NATIONAL LIBRARY
OTTAWA

CANADA

BIBLIOTHÈQUE NATIONALE
OTTAWA

NAME OF AUTHOR John Russell Wheeler

TITLE OF THESIS Some effects of solar levels of ultraviolet radiation on the dissolved organic constituents of surface waters

UNIVERSITY Dalhousie

DEGREE FOR WHICH THESIS WAS PRESENTED Ph.D.

YEAR THIS DEGREE GRANTED 1972

Permission is hereby granted to THE NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

(Signed) [redacted]

PERMANENT ADDRESS:

6297 Yale St.
Halifax, N.S.

DATED October 11 1972

NL-91 (10-68)

SOME EFFECTS OF SOLAR LEVELS OF ULTRAVIOLET RADIATION ON THE DISSOLVED ORGANIC CONSTITUENTS OF SURFACE WATERS

by

John R. Wheeler

Submitted in partial fulfillment of the requirements for the degree
of Doctor of Philosophy in Oceanography at Dalhousie University,
August, 1972

Approved by:

[redacted]

DALHOUSIE UNIVERSITY

Date Sept. 30, 1972

Author John Russell Wheeler

Title Some Effects of Solar Levels of Ultraviolet Radiation on the Dissolved Organic Constituents of Surface Waters

Department or School Oceanography

Degree Ph.D. Convocation - Fall Year 1972

Permission is herewith granted to Dalhousie University to circulate and to have copied, at its discretion, the above title upon the request of individuals or institution.

[redacted]

Signature of Author

CONTENTS

METHODS

Reaction vessels and ultraviolet radiation

Experimental procedure

I. Studies with reaction vessels

RESULTS

Lipids

Proteins

Rate of accumulation of surface pressure

Film dynamics

DISCUSSION

The interaction of proteins with radiation

Lipids

APPENDICES

ABSTRACT

The effects of solar levels of ultraviolet radiation were investigated on selected lipids and proteins in sterile artificial sea water, and on natural and model organic surface films.

Unconjugated linoleic acid, upon irradiation, developed increased absorption at 234 nm, indicating diene conjugation. Unconjugated linolenic acid after irradiation absorbed more intensely at 234 and 269 nm, indicating diene and triene conjugation, respectively. Some formation of particulate material was noted after several hours of irradiation of either lipid, the particles being retained on a filter of 0.8 u pore-size. Oleic acid showed no significant change in its absorption spectrum and no development of filterable particles. A lipid extract of the marine diatom <u>Thalassiosira</u> <u>fluviatilis</u> was also indicated to undergo diene conjugation.

Trypsin and ovalbumen, when irradiated, came out of solution as discrete particles, some of which bore strong resemblence to some organic particulates found in sea water. Their film-like morphology indicated that they were formed at interfaces.

Radiation was found to increase significantly the rate of accumulation of surface pressure in sea surface films mildly enriched with protein. When certain natural and model organic films were subjected to increased surface pressure they tended to collapse, the film organics being transformed into a new state; this state was capable of being disrupted into small particles that tended to sink.

The present research is discussed with regards to the fate of dissolved organics and surface films.

ACKNOWLEDGEMENTS

This research was carried out under the able supervision of Dr. Gordon A. Riley, who also supplied laboratory space and research funds. I also thank Dr. Peter J. Wangersky for the use of equipment and for invaluable discussion.

I am grateful to the following for their assistance: Drs. Robert C. Cooke and Edward R. Baylor provided helpful criticism of the manuscript. Drs. Peter J. Wangersky, Walton Watt and Jonathan H. Sharp were instrumental in the development of the high-temperature still. Shirley A. M. Conover graciously supplied the diatom lipid extract and made several valuable suggestions. Norval Balch, Alan Pease and many other students of the Dalhousie Institute of Oceanography deserve thanks for their useful comments and technical assistance. The Bedford Institute of Oceanography, under the direction of Dr. William Ford, supplied time and space aboard the *C.F.A.V. Sackville*.

I gratefully acknowledge receiving financial support from the National Research Council of Canada and from Dalhousie University during the course of this study.

INTRODUCTION

The organic material of natural waters has been implicated in several significant geochemical processes. Both dissolved and particulate organics, for instance, are a source of the kerogen of sediments, which in turn may be the precursor of petroleum. Duce et al. (1972) have indicated that interfacial organics are involved in sea surface enrichment of certain metals, and this in turn may affect aerosol fractionation. Also, dissolved organics can influence aquatic ecology, and thus alter the distribution of non-conservative elements in the water column.

Despite the importance of dissolved organics in aquatic environments, our knowledge of organic chemical reactions that occur in natural waters is limited. The delineation of organic reactions occurring within a given parcel of sea water is rendered difficult because the dissolved organic substances that are present in sea water are of varied nature and molecular weight; also, most of the organic material has not been characterized. Thus, interpretation of organic processes observed in sea water, in terms of reactions, is usually not possible. It is perhaps more gainful to investigate the reactions of naturally-occurring concentrations of known organics in an artificial sea water medium. As the system would be throughly characterized, observed phenomena could be interpreted in terms of the reactions of the few substances added. However, distilled water, which would serve as the solvent in the sea water medium, frequently contains concentrations of organics equal to or greater than sea water concentrations. These dissolved organics tend to separate from distilled water as particulate material (Wangersky, 1965).

Clearly, if we wish to investigate reactions of naturally-occurring amounts of organics, and especially if we wish to determine whether particulate material is a product of the reactions, a method must be found for cleansing distilled water of its relatively high concentrations of dissolved organic substances. A high-temperature combustion method has been developed for producing water of very low organic content; by careful preparation of salt, it is possible to produce artificial sea water of such low organic content that separation of any inherent substances is so minimal as not to interfere with the observation of particulate reaction products. Thus, particulate material produced in the reaction vessel can be readily interpreted in terms of the reactions of the added reagents. By incorporating a source of simulated solar radiation into the vessel, we may hope to gain insight into solar photochemical processes that may occur at the sea surface.

This research is a study of the effects of simulated solar ultraviolet radiation on the dissolved organics of surface sea water. These effects were investigated at several levels: A. in artificial sea water studies were undertaken of 1) selected lipids, 2) selected proteins, 3) a lipid extract of a marine phytoplankter, 4) lipid and protein together, and 5) protein surface films; B. in natural sea water and lake water studies were carried out on the effects of this radiation on the physical chemistry of natural organic surface films, and on natural films with added organics. By investigating the actions of solar wavelengths and intensities of ultraviolet at several levels of substrate complexity, we hoped to gain an appreciation of the role of this high-energy fraction of sunlight in the carbon cycle of the sea.

The organic composition of the ocean surface

General

The origin of the dissolved organics of sea water is the biosphere. As many biological substances are surface-active, the sea-air interface affords a site for the accumulation of surface water organics. Figure 1 illustrates the distribution of dissolved organic carbon with depth in the ocean. The data for dissolved carbon at 15-20 cm and also in the upper 0.15 mm, are from Williams (1967); the remainder of the data is from Menzel and Ryther (1968). In the laboratory, Jarvis (1967) has shown that the rate of accumulation of surfactants at a sea water-air interface is increased several fold by both stirring and bubbling of the bulk solution; at the ocean surface such an increased rate of accumulation may be effected by turbulence and bubbles produced in breaking waves. Garrett (1967) has found the major chloroform-soluble constituents of the sea-air interface to be fatty acids, fatty acid esters, fatty alcohols, and hydrocarbons. He believes (Garrett, 1970) that proteins may also be significant in modifying the surface properties of sea water.

The sea surface is also enriched in particulate organic carbon (Williams, 1967), which may be transported to the interface or possibly formed in situ. Williams' data for particulate organic carbon, particulate organic nitrogen, and dissolved organic nitrogen in surface films and subsurface waters is given in table 1. In all cases, concentrations are higher (at times ten to twenty fold or more) in the upper 150 u of the sea that at 15-20 cm depth. Also, all concentrations are higher at 15-20 cm than at greater depth (10m - 74m).

Unsaturated lipids

Unsaturated fatty acids constitute a significant fraction of marine organisms in general (Lovern, 1964), and of diatoms in particular (Kates and Volcani, 1966); they are invariably present in sea surface water, and make up a large fraction of the organic material in the sea surface film (Jeffrey et al., 1964). In particular, unsaturated lipids with a chain length of 18 carbon atoms are abundant in marine organisms (Lovern, 1964), in nearshore surface water, and in ocean films (Garrett, 1967; Jeffrey et al., 1964). Garrett (1967), for instance, has determined that the fatty acid 18:2 (probably linoleic acid) and its methyl ester can account for up to 48.5% of the total chloroform-soluble material in the upper 150 u. His analysis of chloroform extracts of film organics from the North Atlantic and North Pacific oceans and from the Gulf of Mexico indicated that 18:2, 18:1, and 16:1 are frequently abundant relative to most other fatty acids at the interface. This abundance of unsaturates did not appear to be correlated with the presence of a visible slick.

Other polyunsaturated acids constitute significant fractions of the lipids of marine organisms. Lovern (1935) reports that 25% of the total lipid of Calanus finmarchicus are 22-carbon chain fatty acids with an average unsaturation of 4 double bonds; 24% are 20-carbon chains with an average of 3.9 double bonds; and 17% are 18-carbon chains with an average of 2.5 double bonds. Ackman et al. (1968) have found that 16:4, 18:3, 18:4, 20:4, 20:5, and 22:6 make up much of the total fatty acids of the marine phytoplankton they investigated. For instance, these acids together account for slightly greater than 50% of the total fatty acids of the chlorophyte Dunaliella tertiolecta.

The polyunsaturated lipids of organisms have largely methylene-interrupted unsaturation (Markley, 1960), and thus the lipids of sea water would also possess unconjugated unsaturation. Fatty acids of marine organisms in general have a greater range of molecular weight and a greater unsaturation than the fatty acids of most terrestrial organisms. It is this variety of unsaturated free fatty acids, and their methyl and glycerol esters, that is the source of much of the surfactant at the sea-air interface.

Organic nitrogen

In addition to long-chain hydrophobic fatty substances, the sea surface film is known to be enriched over subsurface waters in dissolved and particulate organic nitrogen (table 1). Although the nature of this nitrogenous film fraction has not been determined, some of what is present in the dissolved state may be protein.

All proteins are surface-active to some extent; solutions of even the most soluble natural proteins (such as ovalbumen) tend to form monomolecular surface films. The solubility of a protein in water depends upon the distribution and ratio of hydrophilic and hydrophobic residues within the molecule, and upon the resulting dipole moment. When a protein contacts an air-water interface its tertiary structure is modified as a consequence of interfacial forces,

Figure 1. Distribution of dissolved organic carbon with depth.

