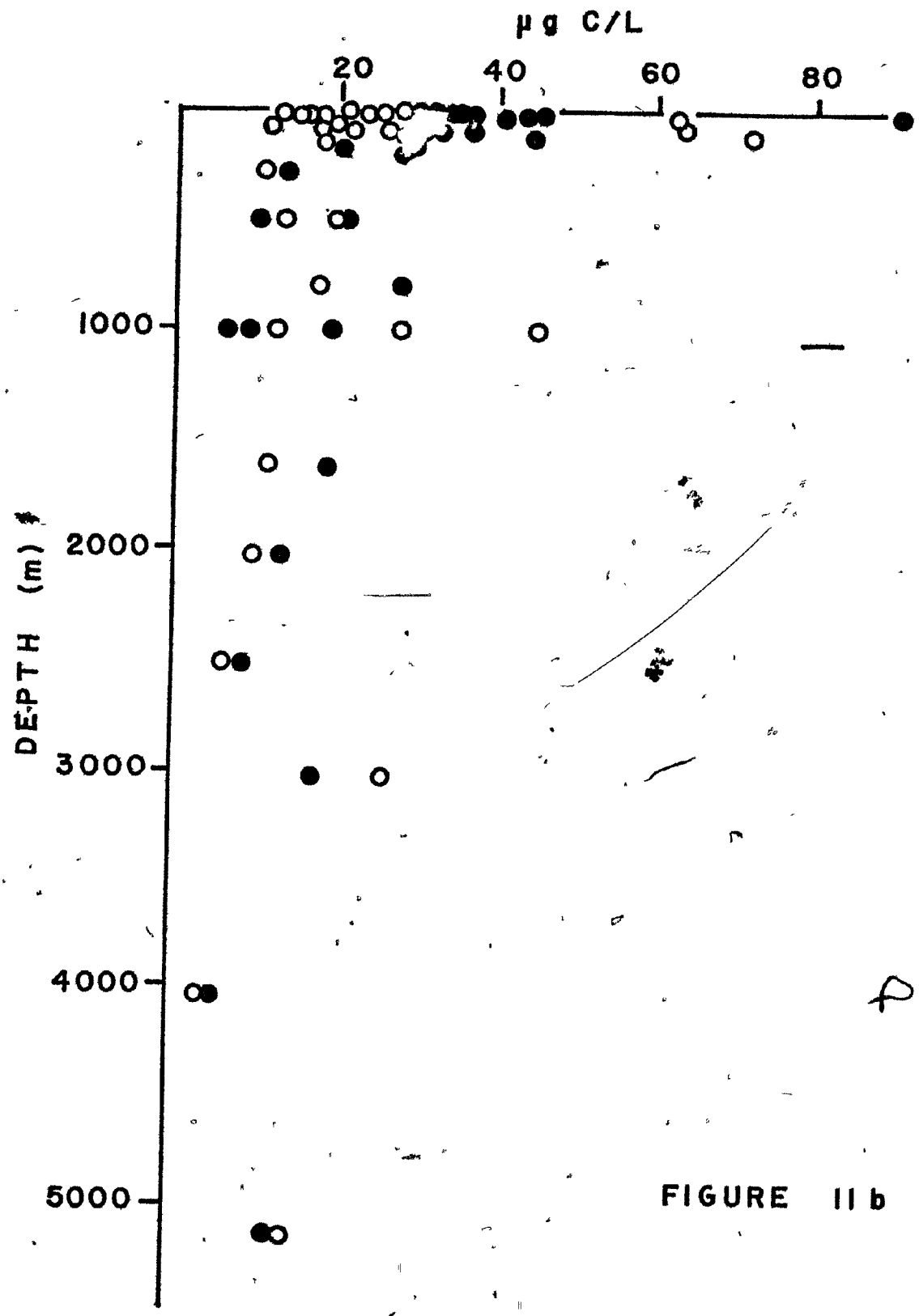


FIGURE 11 b

slides of initial and filtrate-formed samples. Carbohydrate was always considerably more noticeable on these slides. There appears to be a proportional decrease of protein in naturally-occurring particulate matter in aged and in deep seawater while marked changes in proportional amounts of protein in particles formed in filtrates were not noticed. The assertion that protein decreases in deep water is supported by an observed increase in the carbon to nitrogen ratio of particulate organic matter from deep water (Gordon, 1971b).

Returning to Figure 11, there are some exceptions to the general depth trends that were discussed above. Occasionally, concentrations of filtrate-formed particulate carbon were higher than the corresponding concentrations of initial particulate carbon. The figure is separated into "eutrophic" and oceanic groups. A surprising observation resulting from this grouping is the striking difference between the two depth profiles. At depth, the oceanic waters consistently had higher concentrations of initial and filtrate-formed particulate organic carbon than did the "eutrophic" waters. This seems backward from what is known of regional variations of naturally-occurring particulate organic carbon in deep water (Riley, 1970; Nakajima, 1971). Possibly, the apparent difference between the "eutrophic" and oceanic groupings is misleading due to inadequate seasonal sampling. Data from the "eutrophic" region are from autumn while those from the oceanic region are from winter and spring. If the difference were seasonal, then the results would indicate seasonal variation in the potential to form particles as well as seasonal variation in the naturally-occurring particle concentrations.

SOURCE OF FORMED PARTICLES

In the preceding sections, data from replicate samples allowed calculation of experimental errors. Filtrate-formed particulate carbon values that were significantly above experimental errors and microscopic observations of the particulate matter indicated that yields were due to neither contamination nor artifacts. Hence the source of particles is undoubtedly the filter-passing organic matter. Data from the Dawson cruise can be used to make a catalogue of various classes of organic carbon (Table 12). In can be seen immediately that sufficient filter-passing carbon is present in all cases to account for the particulate carbon formed in the filtrates. From Table 12, correlations can be seen of filtrate-formed particulate carbon to both initial particulate and filter-passing fractions. Without statistical refinement these correlations are merely that if comparatively large amounts of carbon are present in particulate and filter-passing form, comparatively large amounts of filtrate-formed carbon are expected. A correlation of this sort between filtrate-formed and colloidal fractions is not shown by the data. However, in all cases, a sufficient amount of colloidal carbon is present to account for all the filtrate-formed particulate carbon. Particle formation is a complex phenomenon and obviously is mediated by several factors. It may be that aggregation of small filter-passing particles into filter-retained particles is a critical step in the phenomenon for the short term experiments reported here. If so, sufficient colloidal carbon was present to account for the small filter-passing particles. Considerably more work must be done on the source of formed particles.

Table 12. Classes of organic carbon. Samples are station number - depth (in meters). All carbon values are in μg/L. Filter-passing values are total minus initial particulate. Colloidal values are 0.003-μm-retention class minus initial particulate unless indicated by asterisk, in which case they are 0.025-μm retention class minus initial particulate.

SAMPLE	FILTER-PASSING	INITIAL	COLLIODAL	FILTRATE-FORMED
17-Sfc	1513	37	403	15
20-Sfc	1240	30	170	15
26-Sfc	1332	28	162	21
19-5	1460	30	10*	14
22-5	1526	44	126	26
23-5	1706	34	46*	15
25-10	1699	41	129	21
24-15	1405	35	55*	17
23-25	1444	46	134	18
23-50	1523	37	103	22
23-75	1477	33	217	22
24-100	1069	21	229	18
25-100	1349	31	179*	72
19-150	1242	28	262	19
22-250	846	14	-24*	11
24-500	738	22	188*	13
22-1000	881	9	201*	12
25-1600	1141	19	181	12
21-2000	796	14	156	10
18-4000	1505	5	245	4
25-5100	1067	13	117*	14

CARBON BALANCE EXPERIMENTS

A possible cause for misinterpretation in experiments on particle formation is contamination of filtrates. Menzel (1966) found in this early experiments that the organic carbon in his filtrates increased markedly from contamination during air bubbling. In the present experiments, filtrates were shaken rather than being bubbled with an external air source and they should not be contaminated. A simple carbon balance experiment could give confirmation of no gross contamination.

A carbon balance was attempted for the experiment on particle formation of Dawson cruise station 10. Measurements were made of the original total organic carbon, the particulate organic carbon collected during both filtrations, and the organic carbon of the filtrate. Since total organic carbon determinations have large analytical errors when compared to the level of those of particulate organic carbon determinations, only the filtrate carbon after both initial and second filtrations was measured. The total organic carbon minus the particulate should equal the organic carbon of the filtrate. The carbon balance is (values in mg C/L with $\pm 2\sigma_x$ in parentheses):

Total	1.36 (0.04)
" minus particulate	1.31
Filtrate	1.04 (0.03)

The amount of carbon removed by the two filtrations significantly exceeded prediction. As additional confirmation, a filtrate from the same experiment in which the two filtrations were through Millipore (rather than silver) filters had a carbon content of 0.98 mg C/L. Obviously, the experiment did not suffer from contamination.

A possible explanation for the excessive carbon removal is that organic matter was lost to the surfaces of the flasks during filtration and shaking. An experiment using water from the Northwest Arm was devised to test this hypothesis. Three replicates were used as in the routine particle formation experiments. Another three setups were used in the same way but immediately prior to the start of the experiment both flasks of each setup were rinsed briefly with the seawater sample. A single seawater sample was taken and four replicate aliquots were poured off for total organic carbon analysis. The vending flasks of the six setups were filled and six filters each for initial and filtrate-formed particulate organic carbon were obtained. Six samples were taken from the double filtrates at the end of the experiment (three replicates each from the routine and the rinsed setups). The average particulate organic carbon values for the two groups of setups were statistically identical. The initial and filtrate-formed values for the routine group were 133 and 40 μg C/L; and for the rinsed group, they were 119 and 47 μg C/L. Thus, if any organic matter was lost to the flask surfaces it did not alter the particulate carbon yields. The carbon balance, in the same form as the previous one, is:

Total	2.50 (0.31)
" minus particulate	2.33
Routine filtrate	2.34 (0.03)
Rinsed flasks filtrate	4.59 (0.32)

The $\pm 2\sigma_{\bar{x}}$ values in this case are not for replicate determinations of a single sample, as in the previous balance, but are for replicate samples. Hence, the total organic carbon value is based upon four replicate determinations each of four replicate samples and the filtrate values are based upon four replicate determinations each of three replicate samples.

The carbon balance with the routine setups meets prediction. It certainly does not meet prediction with the rinsed setups. The large increase of carbon in the filtrates from the rinsed setups is statistically significant and is due to the prior rinsing with the seawater sample. There was no increase in the filtrates from the three routine setups and all six were handled together so there is no reason to suspect contamination from an external source. A possible explanation of this puzzeling finding can be made in light of the algal culture experiments and is discussed later.

The conclusion from the attempts at carbon balancing is that contamination did not add to the carbon yields in the experiments on particle formation. Some organic matter could be lost to the flask surfaces; if so, the carbon yields would be an underestimate rather than too high.

In summary, the particle formation experiments show that particles can be formed in filtered seawater. Experiments that were carefully standardized and that used replicates showed that yields were not due to contamination or artifacts. The amount of particulate matter that can be formed is related to the quantity and quality of organic matter in the seawater. Like the total and particulate organic matter occurring naturally in seawater, the particulate organic matter that can be formed in seawater filtrates is also a somewhat variable quantity and is not entirely predicable in light of our present knowledge.