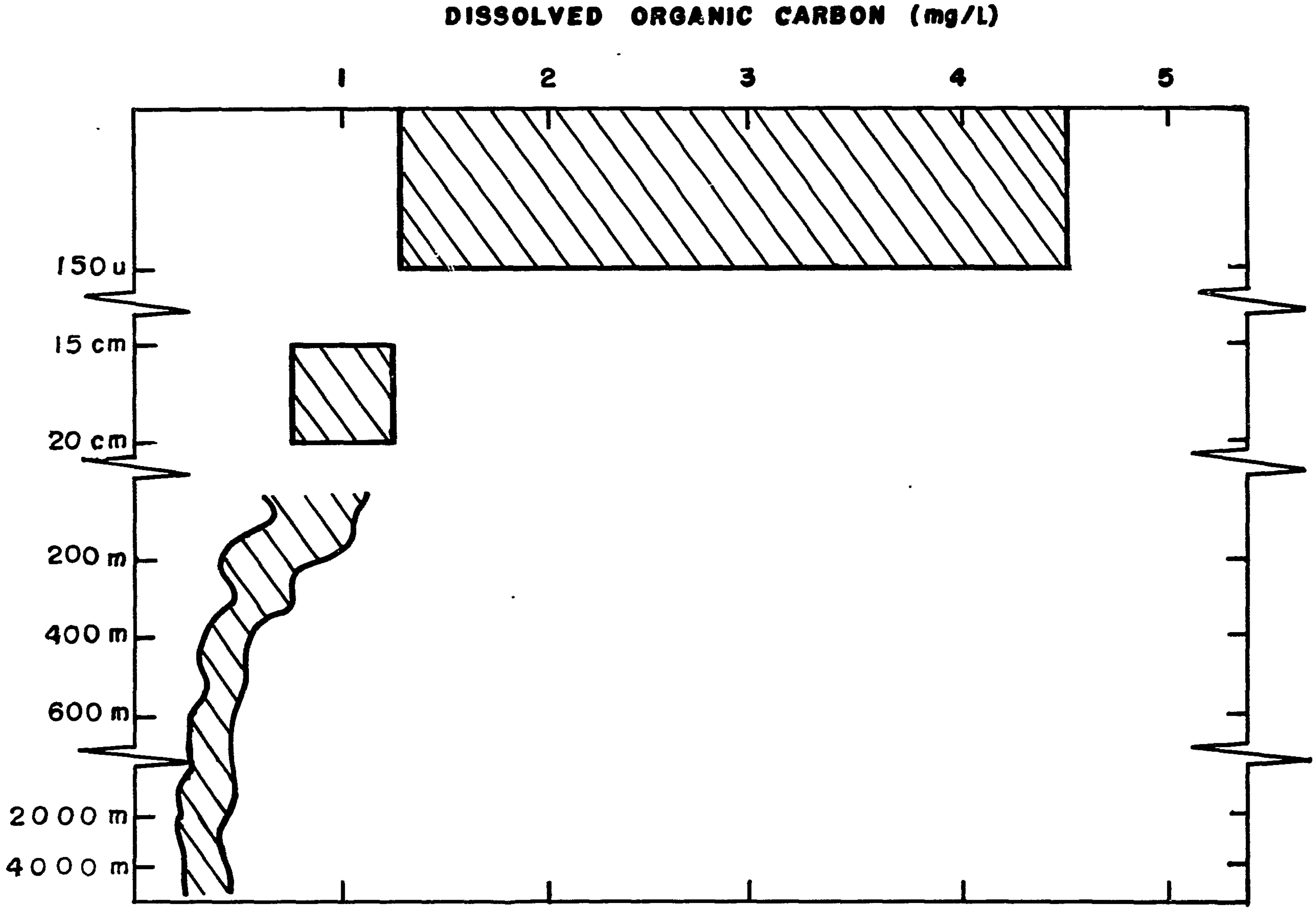

DISSOLVED ORGANIC CARBON (mg/L)
1
2
3
4
5
150 u
15 cm
20 cm
200 m
400 m
600 m
2000 m
4000 m

Table 1. Concentrations (in ug/l) of dissolved and particulate organic nitrogen, and particulate organic carbon in surface films and subsurface waters (from Williams, 1967).

Station	22	29	30	32	33	34	1	2	3
POC surface film	1410	540	450	2500	790	170	830	170	1490
POC subsurface	114	135	62	267	252	30	-	-	-
PON surface film	210	164	134	840	204	293	87	29	189
PON subsurface	24	12	18	65	52	6	-	-	-
DON surface film	381	446	333	547	435	527	122	127	127
DON 15-20 cm	157	175	151	118	115	108	88	78	87
DON subsurface	92	97	106	35	86	96	76	71	74

the result being a partial unfolding of the molecule. This unfolding is indicated to be irreversible, resulting from the breaking of one or more intramolecular bonds (such as a cysteine-cysteine S-S bridge). Once partly unfolded, proteins may have a strong tendency to remain at the interface and thus contribute to the total organic material of the film, and to modification of surface properties as suggested by Garrett (1970).

The flux of solar radiation at the sea-air interface

As many biochemical surfactants absorb sunlight to some extent, solar radiation is an energy input to the sea surface film. The theoretical relationship between energy and wavelength in the vicinity of the solar spectrum is shown in figure 2; in the solar ultraviolet, energies of quanta exceed the dissociation energies of bonds which occur in many biological molecules.

The distribution of solar energy at 32° N latitude is illustrated in figure 3. It is agreed by most workers that the lower limit of solar wavelengths reaching the earth's surface is somewhat less than 3000 Å. However, Roburn (1963) has presented evidence that dieldrin (a chlorinated pesticide that does not absorb at wavelengths greater than 2850 Å) is subject to solar photolysis. Also, Barker (1969) reports that a small but effective fraction of solar radiation between 2000 Å and 2850 Å reaches the earth's surface. Rosen (1971) believes that most of Barker's reported far ultraviolet is concentrated in the 2000 Å - 2200 Å region.

The wavelengths and intensity of solar ultraviolet reaching the surface is largely a function of atmospheric oxygen and ozone concentrations, and of solar altitude. Figure 4 illustrates the large variation in intensity of short-wave ultraviolet with time of day and with season (i.e. with celestial altitude) at 39° North latitude. Ozone is formed above the tropopause from oxygen in a photochemical process at wavelengths shorter than 2424 Å. Ozone itself absorbs strongly between 2200 Å and 2900 Å, and consequently almost all solar radiation within this region that impinges on the atmosphere is absorbed. Absorption at these wavelengths takes place largely in the stratosphere, as a sharp ozone concentration maximum occurs at about 20-30 km altitude. The amount of ozone responsible for this intense absorption is very small, and at STP amounts to a layer only a few mm in depth. Concentrations are greater at high latitudes, and at these altitudes there is a seasonal variation; concentrations also vary with local meteorological conditions, and with time of day. As absorption is an exponential function of the thickness of the ozone layer, small variations in concentration can result in marked changes in the surface intensity of short-wave radiation.

The attenuation of sunlight in sea water is due to absorption and scattering by water, dissolved salts, dissolved organics, and suspended particles. Attenuation of visible and ultraviolet radiation by water and dissolved salts may be relatively unimportant in coastal regions, but would be of greater significance in the open ocean where absorption and scattering by other agents would tend to be less (see Lenoble, 1956; Sullivan, 1963). Jerlov (1955) found that in certain coastal waters absorption by "gelbstoff" was responsible for more than 50% of attenuation at 3800 Å. Burt (1958) and Ivanoff et al. (1961), near Bermuda and the Galapagos, respectively, determined that absorption and scattering by particulates were responsible for 84-100% of the attenuation at 4000 Å. Jerlov (1951, 1964) has conducted the only published studies of the attenuation of short-wave near ultraviolet in sea water. He investigated the transmittance of water from the North and South Atlantic, North and South Pacific, and Indian Oceans at 16 wavelengths from 3100 Å to 7000 Å. He found that sea water transparency to 3100 Å radiation varies with location, but is quite high: transmittance = 50-86%/m. At 3500 Å the range is 71-94%/m; at 3750 Å, 79-96%/m; and at 4000 Å, 84-97%/m.

Figure 2. Relationship between energy and wavelength in the vicinity of the solar spectrum.

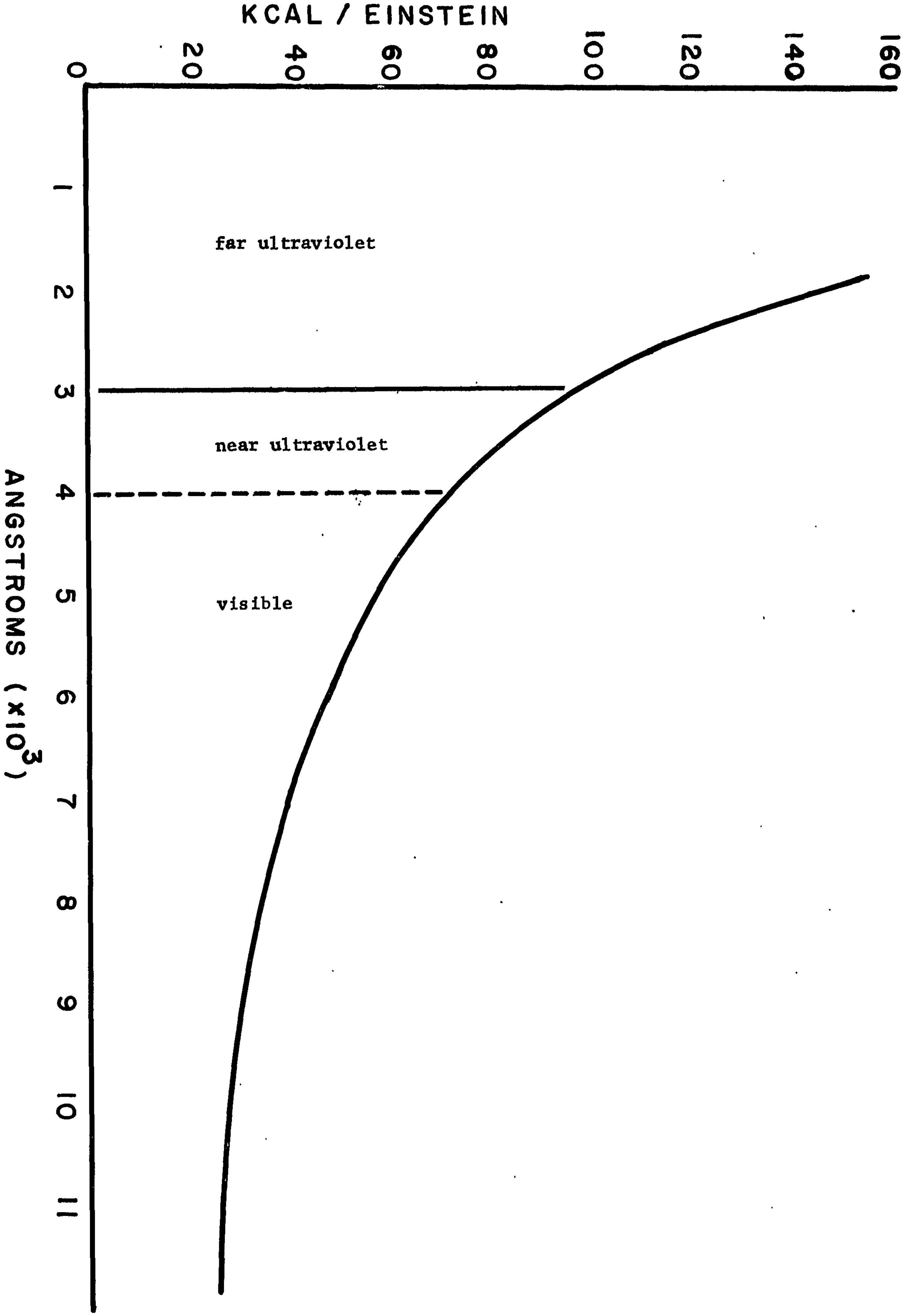

KCAL / EINSTEIN
0
20
40
60
80
100
120
140
160
ANGSTROMS (x10^3)
1
2
3
4
5
6
7
8
9
10
11
far ultraviolet
near ultraviolet
visible

Figure 3. Intensity of direct solar radiation in June at 32° North latitude. Drawn from data in Pettit, 1932.

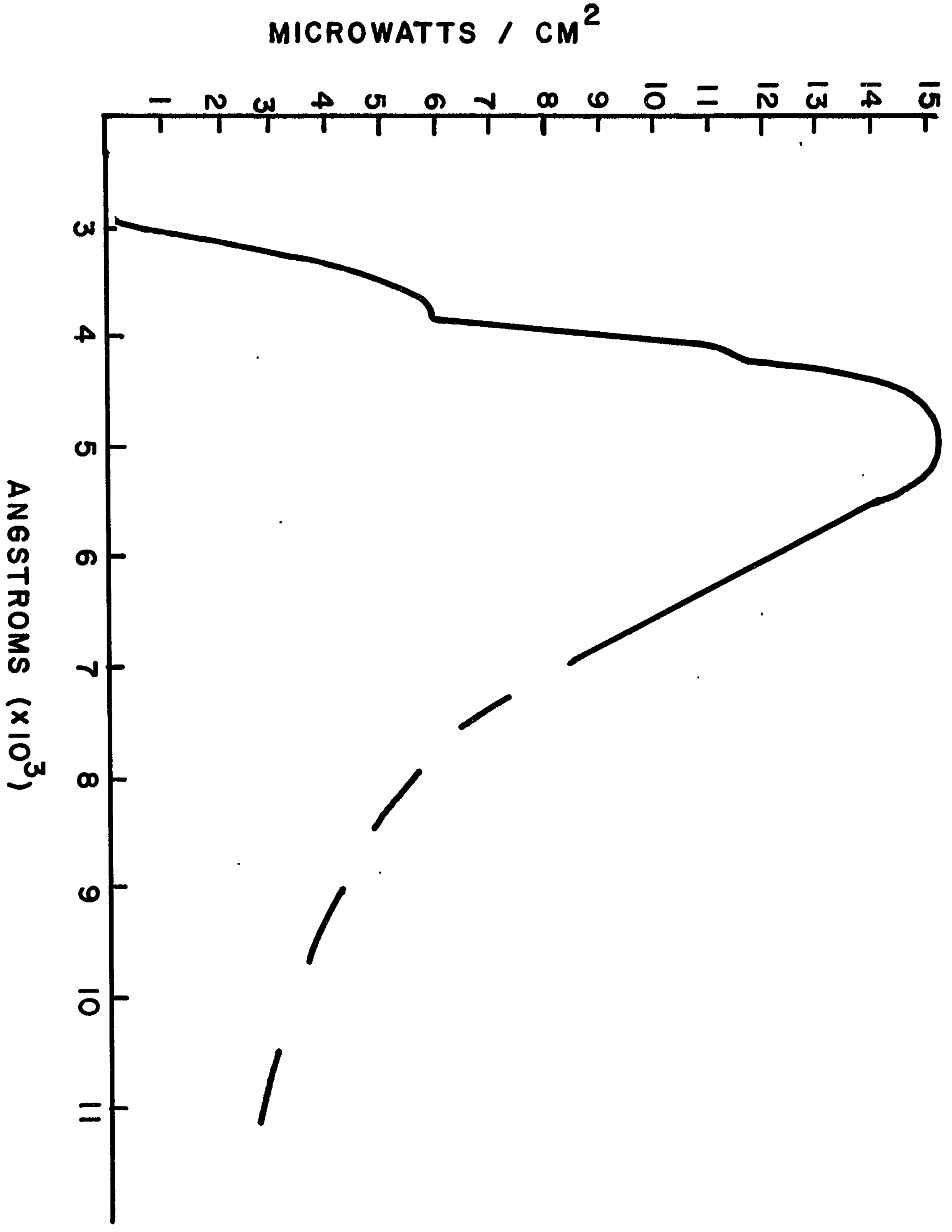
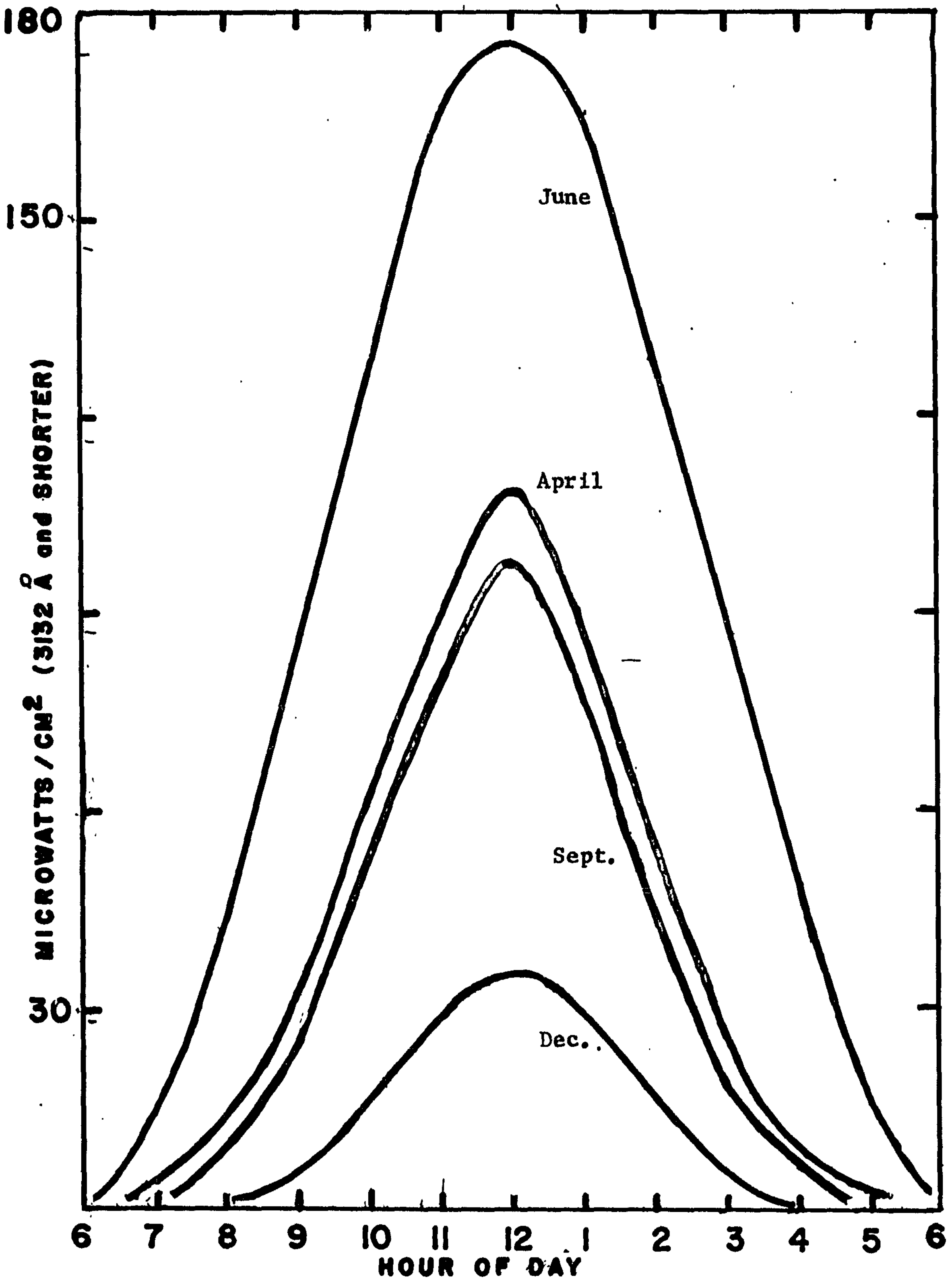

MICROWATTS / CM^2
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
3
4
5
6
7
8
9
10
11
ANGSTROMS (x10^3)

Figure 4. Intensity of solar ultraviolet radiation $\leq$3132Å incident on a horizontal plane at 39° North latitude. From Coblentz and Stair, 1944.


180
150
30
MICROWATTS / CM² (3132 Å and SHORTER)
June
April
Sept.
Dec.
6
7
8
9
10
11
12
1
2
3
4
5
6
HOUR OF DAY

METHODS

Artificial sea water

The production of water of low organic content was accomplished by passing oxygen and vapor from either doubly distilled water or Millipore Super-Q water through a 1000 C^o furnace packed with quartz chips (figure 5). In his analysis of this water, Sharp (1972) detected 0.1-0.2 mg/l of dissolved carbon. Because his high-temperature carbon analyzer was operated at the same temperature as was the high-temperature still, he felt that the carbon detected was not inherent in the vapor from the still, but rather was introduced during subsequent collecting and handling. To insure minimum contamination, we soaked all glass and quartz associated with the still in chromic-sulfuric acid solution for 7 days prior to assembly. In addition, we heated the condenser and receptacle to just below the annealing point of pyrex.

To prevent airborne contamination when water was withdrawn, the air inlet of the receptacle was fitted with an activated charcoal column and filters. Water was transferred from the still via silicone rubber tubing directly into the reaction vessels, which contained known amounts of salts. Re-crystallized NaCl was heated to 650 C^o for 5 days prior to dissolution. Reagent grade KCl, $CaCl_2$, and $MgSO_4$ were re-crystallized in the low-carbon water. The pH of the salt solution was adjusted to 8.0 with NaOH. The concentrations of elements (see table 2) were approximately those of sea water.

Figure 5. High-temperature still used in the production of low-carbon water.

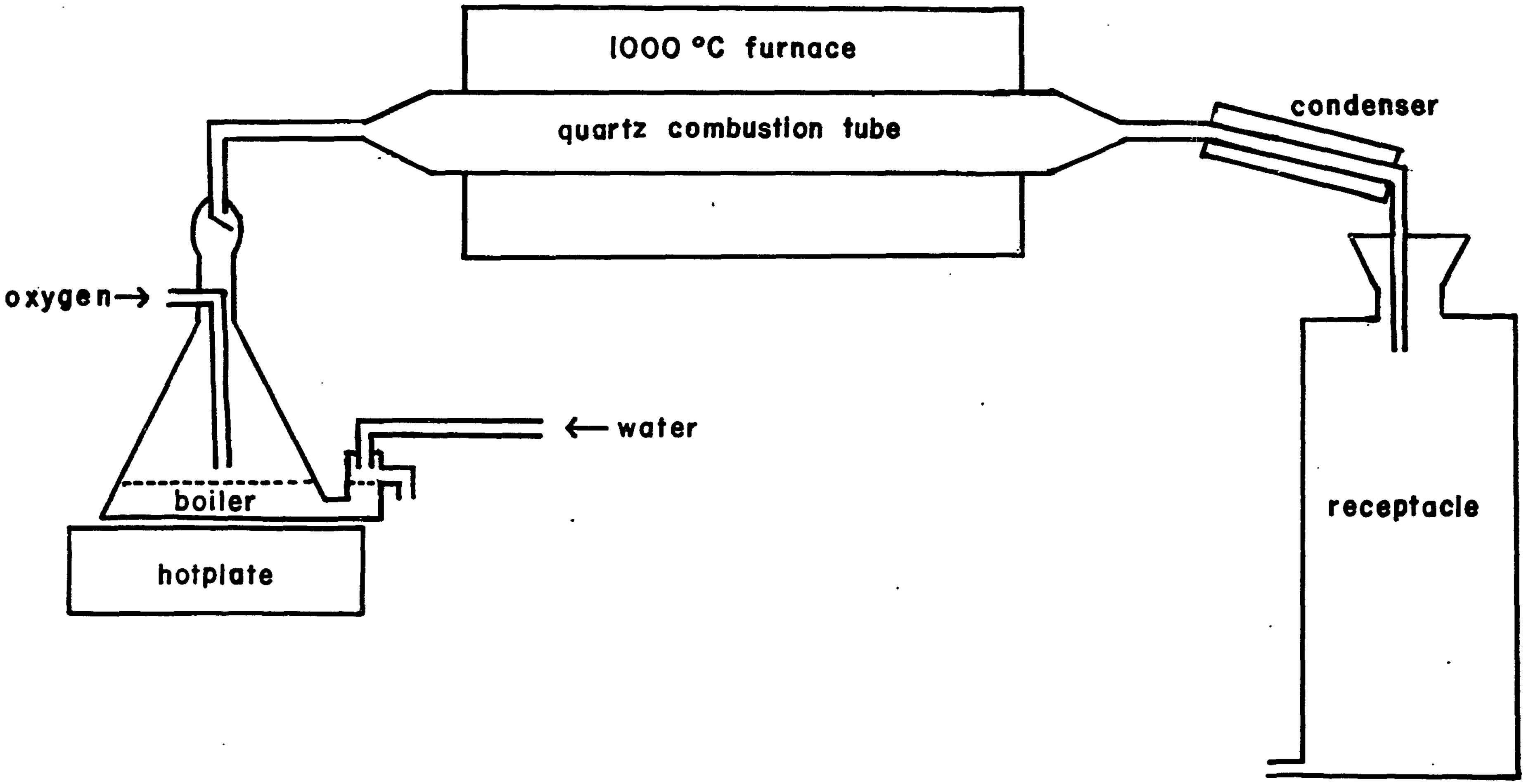

1000 °C furnace
quartz combustion tube
condenser
oxygen →
← water
boiler
hotplate
receptacle