ALGAL CULTURE EXPERIMENTS

PREFACE

Since essentially all the organic matter in the open sea originates from phytoplankton photosynthesis, it is desirable to learn something of the process by which its non-living portions reach the general organic pool. Duursma (1963) made calculations from field measurements or organic carbon and chlorophyll a and somewhat indirectly decided that actively growing phytoplankton were not a major contributor to the "dissolved" organic matter in the sea. He stated, instead, that decaying phytoplankton remnants were the major source. This proposal had been previously made by Krogh (1934). Fogg (1966) concluded that actively growing algae were the major source. His work was based upon extracellular release of C^{14} containing "dissolved" organic carbon from short term incubation experiments. He reconciled the difference between his and Duursma's conclusions with the proposal that the organic matter released from actively growing algae is quickly utilized by bacteria and hence is not that material which accumulates in seawater.

Due to problems in measurement and severe limitations in simulating natural conditions, there have been no thorough studies directly monitoring the organic carbon budget in marine phytoplankton cultures. The little available information from partial or indirect monitoring and from fresh water studies is mentioned in the discussion of results of this section.

An attempt is made in this section to measure the particulate and filter-passing organic carbon of actively growing cultures of a marine diatom and a blue-green alga of marine origin. Some correlative measurements are included of filter-passing organic carbon from old cultures of these

two algae, a marine chrysophyte, and a marine chlorophyte.

MATERIALS AND METHODS

For experiments on actively growing algae, cultures were used of the diatom Thalassiosira fluviatilis (axenic starter from S.M. Conover) and the blue-green alga Schizothrix calcicola (established as an unialgal isolate from surface Sargasso Sea water in November 1968). They were grown as 25-ml cultures in 50-ml micro-Fernbach flasks capped with inverted cutdown 20-ml beakers. Incubation was at 24 C with constant illumination on a glass plate about 6 inches above a fluorescent light bank. T. fluviatilis was grown in an ammonia-nitrogen variant of the Woods Hole medium f/2 (Conover, 1972); S. calcicola was grown in another variant of f/2 (as described by Sharp, 1969, except that the vitamin mixture in this case contained only B_{12} which was added as a commercial sterile serum solution). Sterile flasks of media were innoculated aseptically with equal aliquots of well-mixed starter cultures.

For the axenic T. fluviatilis experiment, 10 flasks were inoculated with 1-ml aliquots from the starter culture which was one month old. This starter was contaminated with a mixed population of marine bacteria, it was well mixed by swirling, and 10 flasks were inoculated with 1-ml aliquots. These were the unialgal cultures of the diatom. A starter culture of S. calcicola that was one and one-half months old was used for the blue-algal experiment. This alga grows as an attached mat; it was detached from the flask surface by swirling the flask; and it was mixed by grinding in a sterile tissue grinder. Aliquots of 0.2-ml from the tissue grinder were used as inocula. Old cultures of the diatom, of S. calcicola, and of

unialgal strains of Chlamydomonas sp. and an unidentified chrysophyte were used as samples of declining growth phases. The latter two algae had been grown in seawater Erd-Schreiber medium and they were provided by R.O. Fournier.

In the active growth experiments, the three sets of cultures were inoculated, and after about two hours one of each was harvested. This initial harvest was denoted time 0; subsequently, one of each was harvested on 1, 2, 4, 6, 8, 10, 15, 20, and 25 days after inoculation. The old cultures were: T. fluviatilis of 39 and 101 days' age; S. calcicola of 27 months; Chlamydomonas and the chrysophyte of 9, 12, 14, and 18 months. They had passed their peaks of growth and had been stored in the light in culture rooms; they were all harvested and processed at one time.

Before harvesting, cell counts of the diatom were made with a Coulter Counter. One-ml samples were taken from the cultures with sterile pipettes and diluted; four counts were made of each.

After the diatom cell counts were made, cultures were checked for bacterial contamination. This was done in two ways, by direct microscopic observations and by plating. A sterile loop was used to transfer a water drop from a culture to an ethanol-cleaned slide which was covered with an ethanol-cleaned 12-mm square coverglass. The slide was examined immediately for evidence of motile bacteria. Following sample filtration (below), the sterile loop was scrapedover a small portion of the filter surface and then streaked on agar. Plates of 5-cm diameter had been prepared with nutrient agar as a2.5% mixture in Erd-Schreiber medium. When streaked, the plates were incubated at about 25 C in the dark and were examined daily for bacterial colonies.

Samples were filtered in Sterifil apparatus through prebaked 0.8-µm pore silver filters directly into the total organic carbon sample bottles.

The methods for analysis of particulate organic carbon on the filters and analysis of filter-passing organic carbon were given in the first section of the dissertation.

RESULTS AND DISCUSSION

The old chlorophyte and chrysophyte cultures were used only for filter-passing organic carbon determinations. The problem of analysis of such high levels of carbon was discussed earlier, and samples are only used here in a gross comparative sense. In both cases, the filter-passing organic carbon increased steadily with age from three-quarters to one and one-half years. The cultures were not bacteria-free, so that the increasing carbon was in excess of that which was utilized by the concomitant bacteria. The other old cultures were used in conjunction with those in a state of active growth and will be discussed with them. The rest of this section deals primarily with results obtained during active growth.

The results of the microscopic checks for sterility were equivocal. The axenic diatom cultures showed occasional bacterial cells, but the few bacteria that were found could be contamination on the slides rather than from the medium. The unialgal diatom and the blue-green algal cultures all had large numbers of motile bacteria throughout the experiments. By the plating technique, the later axenic cultures had some bacteria in them although on days 0, 1, and 2 they were all negative. All the unialgal cultures had bacteria by the standard of this method. The plates from the later axenic diatom cultures had considerably fewer bacteria on them than had those from the unialgal diatom cultures, with only several colonies as compared to many hundreds. Hence it appears that the axenic cultures

started in a bacteria-free condition and became contaminated only after extended incubation.

Data on cell counts and organic carbon are listed in Table 13. The initial samples were originally intended as indicators of conditions at the start of the experiment. However, the very high values for filter-passing organic carbon in these samples seemed anomalous. Hence values for the starting inoculum of the diatom culture are calculated *a posteriori*.

The 39 and 101 day diatom cultures were grown under conditions different from those of the other diatom cultures and were not directly comparable to them. The 39-day culture was comparable to the 30-day starter. They were grown as replicates and the older one was harvested 9 days later than the beginning of the experiment. Values for the inoculum are calculated from properties of the 39-day culture and are compared to the values measured at time zero:

	Calculated	Time-zero
pg C/cell	170	164
cells/ml	9×10^3	17×10^3
particulate mg C/L	1.6	2.8
filter-passing mg C/L	0.6	10.9

The particulate carbon and cell count values calculated for the inoculum underestimate the measured values (by 75 and 90% respectively). If the filter passing carbon value were adjusted proportionately, say by 100%, it would become only 1.2 mg C/L. The cultures at time zero also had organic carbon in them from the medium. For the diatom cultures, the value for the medium should be 3.1 mg C/L which includes an addition of 0.9 mg C/L from the seawater, 0.09 mg C/L from vitamins B_{12}, biotin, and thiamin, and

Table 13. Organic carbon in algal cultures. See text for explanation of cultures. Particulate and filter-passing organic carbon are in mg C/L. Percent is particulate divided by particulate + filter-passing X 100. The pg C/cell column does not necessarily represent cellular carbon content throughout the experimental period but it is probably fairly representative during logarithmic growth.

DAYS GROWTH	CELLS/ml	PARTICULATE	FILTER-PASSING	PERCENT	pg C/CELL
T. fluviatilis (axenic)					
0	$17X10^3$	2.68	10.98	20	159
1	31	4.64	2.95	61	149
2	36	4.72	2.40	66	130
4	38	4.84	2.43	66	127
6	67	8.12	2.94	73	121
8	106	9.84	2.97	77	92
10	128	13.68	3.52	80	106
15	104	21.68	6.97	76	208
20	63	18.44	5.93	76	291
25	39	25.56	6.44	80	659
39	244	39.88	16.40	71	170
101	135	55.12	28.41	66	409
T. fluviatilis (unialgal)					
0	$17X10^3$	2.88	10.73	21	169
1	33	4.05	3.26	55	124
2	35	4.60	3.58	56	132
4	62	7.88	3.66	68	128

DAYS GROWTH	CELLS/ml	PARTICULATE	FILTER-PASSING	PERCENT	pg C/CELL
6	80	8.76	3.20	73	109
8	121	12.04	4.29	74	99
10	109	13.60	3.90	78	125
15	79	12.96	4.84	73	165
20	45	17.88	5.68	76	393
25	31	19.92	4.75	81	644
S. calcicola					
0		6.47	24.33	21	
1		10.96	17.84	38	
2		16.27	14.65	53	
4		21.75	12.48	63	
6		54.13	13.43	80	
8		85.12	15.17	85	
10		130.95	12.53	91	
15		115.56	12.10	90	
20		216.51	14.34	94	
25		233.57	14.02	94	
27 months			76.48		

2.1 mg C/L from the citrate. The derived value for filter-passing carbon thus is 4.3 mg/L. A very large excess of filter-passing carbon was present at time zero and it completely disappeared in the first day. Figure 12 a and b give diagrammatic pictures of the carbon in the diatom cultures.

It is not possible to derive values for the blue-green algal inoculum since no good approximationsof valuesfor the starter culture are available. However, the filter-passing organic carbon remained fairly constant from day-2 to day-25; this level might be reasonable as a value for the starter (45-day culture from similar incubation conditions). If so, the inoculum would account for only0.1 mg C/L of filter-passing carbon. The background for the medium in this case would be 16.6 mg C/L which includes additions of 0.9 mg C/L from the seawater, 0.06 mg C/L from vitamin B_{12}, 12.3 mg C/L from benzyl alcohol preservative in the vitamin serum, and 3.6 mg C/L from EDTA. As with the diatom cultures, a very large excess of filter-passing carbon was present at time zero; it also disappeared by day one. Figure 12 c shows the carbon in the blue-green algal cultures.