Table 2. Composition of artificial sea water (all values in g/1)

Constituent	Artificial Sea Water	Sea Water*
Cl	17.27	19.00
Na	10.50	10.50
$SO_4^=$	3.95	2.83
Mg	1.05	1.35
Ca	0.40	0.40
K	0.38	0.38

* from Goldberg (1965)

Reaction vessels and ultraviolet radiation

a) Reaction vessels

The reaction vessels consisted of either 1) a 3 l spherical flask (figure 6), 2) a 2 l separatory funnel, or 3) a 500 ml erlenmyer flask. All were equipped with a source of radiation, an inlet and outlet for heated compressed air, and an injection port for the sterile introduction of organics. The source of radiation was a 2.4 watt low-pressure mercury arc (Ultraviolet Products, Inc.) which was operated at 80 °C. Radiation from the lamp was passed through a soft glass filter, resulting in exclusion from the reaction vessel of wavelengths shorter than 2940 Å. The transmission of the filter is illustrated in figure 7. The emission of the lamp through the filter was recorded on a Cary 14 spectrophotometer.

b) Actinometry

Energy transmitted to the solution in the 3 l vessel was determined via uranyl oxalate actinometry. In this method oxalic acid is decomposed in a process photosensitized by uranyl ion, the major reaction products being U^{4+}, CO, CO_2, H_2O, and HCO_2H. The amount of oxalic acid lost is determined by the difference in the amounts of $KMnO_4$ required to titrate the oxalate of irradiated and unirradiated solutions. The reaction occurs between 2080 Å and 4350 Å. Liquid actinometry has the advantage that the geometry of the experimental set-up is precisely duplicated during the actinometric procedure.

Three liters of a solution of 3.75 g uranyl oxalate and 6.30 g oxalic acid per liter were added to the vessel in the dark, the lamp was turned on, and 50 ml aliquots were withdrawn over a 60 hour period; titrations were with 0.1 N $KMnO_4$. A control run in the dark for 60 hours resulted in no measurable oxalate decomposition. The volume of $KMnO_4$ equivalent to oxalate loss was plotted against duration of exposure (figure 8). These data yielded a total flux of 5.86 x 10^{-9} Nhv/sec, which was equivalent to a maximum intensity (between 2940 Å and 4350 Å) of about 1040 u watts/cm^2 just below the tip of the lamp. The maximum intensity below 4000 Å was calculated to be 38% that of natural mid-day direct ultraviolet sunlight at 32° N latitude (see appendix 1 for these calculations). The lamp output is compared with natural sunlight in figure 9.

c) Aeration of experimental solutions

Because the present research was an investigation of the effects of a given variable (ultraviolet radiation) on dissolved organics, we decided to limit as much as possible other variables from the reaction vessel. Bubbling has been implicated in the transformation of dissolved organics to the particulate state (Baylor and Sutcliffe, 1963; Riley, 1963; Wangersky, 1965; Riley, 1970). It would thus have been best to eliminate bubbling entirely, so that any particulates produced could be more readily ascribed to the effects of radiation. It was decided, however, that air should be introduced into the vessels, as 1) reactions of the organics may be dependent on a supply of oxygen, and 2) oxygen is available at the sea surface, and it was desired to duplicate sea surface conditions in this regard. Tank compressed air was introduced into the vessel at a minimal rate (once each hour at 10 cc/minute for 30 seconds) in order to supply the system with oxygen while at the same time minimizing bubbling. In an effort to decrease any possible organic contamination, the air was passed through a 1000°C furnace prior to its entry into the vessel. The control experiments (with aeration in the absence of radiation) indicated that the amount of bubbling employed did not induce formation of particulate lipid or protein at the concentrations investigated.

Figure 6. Three liter reaction vessel with the ultraviolet lamp and filter in position.

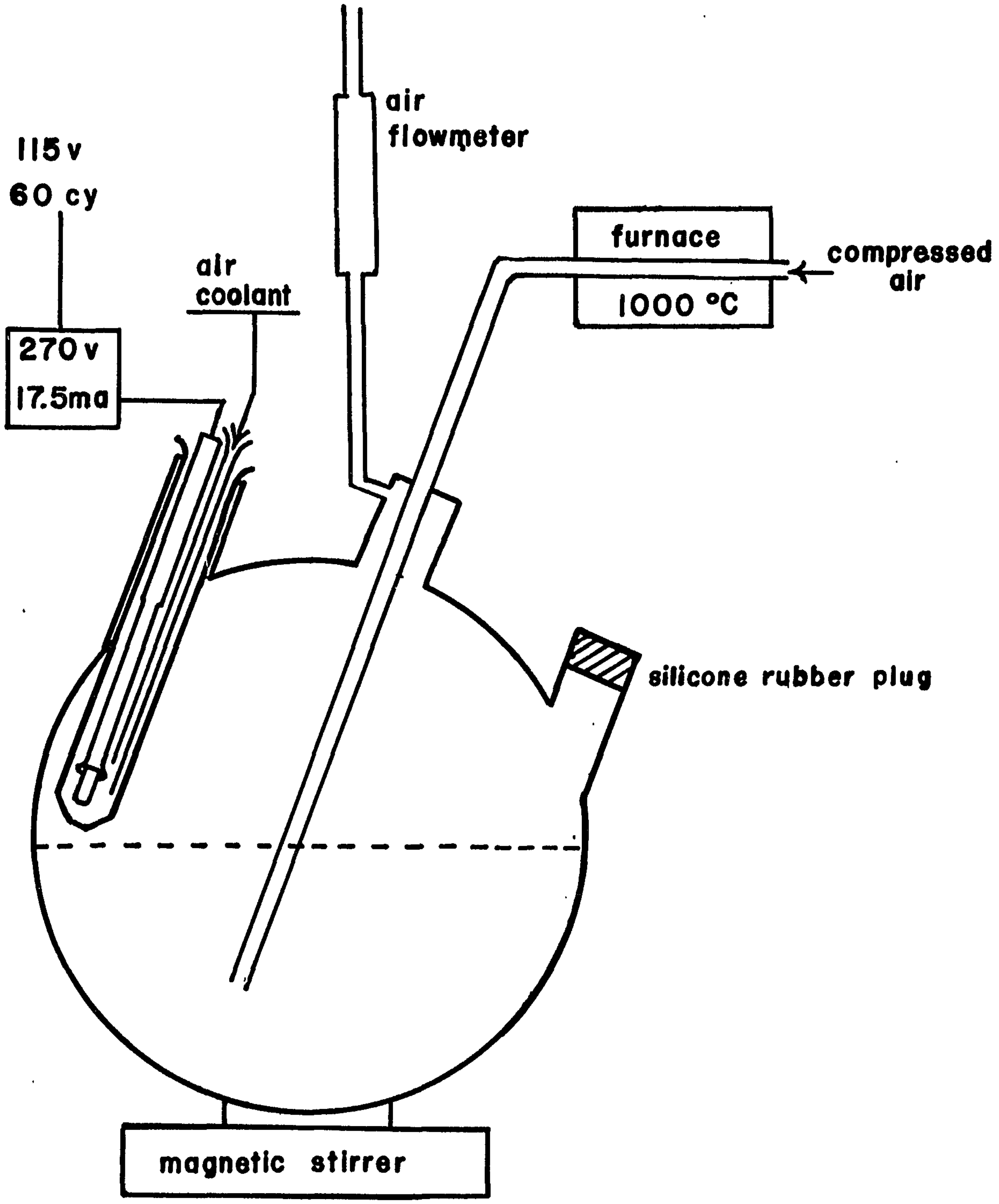

115 v
60 cy
270 v
17.5 ma
air coolant
air flowmeter
furnace
1000 °C
compressed air
silicone rubber plug
magnetic stirrer

Figure 7. Transmission of the filter used in conjunction with the low-pressure arc.

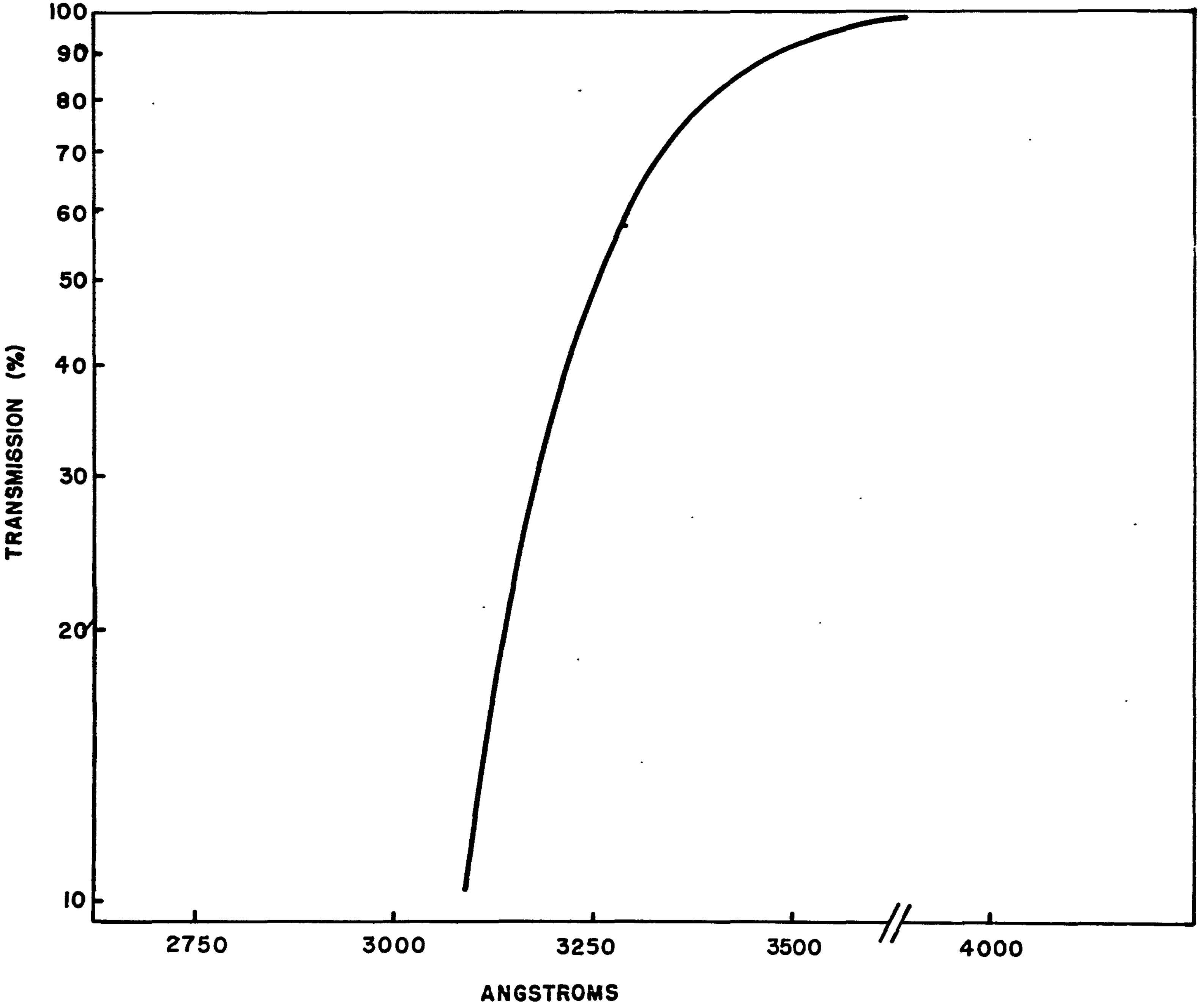

100
90
80
70
60
50
40
30
20
10
TRANSMISSION (%)
2750
3000
3250
3500
4000
ANGSTROMS

Figure 8. Relationship between the decomposition of uranyl oxalate-oxalic acid solution, and its duration of exposure to the filtered radiation.

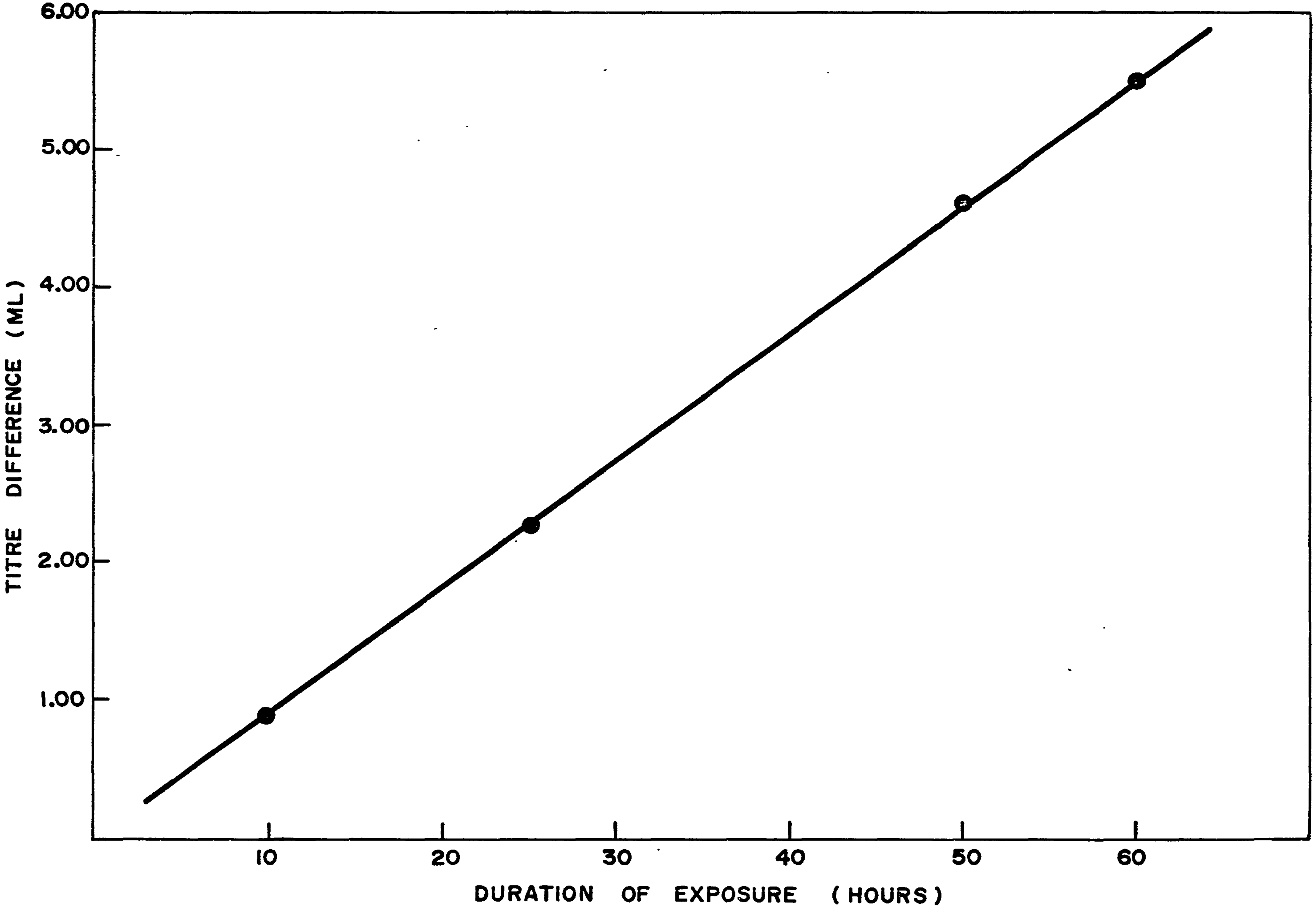
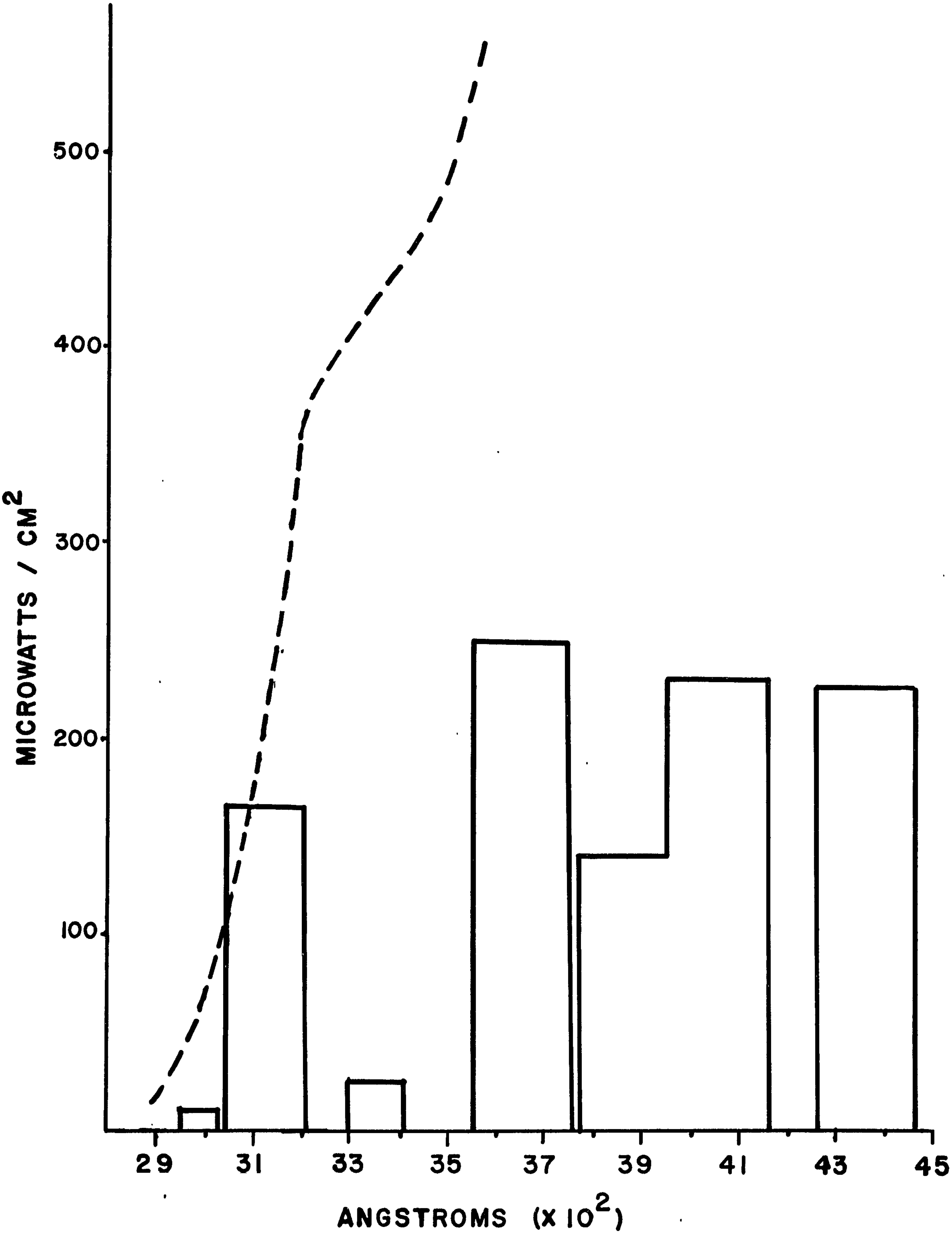

6.00
5.00
4.00
3.00
2.00
1.00
TITRE DIFFERENCE (ML)
10
20
30
40
50
60
DURATION OF EXPOSURE (HOURS)

Figure 9. Intensity of filtered radiation (——) compared with that of direct sunlight (----) at 32° North latitude. The solar energy data is from Pettit, 1932.


MICROWATTS / CM^2
500
400
300
200
100
29
31
33
35
37
39
41
43
45
ANGSTROMS (X 10^2)

Experimental procedure

I. Studies with reaction vessels

a) General

After the vessels and associated glassware had been scrupulously cleaned with chromic-sulfuric acid solution, they were rinsed thoroughly with high-temperature distilled water. The vessel was assembled, the salts were introduced, and it was filled to the mark with low-carbon water. This assembly was then autoclaved at 115 °C for 20 minutes. Also autoclaved were the glassware and artificial sea water used in the sterile transfer of organics. After autoclaving, the vessel and instruments were allowed to cool to room temperature; solutions of the organics investigated were then injected. In the experimental runs, the lamp and filter were placed in position within the reaction vessels. In the controls, the lamp was placed in series with the vessel, irradiating a volume of oxidized compressed air equivalent to that above the water surface in the reaction vessel. This had the effect of introducing ozone into the vessel at about the same concentrations that were present in the experimental runs. Thus the possibility of ozone (rather than radiation) inducing the observed reactions was accounted for in the controls. Blanks consisted of running through the experimental procedure without added organics. Reactions were investigated at 22 ± 4 °C. Sterility was examined at the end of each experiment by incubating a few drops of the artificial sea water in a bacteriological test medium (0.1% peptone + 0.025% yeast extract in artificial sea water).

b) Lipids

Lipids were dissolved in n-hexane, the solvent having been previously purged with nitrogen. They were introduced into the vessels as n-hexane solutions, and the solvent was allowed to evaporate prior to the onset of irradiation. Reactions of the pure lipids were investigated at two concentrations: 338 ug/l and 1.8 mg/l.
1) At the lower concentration, the 3 l vessel was used in the determination of particulate reaction products. The 2 l vessel was used in cyclohexane extraction studies for the determination of changes in absorption spectra induced by the radiation. 2) At the higher concentration the 3 l vessel alone was used, and changes in absorption spectra were not followed. However, particles produced were counted, and their size-frequency distribution was determined.

After irradiation, either of two procedures was followed:
a) the contents of the 3 l vessel were passed through a 0.8 u pore-size filter, or b) the contents of the 2 l vessel were extracted with cyclohexane. After filtration in (a) the filter was rinsed with 20 ml of lipid-pure n-hexane (Applied Science Laboratories). This rinse minimized filter retention of all but hexane-insoluble material. The filter was then stained with OsO_4, a histological lipid stain. The cyclohexane solutions from procedure (b) were analyzed in a Unicam SP8000 recording spectrophotometer.

The lipids investigated were cis-oleic acid, cis-9, cis-12-linoleic acid, and all-cis-9,12,15-linolenic acid (all were 99%

purity from Sigma Chemical Co.). These substrates were stored under nitrogen at -20°C prior to use. A chloroform-methanol extract of T. fluviatilis was re-dissolved in nitrogen-purged cyclohexane, and stored in the dark under nitrogen at 5°C. Its reactivity was investigated at 590 ug C/1.

c) Proteins

Ovalbumen was obtained in 99% purity; bovine pancrease trypsin was obtained twice re-crystallized; both were from Sigma Chemical Co.. After irradiation in the 3 1 vessel at 300 ug/1 (ovalbumen) or 250 ug/1 (trypsin) for 4 hours, the contents of the vessel were passed through a 0.8 u pore-size filter. The filter was then stained with bromphenol blue, which is positive for protein. The stains, staining apparatus, and procedure were identical to those in Sharp (1972).