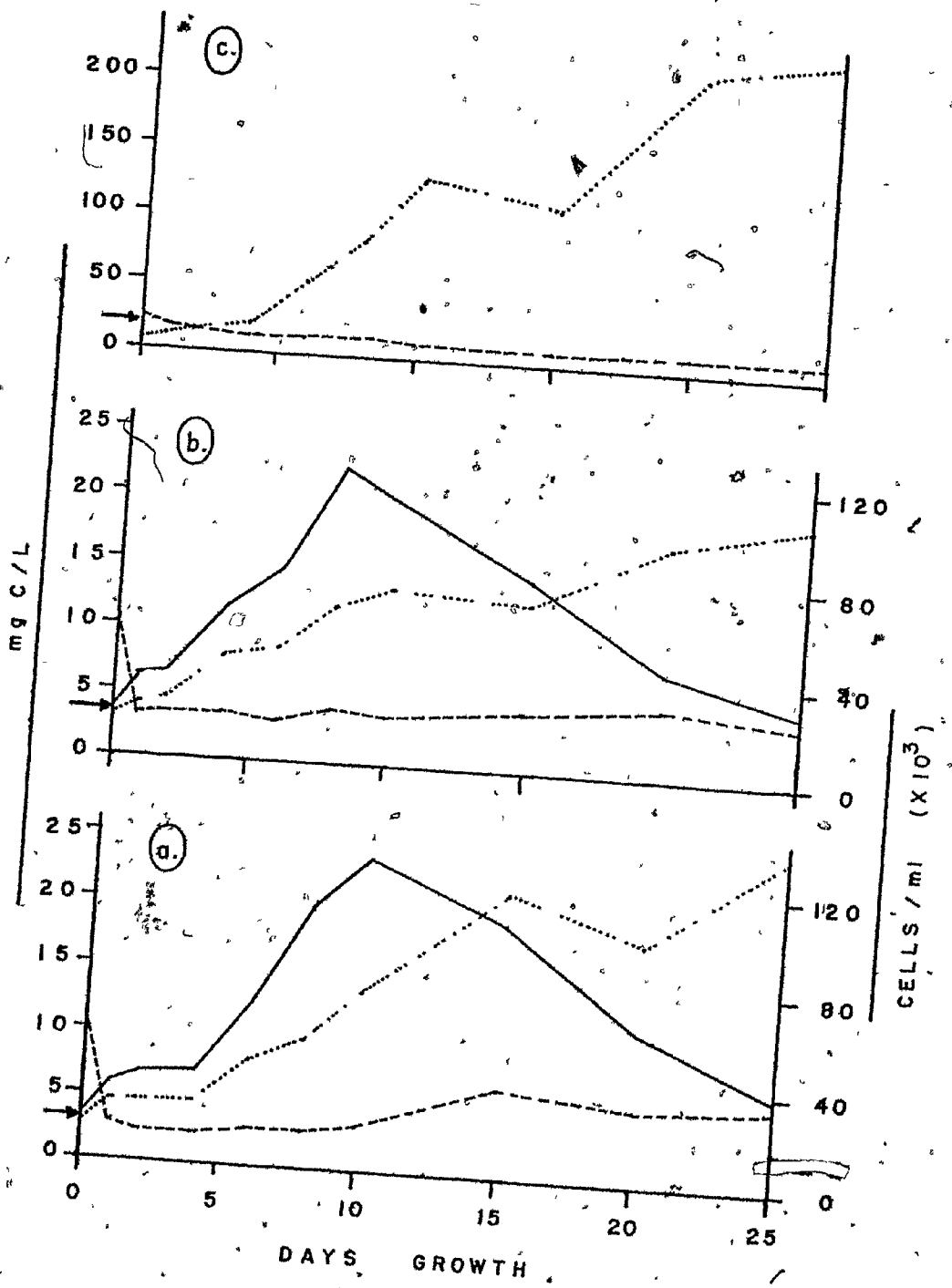
An explanation is desired for the anomaly at the beginning of the algal culture experiments. Very high levels of filter-passing organic carbon were found at time zero. By day one, the levels dropped to normal and remained close to the predicted starting conditions up until day-10 for the diatom cultures and up until the end of the experiment for the blue-green alga. Because all of the samples at time zero had excesses of 6-7 mg C/L, suspicion is aroused of a uniform contamination of the culture media. However, contamination does not seem a satisfactory explanation. The glassware was carefully cleaned with chromic acid, rinsed with doubly distilled water, autoclaved, and stored for four days in a transfer box which was kept sterile by ultraviolet light. The media were carefully prepared, autoclaved, and were also stored in the transfer box (the diatom

Figure 12. Organic carbon from algal culture experiments. Solid lines are diatom cell counts; dotted lines are particulate organic carbon; dashed lines are filter-passing organic carbon. Arrows on the left-hand vertical axes represent the amount of carbon originally in the media.

a. Axenic culture of Thalassiosira fluviatilis.

b. Unialgal culture of T. fluviatilis

c. Unialgal culture of Schizothrix calcicola.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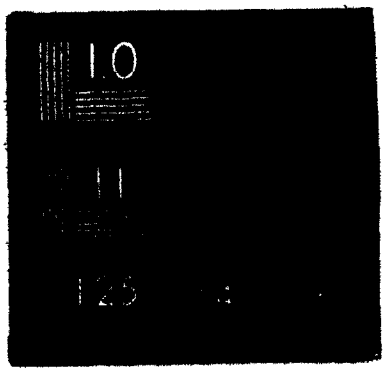

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medium for 4 days, the blue-green algal medium for 7 weeks). Thus, contamination would have to be by volatile organic compounds which could be quickly lost again as volatiles and it would have to have occurred in the transfer box. However, the method of organic carbon analysis excluded volatile carbon since, prior to analysis, samples were acidified to a pH of about 1 and were purged for 5 minutes with nitrogen. Futhermore, it seems unlikely for such a large amount of volatile contamination to occur and to disappear so quickly. Hence there is a question as to whether the anomaly might be explained in terms of algal physiology.

About two hours elapsed between the start of inoculation and the filtering of the samples for the analyses listed as time zero. Thus the excess carbon might represent short term extracellular production by the algae. The disappearance by day one would represent reincorporation by the algae of the organic matter and the ultimate loss of most of it by respiration. As will be discussed below, there is evidence for rapid extracellular production by phytoplankton under conditions that would be considered as cultural shock. In this case, the algae are easily seen to have undergone cultural chock, for between the starter and the new cultures there were large changes in illumination and probably also in nutrient concentrations, pH and salinity.

In short term experiments of 2-5 hours' incubation with freshwater phytoplankton, Watt (1966) found extracellular release of 90% of the photoassimilated C^{14} under conditions of cultural shock. His conditions were dilution of the medium by filtered water, drastic changes in lighting conditions, and illumination of very high or very low intensity during incubation. In the freshwater chlorophyte Chlorella, glycollic acid release was shown to be rapid at first, with a peak at 50-100 minutes of

incubation, and then to drop off, presumably because of reincorporation (Watt and Fogg, 1966). In these experiments, glycollate production was as high as 2 mg C/L in 200 minutes (under contrived changes of lighting and CO_2 concentrations). Experiments have illustrated incorporation of glycollate by the same algae that released it (Fogg, 1966). Guillard and Wangersky (1958), studying carbohydrate production in marine flagellates, reported a sudden release upon inoculation and apparent reincorporation shortly thereafter. Hence, there is evidence that cultural shock can cause extensive short term extracellular production with subsequent reincorporation of the organic matter that was released.

The major problem with the physiological explanation is that the extracellular production necessary to supply the excess carbon would require the photosynthetic uptake of CO_2 at a very high rate. The algae do not have sufficient carbon within their cells at the beginning of the experiment to account for the excess that was found. Also a high respiration rate would be required to release the reincorporated carbon as CO_2. The diatom would have to take up, by photosynthesis, about 3 mg C/L in an hour and excrete almost all of it into the medium as organic carbon. Subsequently, in the next 24 hours, it would have to take back up the released organic carbon and lose it as CO_2. This would amount to an hourly photosynthetic uptake and extracellular release for the diatom of 100% of its cell weight and for the blue-green alga of 50% of its cell weight. The respiratory losses could be explained with hourly rates of about 5%.

The only reference found that gives phytoplankton cell weights, and rates of photosynthesis and excretion is by Eppley and Sloan (1965). They used various ways of estimating photosynthetic and excretion rates in 5 hour incubation studies. Using their most extreme data adjusted to one hour,

a photosynthetic rate of 10% and organic excretion rate of 5% of cell weight can be calculated for the diatom Skeletonema costatum, and this was the highest rate obtained for their data. In their experiments, aliquots of larger cultures were transferred to smaller bottles for incubation under the same conditions, so that probably little cultural shock occurred.

Evidence that possibly corroborates the hypothesis of excess extracellular production in the present algal culture experiments comes from the previously discussed experiments on carbon balance. There an unexplained excess of about 2.3 mg C/L appeared after the three hours' shaking. It could be postulated that phytoplankton adhered to the flasks from the seawater rinse. When put into the filtered seawater with active bubbling under low light intensity, the cells might have photosynthesized and excreted most of the photoassimilated carbon into the medium. This would represent a cultural shock reaction during a short incubation period (3 hours) comparable to that of the algal culture experiments and the result is suggestive of a photosynthetic rate that hardly seems credible. More experimentation is needed before any more than speculation can be given to explain either the anomaly from the experiments on carbon balance or that in the algal experiments.

The rest of the results seem reasonable. Reference is again made to Table 13 and Figure 12. In the diatom experiments, no accumulation of extracellular organic carbon occurred until the end of log growth. The increases in particulate carbon (and in pg C/cell) after the end of log growth probably represent some accumulation of non-living particulate carbon. Also, there were slight increases in the filter-passing carbon after the end of log growth. There was no accumulation of filter-passing organic carbon in the medium throughout the blue-green algal culture experiment. The steady increase in particulate carbon probably represents

a large amount of non-living as well as cellular carbon since this species excretes an abundant amount of mucilaginous sheath material. There was no indication from these experiments of the production of appreciable quantities of filter-passing organic carbon during active growth. In the old cultures there were large increases in filter-passing organic carbon. The lack of extracellular accumulation during active growth by the diatom cannot be explained as heterotrophic utilization by bacteria because the heavily contaminated cultures did not show less accumulation than did the "axenic" ones.

Most of the published work on algal extracellular production has been done by incubating cultures with radioactive bicarbonate for short periods and measuring the radioactive organic carbon in the medium after incubation. A number of authors have expressed misgivings with this approach. As speculated above, the short term C^{14} incubation method could be also affected by cultural shock. Even in very carefully executed experiments, the enclosing of phytoplankton in culture flasks and the addition of the C^{14} might represent cultural shock. Hellebust (1965) has done some C^{14} experiments with marine phytoplankton in which relatively long term incubation was used as well as short periods. Table 14 has been constructed from his data. The table is included to illustrate the effect of incubation time, but effects of differences in light intensity cannot be overlooked. These five species were chosen for the table because they were the only ones that were studied under all three conditions. High light intensity and short term incubation usually showed much greater excretion than the longer incubation. Long term studies monitoring extracellular carbohydrate production in marine flagellates give evidence of no significant accumulation until after the peak of log growth (Guillard and Wangersky, 1958; Marker,

Table 14. Algal extracellular production data from Hellebust (1965); see text. Light intensity is in lux; the excretion is as percent of the photoassimilated carbon; incubation time is in hours.

	INCUBATION TIME	LIGHT INTENSITY	PERCENT EXCRETION
Thalassiosira fluviatilis			
	5	120,000	13
	48	3,000	6.2
	96	3,000	4.9
Skeletonema costatum			
	5	120,000	38
	48	3,000	9
	96	3,000	6.2
Chaetoceros simplex			
	5	120,000	18
	48	3,000	21.2
	96	3,000	22
Pyramimonas sp.			
	5	120,000	20
	48	3,000	9.1
	96	3,000	5.0
Exuviella sp.			
	5	120,000	9
	48	3,000	2.5
	96	3,000	2.4

1965). Two studies with monitoring of total organic carbon in the media of freshwater chlorophytes have been published (based upon chemical oxidation analyses). Allen (1956) found accumulation of organic matter during active growth as determined by dichromate analysis. However, her samples were not filtered. Instead the algal cells were removed by high speed centrifugation, so it is difficult to compare the results with those of measurements in filtrates. Forsberg and Taube (1967) used persulfate oxidation analysis on filtrates of their cultures. They did not find much accumulation of organic carbon in the filtrates until cultures were well into log growth. On several occasions, they found initial decreases below the background carbon levels in the media.

Examining Hellebust's work (1965), only experiments with incubation of one day or longer will be considered for the following discussion. In most of the cultures that he studied, extracellular production was less than 10% of the photoassimilated carbon, but several cultures showed up to 25%. Most of his natural phytoplankton populations also showed low extracellular production (4-16%). In natural populations, values of up to 38% were found only in samples from post bloom conditions. His rates of extracellular production would seem to be higher than the results obtained here from direct monitoring of organic carbon suggest. However, his rates for relatively long term incubation were lower than those which Fogg (1966) used as a basis for his conclusions.

A direct comparison is needed between extracellular production as measured by the C^{14} method and as measured by filter-passing organic carbon. Experiments from this section showed no appreciable accumulation of filter-passing organic carbon during active growth from the one diatom and the one blue-green alga studied (under the culture conditions employed here). Large amounts of filter-passing organic carbon were indicated in old

cultures. Hence, the apparent situation was that the filter-passing carbon seen here originated mainly from the old and dying algae rather than from the actively growing ones. It is possible that much of this material was broken down in time from particulate to colloidal form and then maybe to dissolved form. Hence, rather than excretory loss of dissolved organic carbon from actively growing cells, the phytoplankton in the present study seem to contribute to the organic pool by slow breakdown of particulate organic carbon. Whether these results are fortuitous is not known and should be investigated. If the results are fairly representative of marine phytoplankton, the method of short-term incubation with C^{14} may be generating misleading information.

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APPENDICES

APPENDIX I

SPECIAL REAGENTS AND EQUIPMENT

The experiments and analyses described in this dissertation deal with very low levels of organic matter (parts per million to parts per billion of carbon). Extreme care had to be taken to avoid contamination. Laboratory distilled water contains sufficient organic matter to cause difficulties; "doubly distilled" and "ultrapure" waters were used instead. The doubly distilled water was routinely produced in the laboratory of P.J. Wangersky. It was made by slow redistillation of water from the laboratory supply; condensation was preceded by a long, vertical, reflux column that was packed with glass-chips. The ultrapure water was made in a still adapted from a prototype that had been constructed by W.D. Watt. In the adapted still, steam from doubly distilled water was slowly passed, in a stream of oxygen, through a 1000 C combustion tube packed with quartz chips and was then condensed into an air-locked collection bottle.

The benzoic acid that was used for total organic carbon standards had been recrystallized. All other chemicals were reagent grade.

Most of the equipment that was used is readily available from laboratory supply houses. Special items are referred to in the text by the manufacturer's name or his trade name; the manufacturers of these items are:

Beckman - Beckman Instruments Incorporated, Fullerton, California.

Chromotronix - Chromotronic Incorporated, Berkeley, California.

Coulter Counter - Coulter Electronics Sales Company, Toronto, Ontario.

Coulter Counter - Coulter Electronics Sales Company, Toronto, Ontario.

Diaflo - Amicon Corporation, Lexington, Massachusetts.

Electro-Applications - Electro-Applications Incorporated, Canonsburg, Pennsylvania.

Honeywell - Honeywell Controls Limited, Toronto, Ontario.

Infotronics - Infotronics Corporation, Houstin, Texas.

Millipore - Millipore Limited, Montreal, Quebec.

Nomarski - Carl Zeiss Canada Limited, Montreal Quebec.

Niskin - General Oceanics Incorporated, Miami, Florida.

Selas - Selas Flotronics, Spring House, Pennsylvania.

Sterifil - Millipore Limited, Montreal, Quebec.

Swagelok - Crawford Fittings Canada Limited, Niagara Falls, Ontario.

Swinnex - Millipore Limited, Montreal, Quebec.

Vortex - Vortec Corporation, Cincinnati, Ohio.

Zeiss - Carl Zeiss Canada Limited, Montreal, Quebec.

APPENDIX II

OBSERVATIONS AND ERRATIC RESULTS FROM THE COMBUSTION ANALYZER

As the analyzer is not recommended for routine use in its present form, a rather detailed catalogue is made of problems that have been encountered in its development. These observations are grouped in the following order: oxidation, gas flow, construction materials, inorganic chemistry, water problems, and infrared analyzer.

For accurate carbon analysis; all organic compounds must be completely converted to CO_2. Most organic compounds will decompose at temperatures below 650 C. A higher temperature and catalyst serve primarily to speed decomposition in the relatively short period that the compounds are in the combustion tube. Oxygen, as the carrier gas, helps insure complete oxidation to CO_2. CuO has been used as the catalyst in place of platinized asbestos; no difference in oxidation was detected. With no catalyst in the combustion tube (quartz wool packed in its place), the CO_2 peaks tailed slightly, overall peak areas were slightly reduced, and the machine baseline was less stable. The analyzer behavior with the combustion tube at a lower temperature also indicated slower and incomplete oxidation. The regime of temperature and catalyst chosen seems sufficient to give good reproducible sharp peaks that probably represent total oxidation of all the organic compounds found in seawater.

The fairly high rate of flow of oxygen (225 ml/min) was necessary for rapid and complete flushing of the sample from the injection needle. Lowering the flow rate to about 150 ml/min seemed not to increase the oxidation as determined by running standards and samples at both rates.

A lower rate of flow did, however, give larger peak areas. This was because the evolved CO_2 remained in the infrared analyzer sample cell longer than when the higher rate of flow was used. The rate of 225 ml/min gave fairly sharp narrow peaks that were preferable to the blunter, broader peaks of the lower rate of flow and also gave complete flushing of the injection needle. With a rate of flow higher that that used there was the risk of too rapid a transit of the sample through the combustion tube which could cause reduced oxidation.

The 25-mm OD quartz tubing that was originally used for the combustion tube proved unsatisfactory; it shattered when the furnace was turned off. This was probably due to a combination of devitrification and salt fusion at the injection site, which had a temperature of 1000 C. The 13-mm OD quartz tubing, with the injection site at a temperature below 800 C, was quite satisfactory. The larger diameter tubing served adequately in the oxygen preburner. Stainless steel tubing seemed, overall, to be inferior for this high temperature work. As the oxygen preburner tube, stainless steel packed with CuO and kept below 950 C, lasted about 4 days. Quartz of the same diameter lasted more than a month. At 1000 C, the stainless steel tube disintegrated sufficiently to leak within 24 hours. With platinized asbestos as the packing, the life of the stainless steel tube was slightly extended. Quartz, packed with platinized asbestos, seemed to last indefinitely even at 1000 C. Due to salt and moisture effects, the lifetime of the combustion tube was shorter than that of the preburner tube. However, 13-mm quartz combustion tubes packed with platinized asbestos lasted over a month with little apparent decrepitude. Relative life expectancies of injection needles were similar. A stainless steel needle had to be replaced at least daily if the analyzer was heavily used. The

stainless steel backflow needles lasted at least several months. The lifetime of a quartz injection needle was longer, but variable. It ranged from several days to several weeks. An added attraction of quartz needles is that they broke when worn out and quickly indicated poor condition (peaks suddenly dropped in height and became very broad). The stainless steel needles, as they corroded, slowly began to bend and leak and caused subtly impaired flow. As a result, peaks slowly changed throughout a run so that aliquots of the same sample gave quite different peak areas at the beginning and end. The long 18 gauge needles placed in a horizontal plane, as were used in the original setup, showed impaired flow from needle bending and from salt clogging. The salt clogging problem led to much experimentation with placement and bore of injection needles. When the seawater sample abruptly encountered the high temperature, the resulting expansion produced pressure waves backward and forward. The water vapor primarily went through the tube to its exit. The salt stayed at the injection site when the temperature was below 800 C but it accumulated in a region vertically above the sample entry point. If the needle bore were too large and "sample hangup" occurred, salt also formed inside the needle. When the needle was horizontal, the salt formation also often cemented it to the combustion tube. An ideal injection angle might be vertical from below, but this is impractical. The angle used here gave sufficient slant to overcome the salting problem but still was easily built into a single straight furnace.

NaCl and $MgSO_4$ melt at 801 and 1124 C respectively. When the injection site was kept at 750 C, the majority of the salt in the seawater sample remained there. At 1000 C, some of the salt appeared at the injection site but most of it traveled to the far end of the combustion tube and condensed at that end. Some of the NaCl and $MgSO_4$ were removed at 750 C and various

other salts, especially major ion oxides, were removed; however, the majority of the salt remained at the injection site. It was thus easily removed from the tube. CuO decomposes at 1026 C and if it is used, the combustion tube should not be raised to this temperature. At 950 C, in the presence of chlorides and water, considerable decomposition apparently took place (a green compound, grossly resembling $CuCl_2$, appeared in the condensate). At higher temperatures, there is apparent fusing of copper to the steel or quartz combustion tubes. Pt is thermally quite stable; it melts at 1774 C and is very unreactive except in the presence of chlorides. Since chlorides are present in the combustion tube, some of the platinum is probably stripped away and platinized asbestos should be periodically replaced to insure good catalysis.

On the Hudson, a seawater-cooled condenser was used to remove the water vapor from the combustion products. The analyzer was originally set up in Antarctic waters where the condenser worked well; but in the tropics, with surface seawater temperatures near 30 C, the cooling was inadequate. With inadequate cooling, water vapor became a serious problem. The Vortex Tube, used with laboratory compressed air (a gauge pressure of about one kg/cm^2) gave a fairly constant flow of air at about 12 C. Good water removal resulted. Minimal contact of the carrier gas with water will also aid in keeping water vapor low. The condenser water collector allowed periodic flushing which reduced water contact. The $Mg(ClO_4)_2$ drying column was necessary since appreciable amounts of water passed the cold air condenser. Some water also passed the drying column (as observed by trapping it in a dry ice trap), but not enough to affect the analysis adversely. The infrared analyzer dectors were filled with dry CO_2 to give specific detection. However, there are minor overlaps in the H_2O and CO_2 infrared absorption

spectra; so H_2O will give slight interference. For accurate analysis of small amounts of CO_2, a minimal and constant H_2O level is desired.