The 500 ml reaction vessel was used in conjunction with particulate carbon analysis. After irradiation of ovalbumen at 750 ug/1, the vessel's contents were passed through a 0.8 u pore-size silver filter (Selas Flotronics, Inc.). The filters were heated, prior to use, to 440°C in a muffle furnace and remained at that temperature for 24 hours. The heating had the effect of reducing the amount of carbon inherent on the filters. After use, the experimental filters were dessicated at room temperature. "Zero time" samples were taken by introducing the ovalbumen into the vessel and immediately filtering the resulting solution; these were also dessicated, as were filters for determination of filter blank. The carbon and nitrogen retained on each filter was determined with a Hewlett-Packard F & M model 185 CHN analyzer. See appendix 2 for details of the analyzer and its calibration.

d) Lipid with protein

The reactivity of linoleic acid with ovalbumen, each at 1 mg/l, was investigated in the 3 l vessel. After 10 hours irradiation, the contents were filtered through a 0.8 μ pore-size Millipore filter, and stained with either OsO_4 or bromphenol blue.

II. Surface film studies

Monomolecular surface films exert a pressure on barriers which confine them; this "surface pressure" is due to intermolecular repulsion within the film, and is numerically equal to the decrease in surface tension due to the film's presence.

In this work surface tension was measured directly with a Surface Tensiomat (Fisher Scientific Co.). The platinum ring was cleaned in chromic-sulfuric acid solution, and rinsed in artificial sea water prior to each reading. The trough was 29.8 cm x 18.8 cm x 4.6 cm deep, and was equipped with moveable glass barriers (figure 10). The trough and barriers were rinsed in a solution of paraffin in octane, and allowed to dry. The resulting paraffin-coated apparatus provided the hydrophobic surfaces necessary for the proper functioning of the trough. The waxed barriers could be glided across the trough, increasing or decreasing the area of the film.

Studies were undertaken of the effects of ultraviolet on the rate of accumulation of surface pressure and on the collapse pressures of artificial and natural films. In view of the findings regarding the effects of radiation on the rate of accumulation of pressure, studies were subsequently made of the effect of lateral surface pressure on the transformation of dissolved carbon to the particulate state.

The trough was filled to near-overflowing with either 1) a solution of ovalbumen in artificial sea water (1.71 mg/l), 2) Sargasso surface sea water or surface lake water (both bucket samples), or 3) the Sargasso sea water spiked with ovalbumen (1.71 mg/l). In the determination of surface pressures at varying film areas, the waxed barrier was moved so as to decrease the area in certain decrements. After each adjustment of the barrier the film was allowed to equilibrate for 5 minutes prior to determining surface tension. When used, the ultraviolet lamp and filter were placed 2 cm above the centre of the trough solution to give a maximum ultraviolet intensity of approximately 1040 u watts/cm^2.

The data were plotted as force vs. area curves (ΔF vs. A), where ΔF (equivalent to film pressure) was calculated from surface tension data:

ΔF = surface tension at original area — surface tension after film compression

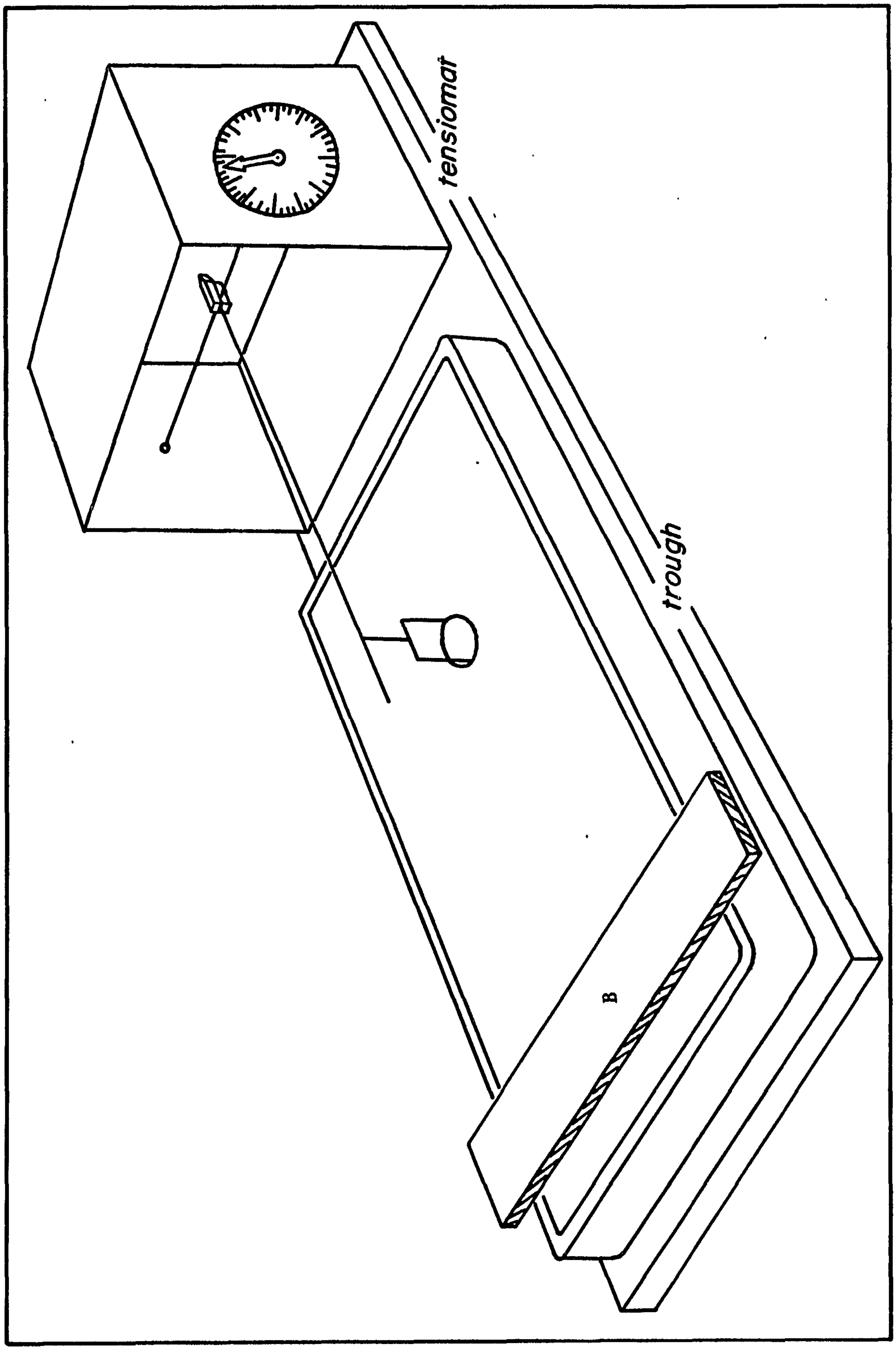
When studying rates of accumulation of surface pressure, the area of the film remained constant and ΔF was plotted against time, where

ΔF = surface tension at zero time — surface tension at time of reading

The natural waters were filtered through a 0.8 u pore-size filter prior to introduction into the trough.

In determining the effect of increased surface pressure on the transformation of film organics to the particulate state, films were allowed to accumulate for various periods, and then compressed at the rate of approximately 19 cm^2/sec to an area of 18.8 cm^2 from 560.2 cm^2. The barrier was held for 3 minutes, and then released to allow the film to re-expand at the interface. The extent of re-expansion was measured as subsequent decrease in surface tension. Compressed film which did not re-expand was collected on Millipore filter pads, and examined microscopically to determine film collapse and the formation of particulate carbon from interfacial organics.

Figure 10. Trough and tensiomat used in the determinations of surface tension. The barrier (B) can be moved across the trough, increasing or decreasing the area of the film. The arm holding the platinum ring is clamped to the horizontal torsion wire within the tensiomat. A force pulling the ring up out of the surface is mediated through the torsion wire when the dial on the face of the tensiomat is turned clockwise. When this force on the arm just exceeds the force holding the ring in the water surface, the ring will break through the interface: surface tension can then be read directly on the dial.


tensiomat
trough
B

RESULTS

Lipids

Linoleic acid

Upon irradiation in aerated artificial sea water at 338 ug/l, linoleic acid developed intense absorption at 234 nm, indicative of conjugation isomerization of the double bonds (figure 11). Upon exposure in the dark to aerated sea water, absorption in this region increased moderately. A very slight decrease in absorption at 234 nm developed when this lipid was exposed to nitrogen-purged artificial sea water (i.e. artificial sea water without oxygen and radiation). Particulate reaction products were noted in the vessel after 7 hours of irradiation in aerated sea water. These particles were retained by a 0.8 u pore-size filter and stained readily with OsO_4 (figure 12). This positively staining particulate matter of characteristic morphology was not produced when control artificial sea water (without added lipid) was irradiated.

Upon irradiation at 1.8 mg/l, positively-staining particles were noted in the vessel after 1 hour. It appeared from microscopic observation of the filters (and the filtered linolenic acid reaction products--to be discussed) that much of this high concentration of lipid was dispersed through the 3 l of artificial sea water as very small droplets, ranging in diameter from about 1 u to over 100 u. Initially it appeared that all of these droplets passed through the filter, as after the hexane rinse no trace of lipid remained on the filter surface. After 1 hour, however, portions of some of these droplets remained on the surface, appearing as positively-staining circular discs. In addition, definite particles were present after several hours of irradiation (figure 13). Microscopic observation of filter-retained material suggested the transition: "filter-passing droplet → partially retained droplet → particle" (possibly gradual polymerization). It is difficult to relate the morphology of filtered material to the history of lipid in the reaction vessel, and the above proposed transition is hypothetical. However, with increasing periods of irradiation, the discs increased in number, appeared to stain more intensely, and there was a greater number of particles within the disc areas. These observations support the above mechanism.

It was not possible to distinguish some opaque discs from particles, and it was decided to include all lipid material on the filter in the "particle count" to which each filter was subjected. In 5 separate experiments, linoleic acid was irradiated at 1.8 mg/l in the 3 l vessel, and after either 1,2,4,7, or 13 hours, the entire 3 l volume was filtered; the filter was rinsed with hexane, and stained. Each of the 5 resulting filters was then examined with a Zeiss microscope equipped with Nemarsky interference optics. On each filter 31 fields were examined, the number of discs plus particles were counted, and their diameter or greatest dimension was recorded. From the number of discs plus particles observed, the total number on the filter was estimated (see appendix 3). This estimate was representative of the total amount of hexane-insoluble filter-retained lipid. These estimates and their 95% confidence intervals are plotted against time of exposure of the lipid to radiation in figure 14. When 1.8 mg/l of this lipid were exposed to ozonated artificial sea water for 7 hours (control) and filtered through a 0.8 u millipore filter, no positively-staining hexane-insoluble material was present on the filter. This contrasted with 13,436 ± 2,786 discs plus particles retained on the experimental filter after 7 hours, indicating that the lipid was retained on the filter due to its previous subjection to radiation.

Figure 15 illustrates size-frequency distributions of the filter-retained material after exposure of the lipid to radiation for 4, 7, and 13 hours.

Oleic acid

When irradiated for 12 hours in aerated artificial sea water at 338 ug/l, oleic acid showed a slight decrease in extinction at 234 nm (figure 16). No development of particulate material was observed. Also, no formation of particulate material was evident after 12.5 hours of irradiation at 1.8 mg/l.

Linolenic acid

In aerated sea water under irradiation, linolenic acid developed absorption maxima at 234 nm and 269 nm, indicating diene and triene conjugation respectively (figure 17). As was the case with linoleic acid, positively-staining reaction products were filterable after 7 hours of irradiation. A small but significant increase in absorption at 234 nm developed when the lipid was subjected to the aerated sea water without irradiation.