Since the infrared analyzer is used as a flow-through machine, it should be possible to compensate for background CO_2 and H_2O in the carrier gas. This is true only to a limited extent. The pressure surge from gas expansion in the combustion tube causes irregularities of flow. Such irregularities cause a broken baseline if there is much contamination in the carrier gas. The CO_2 from an injected sample was detected in the analyzer sample cell for about 1.2 minutes. By calculation, a 2 mg C/L sample would represent a detected peak of an average concentration of 1.4 ppm CO_2. Precise detection of such a small amount of CO_2 as a short discrete peak is not easily within the sensitivity of the Beckman analyzer. The analyzer has a ten turn potentiometer allowing 1000 unit readout. In early work, a gain of 1000 or 750 was used but 300 was adopted for the final setup. With a gain of 300, a 2 mg C/L sample gave a deflection of about 1% full scale on the analyzer ammeter whereas a sample of the same concentration in the persulfate oxidation setup, with a gain of about 50, gave about 20% deflection. Because of the sensitivity problem, the combustion carbon analyzer must be considered an unstable instrument. The CO_2 peaks from samples were tiny against the electronic background of the infrared analyzer. Voltage spikes, frequency shifts, and transient line noise made the analyzer practically unusable; the best performance was found late at night when domestic electrical consumption was at a minimum. In addition to the electrical vulnerability of the infrared analyzer, it is vulnerable to vibration. This limited its shipboard use. On the Dawson, when the ship ran full speed in minor swell, the vibration from the motors prohibited use of the analyzer; at lower speeds and when on station, it was satisfactory.

There were a number of serious sources of error in the carbon analyzer and because of them a great deal of experience with the instrument was needed before it could be used reliably. A new needle or quartz plug in the combustion tube, if improperly positioned, could give erratic results. If the drying column allowed too much water to pass, overly high values could occur. A slight leak in the plumbing could cause a quite large eccentricity in results. In addition to necessitating experience with the machine, the above inconsistencies contributed to the need for running standards with every sample run. By observing the variations in over 60 standard curves that have been run, this necessity is obvious. To minimize any progressive error occurring during a run, standards were always interspersed throughout the samples. After extensive experience with the carbon analyzer, it was possible to detect and remedy most errors and when necessary, to reject a day's run as having been too variable, and to reject and repeat individual injections that exceeded normal replicate variability. It was thus possible to use the combustion method reliably with sufficient experience.

APPENDIX III

STANDARDIZATION FOR TOTAL ORGANIC CARBON

Standards should be in the same form as samples for continuity. Benzoic acid in solution was used for the organic carbon analyses. This chemical was chosen because it was available as a reagent of high purity. Also, it should be sufficiently bacteriostatic to serve as a preservative, so that standards could be stored for long term use. In early work, standards were made in ultrapure distilled water (see Appendix I). Analysis by the combustion method showed that this water was not free of carbon (0.1 to 0.2 mg C/l was measured, but with very poor precision, since this was at the detection threshold of the analyzer). It is unlikely that any carbon remaining in the water after production in the ultrapure water still could be detected by the combustion method. Any carbon detected in this water must be re-introduced in condensation, collection, and handling. Water from a Millipore Super-Q purification system showed carbon values at about the same level as the ultrapure water. It is probably not feasible to produce water of greater purity without working in a controlled atmosphere. Once the nature of the carbon analyzer was well understood, it was possible to compensate for carbon blanks, and a standard solvent with low carbon was no longer necessary. Aged Sargasso seawater was used subsequently.

Sets of benzoic acid standards were made by weighing out benzoic acid and adding it to the solvent in a volumetric flask; this was the primary standard. After the benzoic acid was dissolved, a second standard was made by dilution of some of the primary standard in a volumetric flask. A set of standards consisted of two primaries, two secondaries, and a

solvent zero. Agreement of the standards in a set insured that no gross weighing or volumetric error had occurred. In a two year period, 28 standards were made and checked against each other for consistency. In the absence of contamination, the standards were stable for a long period of time (with the exception of the seawater solvent zero standards which decreased in carbon content in several months).

The regression formula for standards from a specific run was used for calculation of carbon in samples from the same run. With one exception, all regressions were linear with correlation coefficients of 0.994 or higher. A persulfate oxidation standard run of high values (2-140 mg C/L) was fitted to a parabolic regression curve.

A linear regression curve of carbon content against peak area should, within statistical limits, pass through the origin. Since the infrared analyzer obeys Beer's Law, failure of a standard line to have a zero intercept could indicate that the data was force-fitted to a straight line. With the single above exception, all standard curves considered here represented infrared analyzer response that required only a short range of the analyzer output scale and that was below optical saturation, and the curves were unquestionably linear. Therefore, the y-intercept represented a combination of solvent and machine blanks. As mentioned earlier, a solvent blank was inevitable. It can, however, becompensated for mathematically by determining its value and adjusting the carbon content of the standards to include it. This was done with standards on the combustion analyzer. The machine blank was found by running a regression of different volumes of a standard against peak areas (in this case, there can be no solvent blank). At the same time a normal regression was run, and the machine blank was subtracted from the total blank. A determination of the solvent

blank was made with a set of 5 seawater standards and a set of 3 ultrapure water standards run on three separate days. The solvent content in each of the seawater standards was calculated from the two regression formulae (this gave a total of 30 values, each being the mean of 4 replicate injections). The average of the 30 was 0.86 mg C/L. The nominal values of the standards (amount of benzoic acid added) were then superseded by the total carbon values. All other sets of standards were run against this set and their total carbon contents were computed. All standard curves for calculations of sample carboncontent were made using total carbon contents of the standards. Thereafter, variation in regression curves represented only the machine blank effect.

Experimentation with the combustion analyzer and the persulfate oxidation analyzer revealed that the machine blank varied considerably from day to day in both slope and y-intercept. The machine blank can be better understood after examining some work done with atmospheric CO_2 determinations (Sharp, unpublished data). In that work, a carbon dioxide analyzer was assembled that took discrete gas injections. It consisted of a valved sample loop, a drying column, the Beckman infrared analyzer, the Infotronics integrator, and the Honeywell recorder. The detection and readout were the same as those of the organic carbon analyzers. Standardization was done with discrete injections of known CO_2 content gas standards. For 26 standard curves run in about 20 days, a great deal of variability was found. The regression lines varied in slope and y-intercept and the intercepts ranged from posititve to negative (t-tests showed the intercepts to be significant sometimes and sometimes not). The Beckman analyzer output obviously was varying from day to day. This can be confirmed by observations from particulate organic carbon work. There, in spanning the analyzer with

carrier gas as zero and a CO_2 standard as an upscale limit, the zero and gain settings had to be varied from day to day. The variability of the Beckman output is probably due to a slight drift in the oscillator unit, slight contamination to the surface of the sample cell-detector window, and an inability to reproduce the flow rate of the carrier gas exactly. The variability seen in the organic carbon regression curves is probably also from the machine blank of the infrared analyzer.

The discrete CO_2 injection technique might seem a good way to standardize the organic carbon analyzers also. However, it will work only if the peaks from the gas injections are the same shape as those from the liquid standards. A three way valve was put in line before the ampoule in the persulfate oxidation analyzer. In this way, the gas bubbled through the water in the ampoule before entering the infrared analyzer. The peak shapes from gas injections closely resembled those from liquid standards, and standard curves run on the same days were similar in that their slopes did not differ significantly, but the intercepts did. The combustion analyzer was adapted for gas injections by putting the three way valve in front of the injection needle, so that gas samples entered the combustion tube in the same fashion as liquid samples. In this case, the gas peaks were sharper and higher than the liquid peaks, and standard curves derived by the two methods were significantly different in respect to both their slopes and intercepts. However, the curves were similar enough to show that both standardizations were roughly the same. Certainly the comparison of gas standardization to that of liquid indicates that liquid standardization in both analyzers is accurate, for the expected amounts of CO_2 are evolved from the liquid, and liquid standards are more appropriate for use in analyses of liquid samples.

APPENDIX IV

DATA ON TOTAL ORGANIC CARBON

Listings are given for replicate analyses of aliquots from single samples by high temperature combustion and by persulfate oxidation. Samples are from stations on cruises of the Hudson, Dawson, and Sackville and from cultures of the following algae: Thalassiosira fluviatilis (axenic), T. fluviatilis (unialgal), Schizothrix calcicola, Chlamydomonas sp., and an undentified chrysophyte. Samples from cruises are listed by station number and depth (in meters); those from the Dawson cruise additionally have two filtrates from 0.025-μm and 0.003-μm cutoffs (listed respectively as 25 and 3) - see text. Algal culture samples are listed in months' or days' age, as indicated. Combustion and persulfate oxidation values are in mg C/L; errors are percent relative standard error ($\pm 2\sigma_{\bar{x}} / \bar{X}$ times 100). Combustion values are means of four replicates unless otherwise indicated in parentheses; persulfate oxidation values are means of three replicates unless otherwise indicated in parentheses. An asterisk indicates that the sample value is erroneously high.

SAMPLE	PERSULFATE	ERROR	COMBUSTION	ERROR
HUDSON				
9-5	1.16	9.43	1.69 (3)	6.96
-480	0.94	4.88	1.81 (3)	3.38
-4580	0.63 (1)	10.00	2.93 (2)	1.68
10-5	1.05 (1)	10.00	1.87 (3)	4.10
-3480	0.68 (1)	10.00	1.89 (2)	8.30
11-5	0.98 (2)	3.34	1.35	4.23
-4780	0.70	4.00	1.33 (3)	2.24
12-25	1.13 (2)	8.23	2.22	6.14
-90	0.91	9.09	0.64	7.40
-680	0.88 (2)	10.04	2.14	10.04
-2980	0.72 (2)	2.23	1.34	11.07
-4280	0.75	4.75	1.40 (3)	9.90
13-5	0.78	5.90	0.80 (5)	9.85
-4980	0.63	5.14	2.26 (5)	4.21
14-5	0.80	7.36	2.19	1.63
-4980	0.56	4.75	1.88 (3)	3.63
15-15	0.67 (2)	2.73	2.25	4.82
-4380	0.31 (1)	10.00	1.38 (3)	2.35
16-15	0.75 (2)	1.91	1.25	4.35
DAWSON				
17-Sfc	1.03 (2)	2.64	1.55	8.14
-25	0.88	7.58	1.48	4.81
-3	----	----	1.11	3.11

SAMPLE	PERSULFATE	ERROR	COMBUSTION	ERROR
DAWSON				
18-4000	0.91	6.32	1.51	5.57
-25	0.96	7.80	1.41	7.17
-3	0.89 (2)	3.80	1.26	12.07
19-5	1.22	4.23	1.29	7.78
-25	1.24	0.79	1.25	8.98
-3	1.15 (2)	8.68	1.29	3.02
19-150	----	----	1.27	8.85
-25	1.05	7.07	1.03	6.98
-3	0.94	3.61	0.98	7.57
20-Sfc	1.18	4.48	1.27	5.02
-25	1.24 (2)	3.91	1.16	9.95
-3	1.15	1.20	1.07	12.84
21-2000	0.76	2.38	0.81	3.50
-25	0.79	1.50	0.69	6.69
-3	0.81 (2)	6.55	0.64	5.83
22-5	1.21	4.08	1.57	2.24
-25	1.14 (2)	4.51	----	----
-3	1.17	1.57	1.40	2.31
22-250	0.69	5.12	0.86	4.12
-25	0.70	0.29	0.90	3.51
-3	0.71	7.02	0.87	1.17
22-1000	0.63	3.14	0.89	2.89
-25	0.63 (2)	0.25	0.97*	1.69
-3	0.76* (1)	10.00	0.68	7.95
23 -5	1.08	6.79	1.74	7.07
-25	1.48 (2)	0.92	1.66	3.72
-3	1.26 (1)	10.00	1.77	7.61

SAMPLE	PERSULFATE	ERROR	COMBUSTION	ERROR
DAWSON				
23-25	1.10 (1)	10.00	1.49	7.45
-25	1.36* (2)	6.87	1.37	7.62
-3	0.99 (2)	0.59	1.21	10.30
23-50	1.02 (2)	3.34	1.56	6.38
-25	1.35* (2)	8.28	1.48	10.52
-3	0.97 (2)	5.21	1.42	9.08
23-75	1.07 (2)	5.33	1.51	11.62
-25	1.06	1.46	1.46	5.83
-3	1.09	4.81	1.26	11.31
24-15	1.28	4.13	1.44	4.82
-25	1.36 (2)	9.91	1.35	3.58
-3	1.24 (1)	10.00	1.47	3.88
24-100	1.14	9.02	1.09	10.49
-25	1.43*	3.09	0.96	6.36
-3	1.17	7.95	0.84	12.17
24-500	0.70	6.73	0.78	9.50
-25	0.72	1.49	0.57	14.43
-3	0.71 (2)	7.06	0.76	13.70
25-10	1.22	3.11	1.74	5.50
-25	1.38* (1)	10.00	1.51	5.02
-3	1.26 (2)	3.43	1.57	2.61
25-100	1.08 (2)	1.54	1.38	1.24
-25	1.25	9.91	1.17	3.88
-3	1.17	5.48	1.67*	5.66

SAMPLE	PERSULFATE	ERROR	COMBUSTION	ERROR
DAWSON				
25-1600	0.79	8.11	1.16	3.59
-25	0.80 (2)	6.87	1.01	6.23
-3	0.66	7.53	0.96	1.90
25-5100	0.64 (2)	4.09	1.08	6.25
-25	0.64	8.53	0.95	5.72
-3	0.61	3.94	1.70*	1.87
26 -Sfc	1.01	6.95	1.36	2.69
-25	1.06 (1)	10.00	1.34	4.08
-3	0.98	8.53	1.17	4.09
-Ag	1.07 (1)	10.00	1.04	3.11
-MF	0.93	1.77	0.98	4.33
SACKVILLE				
27-5	1.39 (2)	6.31	1.47	3.58
-25	1.44	8.38	1.38	3.37
-50	1.35 (2)	8.03	1.25	5.51
-75	1.04 (2)	3.03	1.18	4.53
-100	1.10 (1)	10.00	1.23	5.98
-1500	0.86 (2)	3.11	1.07	3.34
-2000	0.91 (1)	10.00	1.18	0.35
-2500	0.85 (2)	5.67	1.14	6.32
28-5	1.16	0.94	1.50	3.41
-25	1.15	6.13	1.13	2.84
-50	1.14 (2)	6.51	1.27	1.94
-100	1.02	7.81	0.83	3.17

	SAMPLE	PERSULFATE	ERROR	COMBUSTION	ERROR
	SACKVILLE				
29	-500	0.83	6.09	0.91	5.39
	-1000	0.86 (1)	10.00	0.94	3.31
	-2000	0.83	4.05	0.80	6.12
	-3000	0.80	8.82	0.84	2.34
	-4000	0.80 (2)	1.77	1.03	3.58
	-4500	0.80 (2)	2.88	1.09	1.50
30	-10	1.29 (2)	3.15	1.35	3.10
	-50	1.27	6.47	1.16	4.24
	-500	0.92	8.76	1.04	4.42
	-1000	0.87	6.54	0.83	5.80
	-1500	0.86	2.81	0.86	5.54
	-2000	0.95	5.86	0.91	3.34
	-2500	0.75	6.94	0.76	3.89
	-3000	0.89	2.77	0.95	5.72
	-3450	0.99 (1)	10.00	0.96	2.99
	-3950	0.99 (2)	2.56	0.77	5.54
31	-200	0.91	6.04	1.53	5.15
	-300	0.85	5.66	1.54	5.47
	-400	0.78	2.61	1.35	2.17
	-500	0.73	1.74	1.53	2.27
	-600	0.72	4.12	1.39	8.05
	-1000	0.79	8.73	1.34	2.38
	-1500	0.78	6.59	1.43	3.68
	-1600	0.71	1.02	1.39	2.31
	-1700	0.76	9.64	1.52	6.37

SAMPLE	PERSULFATE	ERROR	COMBUSTION	ERROR
SACKVILLE				
31-1800	0.76	9.68	1.42	2.75
32-10	1.28 (2)	7.89	1.49	2.42
-50	1.11	2.97	1.38	6.01
-100	0.85	5.36	1.22	4.34
-500	0.76 (2)	7.54	1.16	5.15
-1000	0.80	2.78	1.08	8.09
-1200	0.68	6.55	1.14	5.16
-1400	0.79	4.06	1.07	4.84
-1600	0.74	4.49	1.00	8.25
-1800	0.72 (2)	9.31	1.24	7.18
-2000	0.70	4.79	1.07	5.08
T. fluviatilis (axenic)				
0 days	10.28	1.35	10.98	0.71
1	2.47	7.99	2.95	4.24
2	2.58	4.35	2.40	4.34
4	2.69 (2)	5.30	2.43	2.88
6	2.80 (2)	4.00	2.90	6.12
8	2.90 (2)	0.24	2.97	1.63
10	4.80 (2)	0.83	3.52	6.03
15	6.65	1.92	6.97	1.96
20	6.10	2.55	5.93	3.16
25	8.32	5.65	6.44	0.94
39	19.26	3.87	16.40	3.09
101	28.21 (2)	8.46	28.41	1.52

SAMPLE	PERSULFATE	ERROR	COMBUSTION	ERROR
T. fluviatilis (unialgal)				
0 days	11.33	3.83	10.73	0.41
1	3.51	5.79	3.26	4.56
2	3.16 (2)	3.32	3.58	1.46
4	3.36	2.48	3.66	3.30
6	3.92 (1)	10.00	3.20	1.15
8	4.56 (2)	5.70	4.29	3.77
10	6.77	6.47	3.90	5.02
15	5.03 (2)	1.17	4.84	1.81
20	4.72 (2)	1.68	5.68	2.44
25	4.85	5.98	4.75	1.53
S. calcicola				
0 days	24.66 (1)	10.00	24.33	0.56
1	16.37	2.25	17.84	0.87
2	14.36 (1)	10.00	14.74	1.29
4	14.06	5.56	12.48	1.79
6	16.82	2.21	13.43	1.06
8	14.60 (2)	6.80	15.17	0.66
10	14.50	3.98	12.53	3.31
15	13.86	1.36	12.10	0.73
20	14.82	2.38	14.34	0.74
25	15.82 (2)	4.46	14.02	1.48
27 months	79.08 (2)	0.66	76.48	0.86

SAMPLE	PERSULFATE	ERROR	COMBUSTION	ERROR
Chlamydomonas sp.				
9 months	119.03	3.13	110.38	1.49
12	128.45	4.00	109.35	1.06
14	151.45 (2)	9.67	134.77	0.80
18	162.20	3.97	136.47	0.47
unidentified chrysophyte				
9 months	169.55	5.50	143.97	1.29
12	234.02	1.63	186.33	1.17
14	239.53	2.25	204.72	0.99
18	241.45	3.06	201.60	0.85

APPENDIX V

STAINING AND MICROSCOPY

In experiments on particle formation, replicates for microscopy usually consisted of three stained filters mounted on slides. The third replicates from the Dawson cruise samples were unstained and were mounted directly from the sample filter holders. Staining procedures were similar to those of Gordon(1970). His periodic acid-Schiff's reagent solutions and regime were used. The bromophenol blue stain was used with suggestions by Mazia, Brewer, and Alfert (1953) and Kunkel and Tiselius (1951); the aqueous staining solution was used rather than the alcoholic one employed by Gordon. Additionally, an osmic acid staining regime was used (after Gurr, 1962). The staining reagents and regimes are given in Table 15. The osmic acid stain was re-used; all other reagents were discarded upon being flushed from the staining apparatus. Bottles for staining solutions were cleaned with chromic acid, and new staining solutions were made often. Staining apparatuses were cleaned in a manner similar to the filtering setups used in the experiments on particle formation. All Millipore filters were pretreated by boiling in doubly distilled water and rinsing with the same before placing in filter holders. After sample filtration, a filter was transferred to a staining apparatus and stained; one apparatus was used throughout for each staining regime.

A staining apparatus somewhat different from that used by Gordon (1970) is illustrated in Figure 13. The teflon gasket was needed because the Swinnex-25 filter holder, used for sample filtration, had a larger surface area than the 25-mm glass one that was used for staining. The staining

Table 15. Solutions and regimes for staining. Solutions were stored at room temperature unless otherwise noted; all water was doubly distilled.

Periodic acid-Schiff's Reagent

Acid

1 gm H_5IO_6 in 100 ml H_2O

Stain

1 gm basic Fuchsin in 100 ml boiling H_2O, cool to about 50 C

add 1 ml conc HCl

add 2 gm $K_2S_2O_5$,

leave at room temperature overnight

decolorize with 0.5 gm activated charcoal, filter through Whatman #1 paper

store in refrigerated dark bottle

Regime

2.5 ml acid for 10 min

5 ml H_2O rinse

2.5 ml stain for 10 min

5 ml H_2O rinse

Bromophenol blue

Stain

10 gm $HgCl_2$ in 100 ml H_2O, heat to enhance solution

add 2 ml conc acetic acid

add 100 mg bromophenol blue, cool to room temperature

filter through Whatman #1 paper

Acid

1 ml conc acetic acid in 100 ml H_2O

Regime

2.5 ml stain for 10 min

2.5 ml acid for 5 min

5 ml H_2O rinse

5 ml H_2O for 3 min

Osmic acid

Stain

0.25 gm OsO_4 in 25 ml H_2O

store in refrigerated dark bottle

Regime

heat about 2.5 ml stain in test tube until boiling

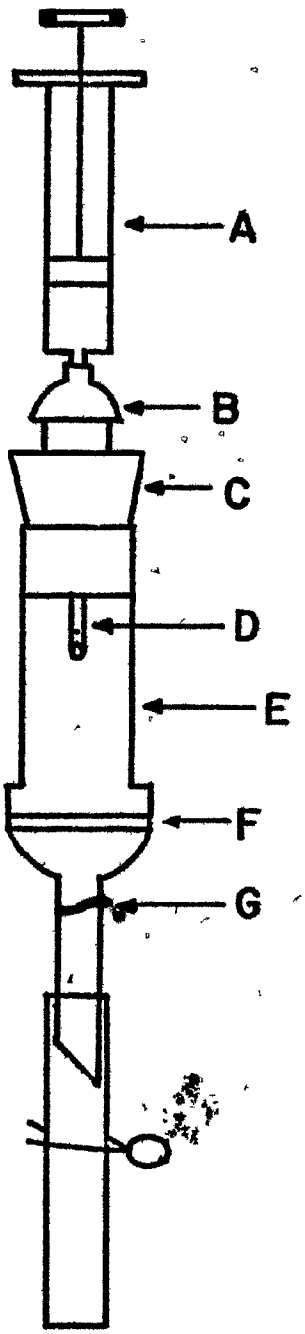
2.5 ml stain for 5 min

5 ml H_2O rinse

CAUTION

The osmic acid staining solution was always made up in a fume hood. Heating and staining by osmic acid was always done also in a hood.