At 1.8 mg/l, reaction products were retained on a 0.8 u pore-size filter after 3 hours of irradiation. These products (discs and particles) were indistinguishable from those of linoleic acid irradiation; however, they appeared to be formed at a lesser rate. The products were subjected to the same numerical analyses as the linoleic acid products: filter-retained lipid vs. time of exposure is illustrated in figure 18; the size-frequency distributions of the retained lipid after 7 and 12 hours of irradiation are shown in figure 19.

Diatom lipid extract

The absorption spectrum of a lipid extract of <u>Thalassiosira fluviatilis</u> is shown in figure 20. Upon irradiation in the presence of air, an absorption maximum developed in the conjugated diene region (figure 21). After 12 hours, positively staining disc areas (similar to those due to irradiation of the pure lipids) were observed after filtering the contents of the 3 l vessel.

Increased absorption in the near-ultraviolet

Coincident with the conversion of methylene-interrupted unsaturation to conjugated unsaturation upon irradiation, was the development of increased absorption at wavelengths longer than 2900 Å (figures 11, 17, and 21). Thus, the reaction products absorb high-energy solar ultraviolet more intensely than do the lipid substrates. Such absorption may lead to further photochemical reaction at the sea surface.

Figure 11. Absorption spectrum of linoleic acid after 0 (——), 4 (----), and 12 (····) hours of irradiation in aerated artificial sea water.

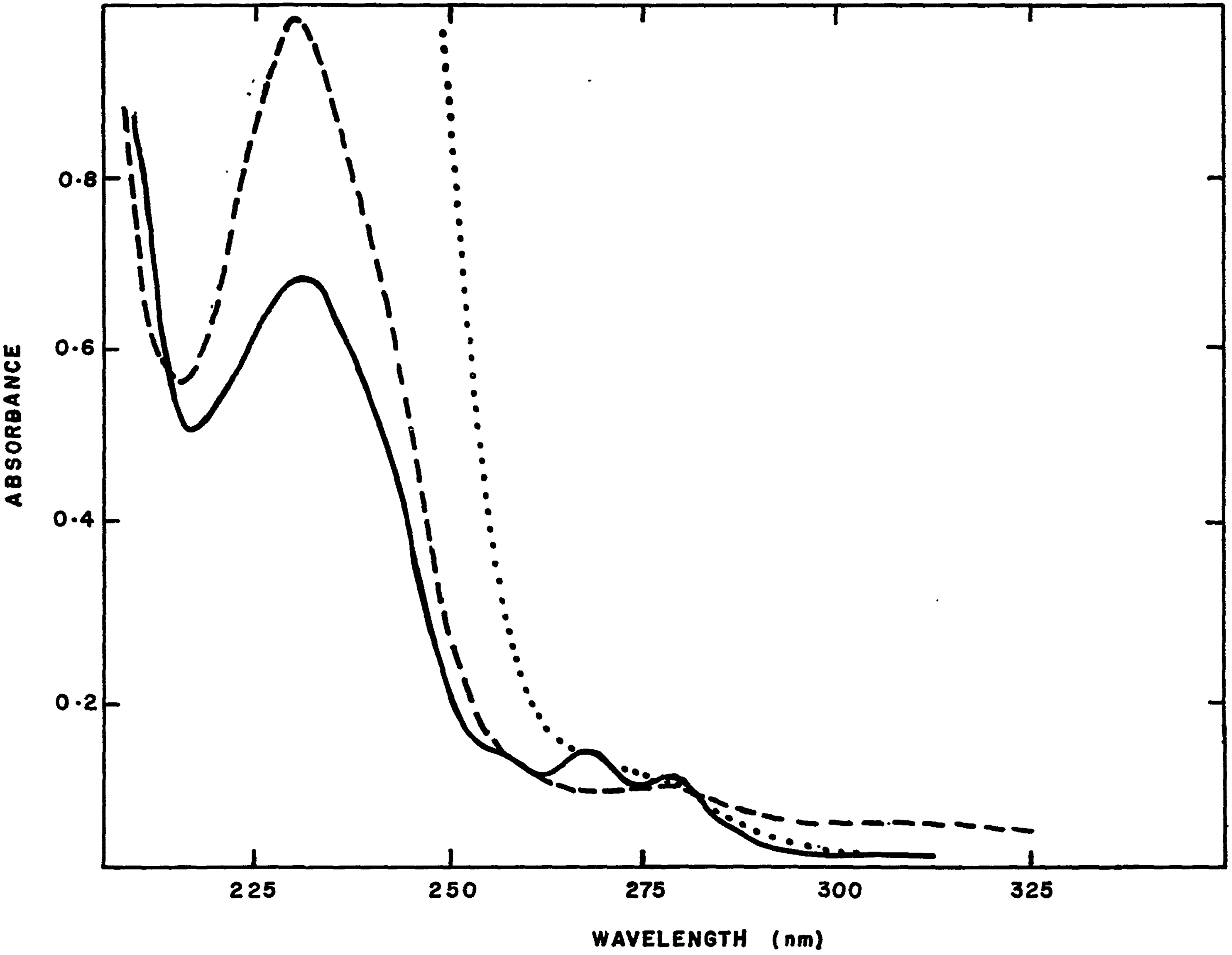
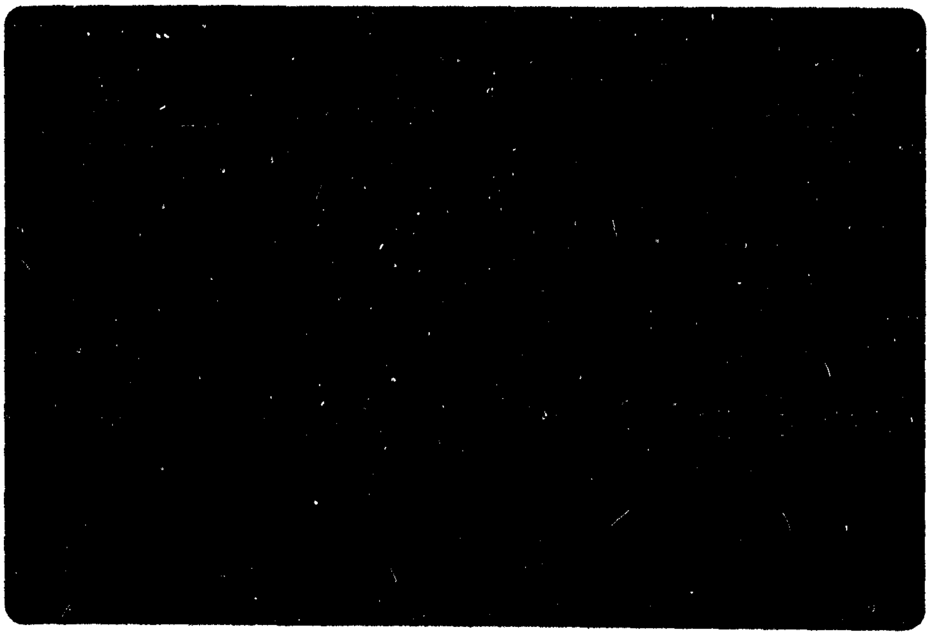
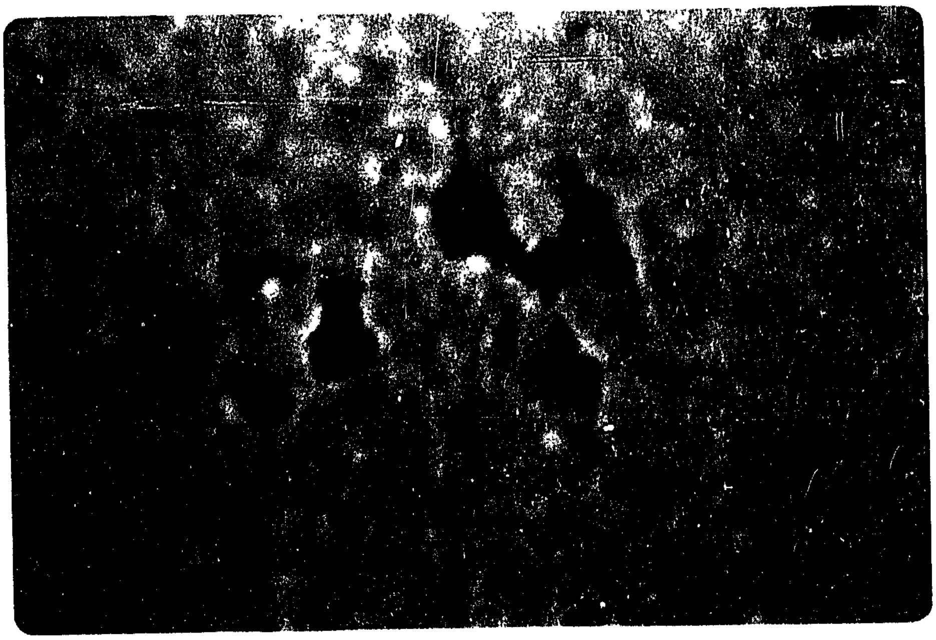

ABSORBANCE
0·8
0·6
0·4
0·2
225
250
275
300
325
WAVELENGTH (nm)

Figure 12. Photomicrographs of some particulate lipid resulting from irradiation of linoleic acid at 338 ug/l in artificial sea water for 7 hours. x1000