Figure 13. Staining apparatus. A-hypodermic syringe (Glass with rubber plunger). B-Swinnex-13 filter holder with 0.22-μm filter. C-number 0 silicone rubber stopper. D-3-mm OD silicone rubber tubing. E-top of 25-mm diameter Millipore glass filter holder. F-teflon gasket. G-bottom of the filter holder. E and G are held together with a spring clamp; G is closed at the bottom by a pinch clamp on 6.4-mm OD silicone rubber tubing.


A
B
C
D
E
F
G

procedure follows: With the bottom pinch clamp closed and the silicone stopper not tight, a staining solution was introduced by syringe through the filter in the Swinnex-13 holder onto the sample filter. The stopper was then pushed in tightly. For flushing, the pinch clamp was opened and the staining solution was expelled by pressure from the air-filled syringe. After staining, filters were transferred to ethanol-cleaned microscope slides, dried in a 60 C oven with the filter on a slide in a closed Petri dish, and were mounted with Permount under ethanol-cleaned 22-mm square coverslips. Numerous filter blanks were made.

Sample filtration by gravity caused only mild pressure on the filter (about 0.1 kg/cm^2 gauge pressure) so that particle distortion by filtering pressure was minimal. Unfortunately, extensive flattening of non-rigid spherical objects on the filters was unavoidable. In an attempt to normalize chemical alteration of organic matter on the filters, all stains were used as aqueous solutions. Some distortion did probably occur from alterations by fixatives (the periodic acid, mercuric chloride, and osmic acid act as fixatives). Also, alteration of material on the filters was caused by the acidic solutions that were included in each of the staining regimes. Thus, all calcareous material was removed (abundant coccoliths were found both loose and attached to coccolithophorids on unstained slides while none were ever found on stained slides). Extreme care was taken to avoid contaminating the filters with materials or by procedures used in the filtration of the samples and staining. Blanks were needed nonetheless.

Because particle formation can occur in filtrates even without bubbling, the use of filtered seawater for making filter blanks was not satisfactory. Blanks were usually made by treating blank filters like sample filters except for seawater filtration. Blanks made by passing about 25 ml of

dialyzed seawater through the filter indicate that there is no noticable salt effect; therefore, dry filter blanks are acceptable. All the staining solutions were filtered through 0.22-μm filters immediately prior to contact with the sample filters, and the sample filters had been carefully cleaned with boiling distilled water prior to use. In spite of these precautions, all blanks (including unstained ones) had some particulate matter on them which probably originated mainly from handling. In the osmic acid staining procedure, apparently some degradation of the staining apparatus also contributed to the organic matter. In particulate organic carbon analysis, filter blank values were subtracted from sample values. Since most of the microscopic work was qualitative, precise blank subtraction could not be done. A rough visual adjustment was attempted when examining sample filters.

For microscopic examination and photography of material on the filters, a Zeiss Universal microscope with attached camera was used. From the beginning of the use of membrane filters in oceanographic research, attempts have been made to improve contrast of retained material for microscopic viewing. Zeiss' Nomarski differential interference contrast optics offer a great advantage in this endeavor. Padawer (1968) discussed details and pitfalls of the principle of Nomarski optics. The advantage of this system for the present work is that very high resolution and good contrast can be afforded for even very thin ephemeral matter on the filters. These optics were specifically designed for enhanced visibility of transparent objects; the physics of differential interference insures increased discrimination of slight differences in refractive indices. The cleared Millipore filter has approximately, but not exactly, the same index of refraction as the glass slide and coverslip. The slight difference between the slide and filter indices causes the filter to be an effective light diffuser so that

resolution of objects above the filter is degraded. Both bright field and phase-contrast optics depend upon a depth-of-focus plane sufficiently thick so that this diffusional resolution loss is inescapable. Nomarski optics allow an effective plane of focus thin enough so that objects can be focused upon sufficiently above the filter to escape most of this diffusional effect. Additionally, due to the greater discrimination of refractive indices, the filter can be clearly focused upon with Nomarski. It must be noted that the improved resolution of Nomarski over other optical systems applies to the case of material on the filters and that it is not necessarily an inherent quality of the optics. The reason for this is that Nomarski optics avoid resolution loss that the other optical systems cannot avoid. Optimally, Nomarski optics can be employed for distinction between planes of focus of less than 10 Angstroms (Padawer, 1968). With the great object discrimination plus the very thin plane of focus, it is possible effectively to slice optical sections through an object on the filter by changing the fine focus. As Padawer has cautioned, the apparent bas-relief image from Nomarski is somewhat anomalous; the relief is dependent upon both thickness and refractive index of the object and hence is not strictly geometric. Additionally, one cannot usually differentiate between a protrusion and an inclusion on the surface of an object. With acknowledgement of these limitations, Nomarski optics can be used to view the fine details of very thin and transparent objects on Millipore filters. Figure 14 shows the same microscope field viewed with bright field, phase-contrast, and Nomarski optics. As well as greater discrimination of details of objects on the filter, the filter itself is better viewed with the Nomarski optics.

The three staining procedures were used in an attempt to chemically characterize the organic particles. The periodic acid-Schiff's Reagent

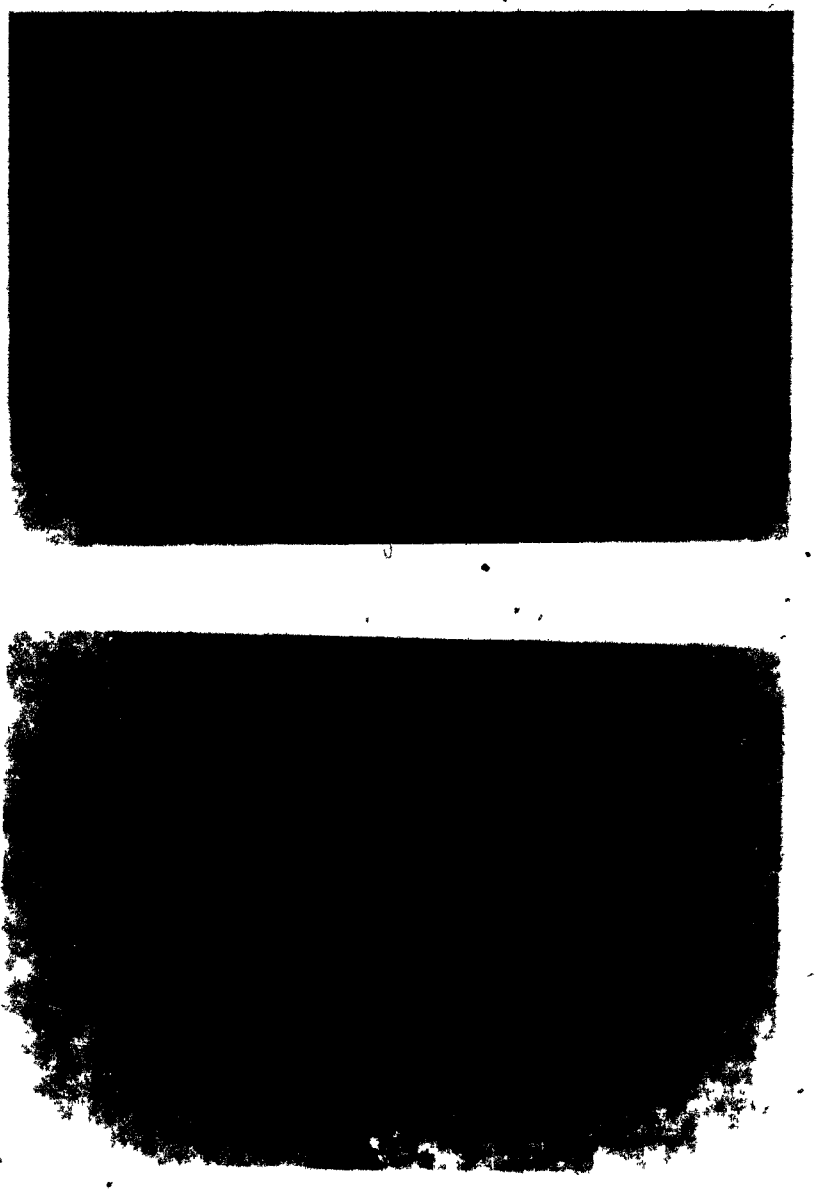


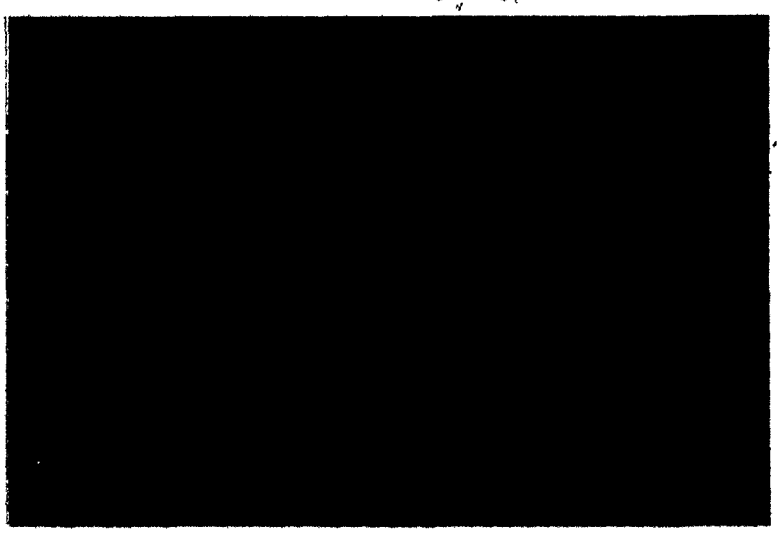
Figure 14. Unstained aggregate particle with diatom and coccolithophorid remnants and coccoid cells (from deep water). Bars represent 2 μm. A-bright field, B-phase contrast. C & D-Nomarski, two optical planes.

C

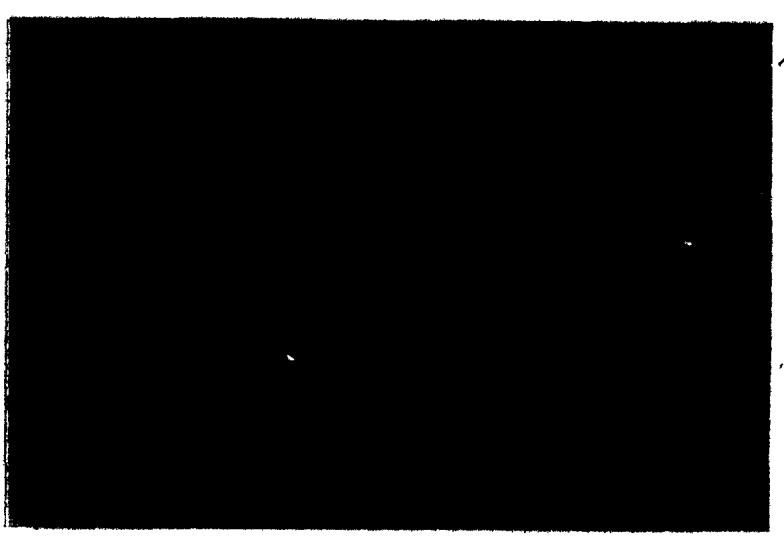
D

Figure 14. Continued.

should be specific for carbohydrate, bromophenol blue for protein, and osmic acid for lipid. Cytochemical specificity is usually stated for known regions of tissue rather than for amorphous non-cellular matter on membrane filters. The morphology of the particlulate organic matter is quite variable; one would expect the chemical composition of particles also to be rather variable. Hence, a single particle or a single region in a particle could easily give positive reactions to more than one specific chemical test. Because of this heterogeneity, one must interpret qualitative cytochemical results with caution; quantitative conclusions are impossible. In deciding upon criteria for positive staining diagnoses, phytoplankton and zooplankton on filters were examined. Since these are better known biochemically than non-living particles, their staining behavior should give some information on how the stains react on material retained on the Millipore filters. Figure 15 shows the dinoflagellate Ceratium sp. stained with the three stains and unstained. The unstained speciman is a poor one and not form the same collection as the three stained ones (they are all from aliquots of the freshly collected sample of series 2 of Table 1[illegible] so it may be a different species and of different physiological condition than the others. The periodic acid-Schiff's Reagent stained the cellulose exoskeleton, membraneous webbing within the horns, and the central protoplasm all red. The bromophenol blue stained discrete protoplasmic arms within the horns and central protoplasm green; also the nucleus is clearly seen as a more intensely stained area (red). Though the osmic acid has grey-brown staining of material similar to that of the periodic acid-Schiff's Reagent, probably the only positively stained materials are circular inclusions within the central protoplasm (the dinoflagellates presumably were in a late bloom condition and had built up some lipid stores). With these and other



A



B

Figure 15. Ceratium sp. on Millipore filters. Bars represent 5 μm. A-unstained. B-stained with periodic acid-Schiff's Reagent. C-stained with bromophenol blue. D-stained with osmic acid.

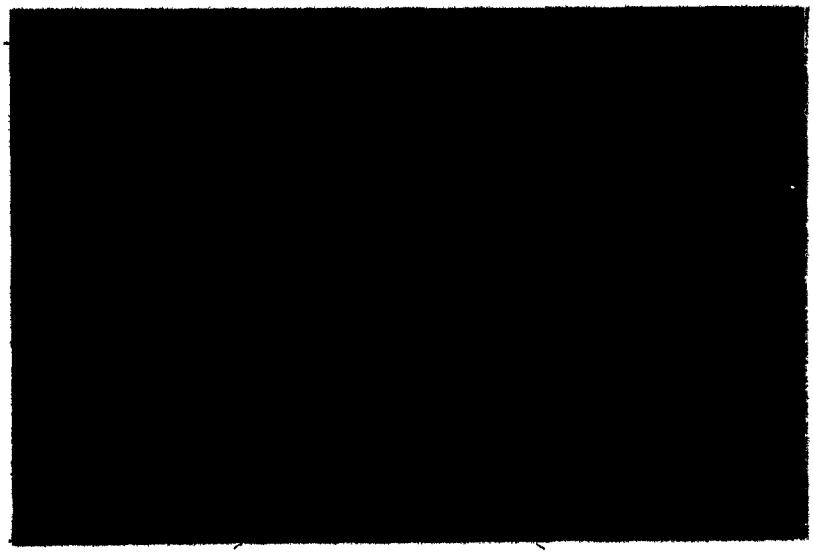
C

D

Figure 15. Continued.

observations, the interpretation of positive staining behavior was: carbohydrate - red with periodic acid-Schiff's Reagent; protein - green or blue (very intense, staining becomes red, orange, or purple) with bromophenol blue; lipid - intense black with osmic acid. The lack of positive staining is also important for cytochemical specificity. Thus with the advantage of the Nomarski optics, transparent unstained matter was found on slides that were stained with each of the procedures. With bromophenol blue, straw yellow staining was not considered positive and with osmic acid, grey-brown staining was not considered positive. Figure 16 shows naturally occurring particles unstained and with positive staining by the three procedures.

In many of the experiments in the section on particle formation, replicates for microscopic examination were made in addition to replciates for carbon analysis. From these, some general points can be made. Carbohydrate was the most common chemical grouping in all cases; protein was usually found in both naturally occurring organic matter and in particles formed in filtrates; lipid was often found in organisms and in their fragments, but rarely was found in particles other than as inclusions, most of which probably were bacterial cells. This chemical characterization is similar to that reported by Gordon (1970) for naturally occurring particulate matter. As discussed by him, "flake" particles seemed to be predominately protein and "aggregates" were predominately carbohydrate (though both types stained for both chemicals in some cases). The indistinct blotches, mentioned in the main text, stained primarily as carbohydrate with a little protein. Of the particulate matter formed in filtrates, the flakes were the most abundant and they were made of protein and carbohydrate; aggregates that were mainly carbohydrate were also formed. The formed particulate matter did not include organisms other than a few distinct circular objects that were probably bacteria.



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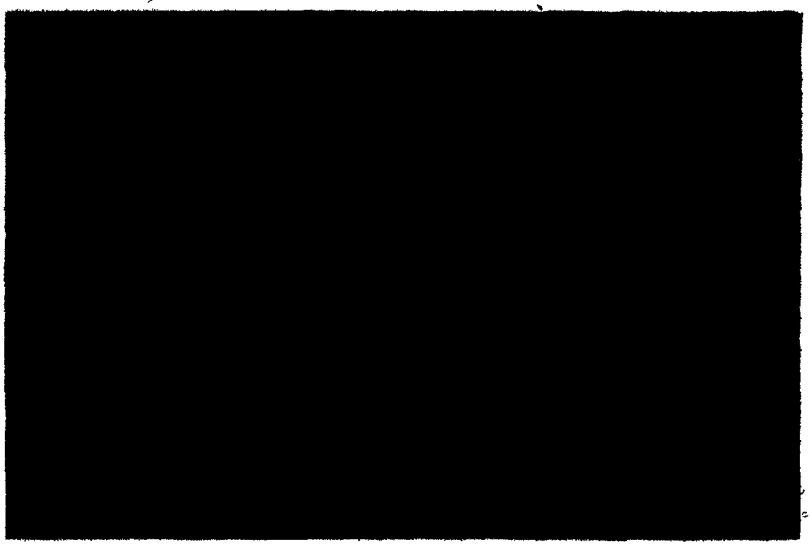
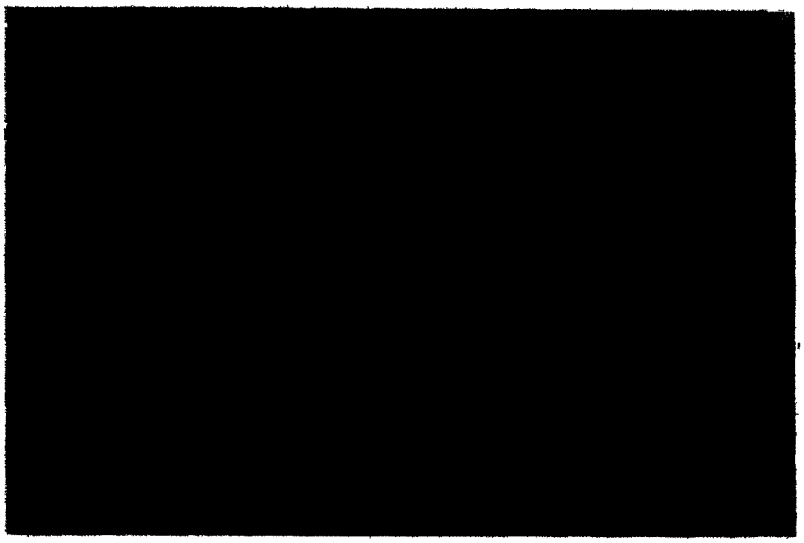


B

Figure 16. Naturally occurring organic particles. Bars represent 2 μm. A-unstained flake. B-aggregate stained with periodic acid-Schiff's Reagent. C-flake stained with bromophenol blue. D-aggregate stained with osmic acid.

C



D

Figure 16. Continued.

APPENDIX VI

DATA FROM PARTICLE FORMATION EXPERIMENTS DONE AT SEA

Particulate organic carbon yields are in µg C/L. Standard errors of the mean and number of replicates upon which they are based are given in parentheses ($\pm 2\,\delta_{\bar{x}}$ / n).

SAMPLE	INITIAL	FILTRATE FORMED
St. Margaret's Bay		
1. September	92.0 (18.8/3)	24.0 (8.5/3)
2. October	78.0	28.7
Cruise stations		
1. Sfc Gulf Stream	90.0 (11.1/3)	27.7
2. Sfc·Sargasso Sea	45.4 (1.6/3)	28.0
3. Sfc Sargasso Sea	32.3 (4.2/3)	24.1
4. 1000 m Sargasso Sea	6.3 (1.7/3)	45.4
5. 1500 m slope	5.6 (2 5/2)	-0.8 (3.6/3)
6. 10 m shelf	37.0 (3.0/3)	21.8 (7.0/3)
7. Slope		
3 m	31.8	7.4
100	6.7	-0.5
500	2.2	0.2
1000	1.8	-2.8
1500	3.5	-4.1
2000	3.5	0.2

SAMPLE	INITIAL	FILTRATE-FORMED
Cruise stations		
8. Shelf		
Sfc	32.6	22.2
10 m	23.0	27.6
25	30.0	13.0
50	32.3	21.4
75	22.4	1.2
100	15.0	0.6
17. Sfc Sargasso	37.1 (9.4/3)	14.6 (1.1/3)
18. 4000 m Sargasso	5.2 (2.5/3)	4.4 (3.7/3)
19. Caribbean		
5 m	30.5	14.4
25	35.6	12.2
75	37.2	21.1
100	28.9	17.5
150	28.1	19.0
20. Sfc Caribbean	29.7 (9.3/3)	15.3 (7.4/3)
21. 2000 m Caribbean	13.5 (1.2/3)	10.0 (2.6/3)
22. Caribbean		
5 m	44.5	26.5
50	37.8	26.3
250	13.5	10.9
500	10.6	21.1
1000	9.0	12.2
2500	8.6	7.0

SAMPLE	INITIAL	FILTRATE-FORMED
Cruise stations		
23. Caribbean		
5 m	34.5	15.3
25	45.8	17.7
50	36.8	21.9
75	32.9	22.0
100	44.8	17.6
800	28.3	17.4
24. Caribbean		
15 m	35.0	16.7
100	21.3	18.4
500	21.6	13.2
25. Puerto Rico Trench		
10 m	40.8	21.0
100	31.2	72.5
1600	19.1	11.7
5100	13.3	14.5
26. Sfc Sargasso	28.0 (0.7/2)	21.3
27. Sargasso		
Sfc	26.8	26.6
10 m	31.2	63.3
50	29.8	64.1
1000	19.6	28.3
3000	18.0	26.5

END

28 • 11 • 74

FIN