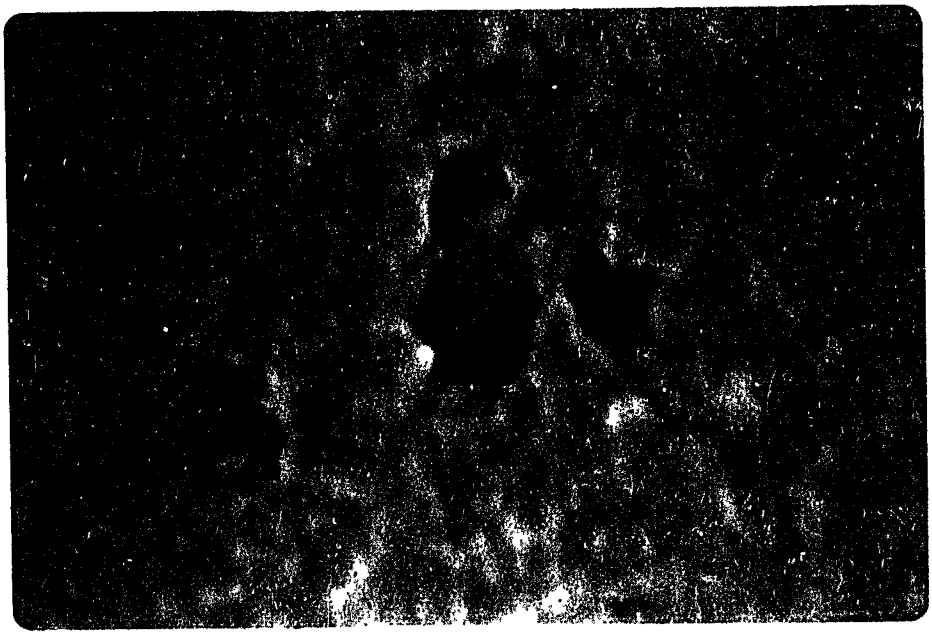
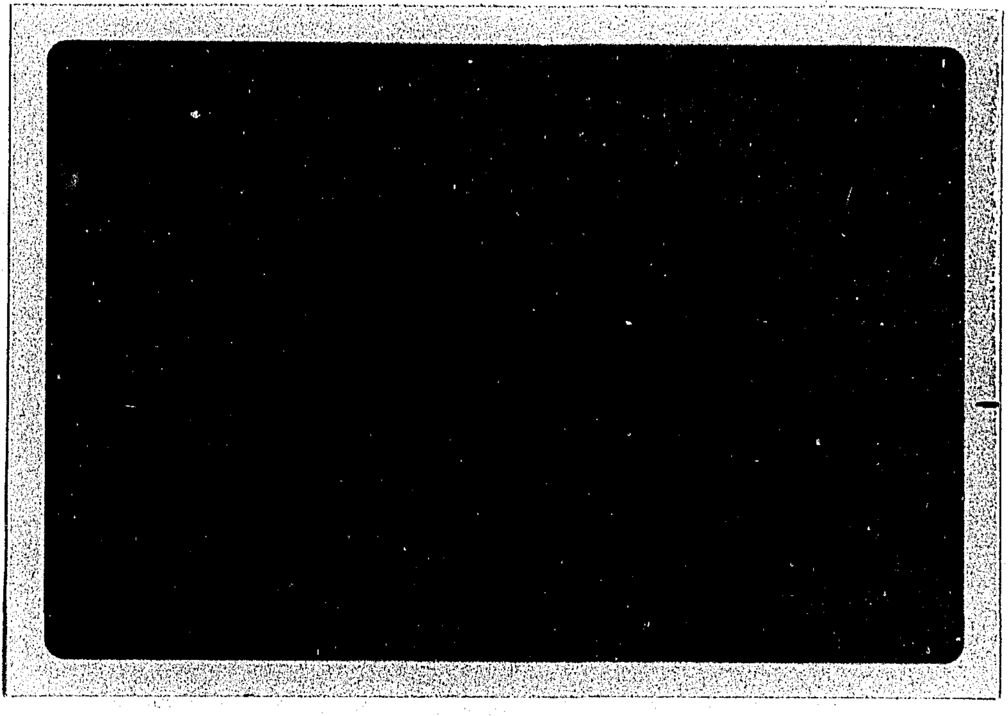
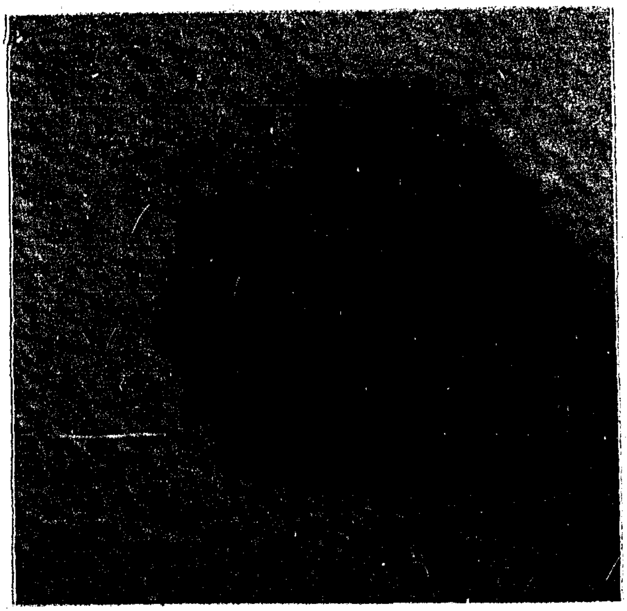
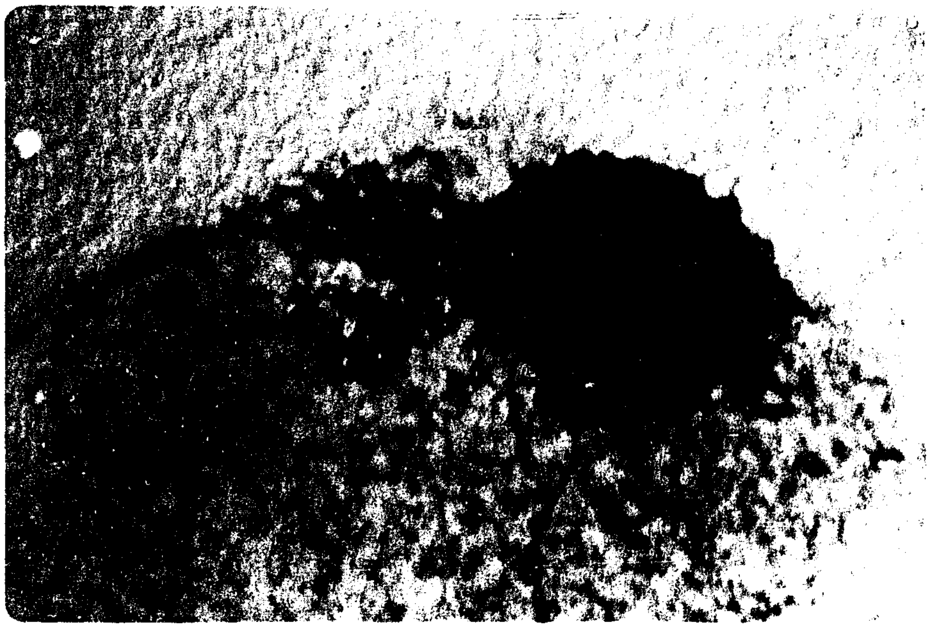


Figure 13. Photomicrographs of some particulate lipid resulting from irradiation of linoleic acid at 1.8 mg/l in artificial sea water for 7 hours. x400







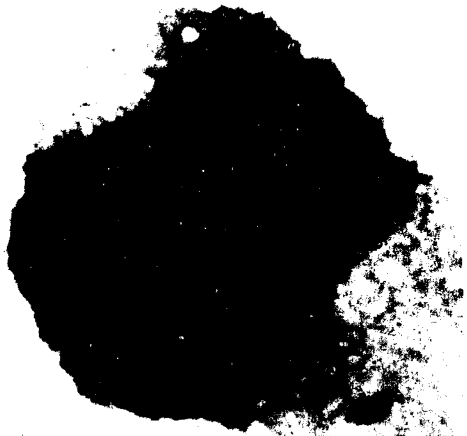


Figure 14. Filter-retained lipid (discs plus particles) resulting from irradiation of linoleic acid.

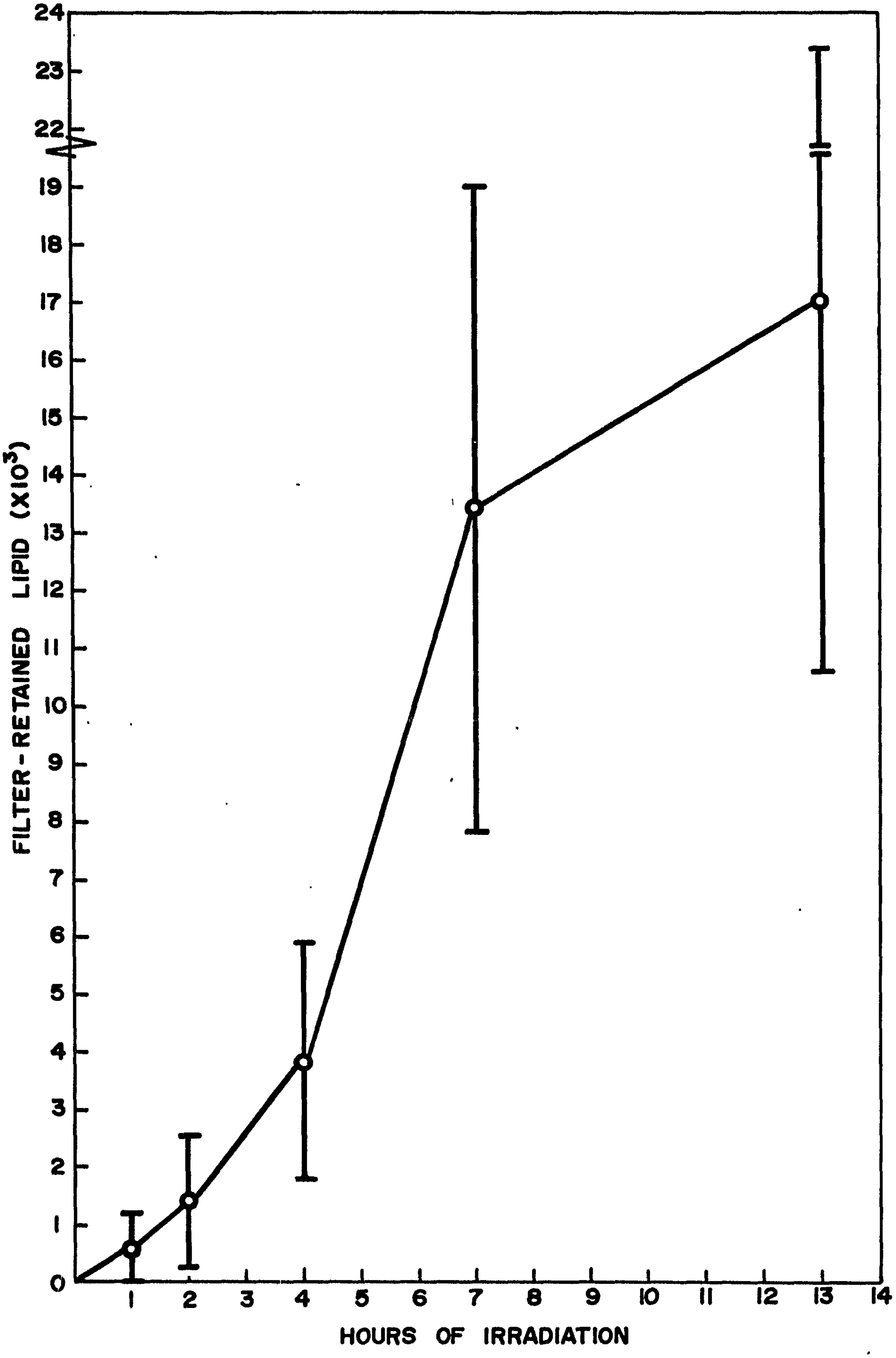
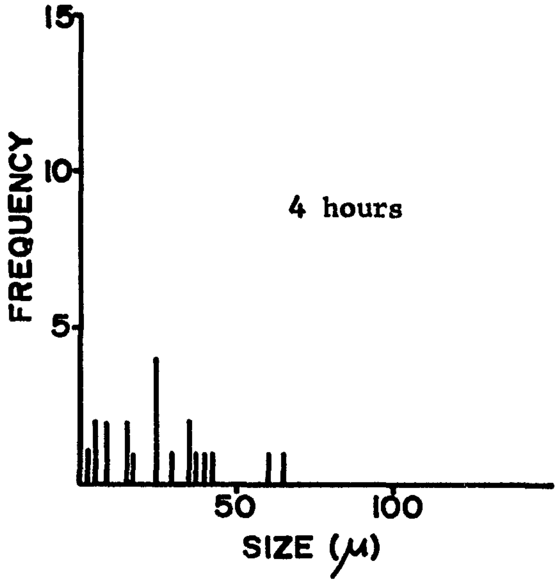
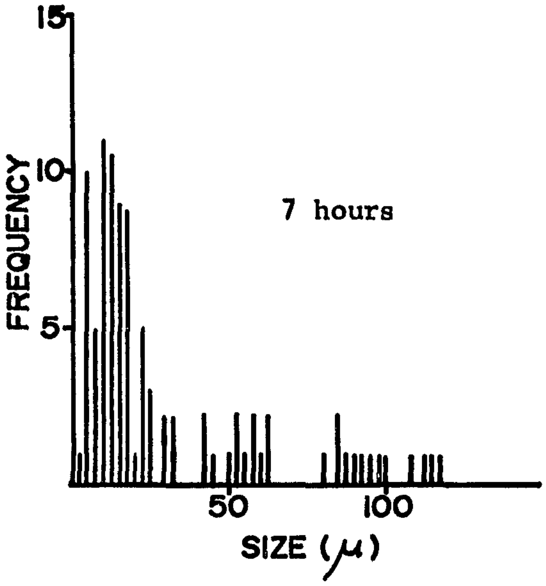

FILTER-RETAINED LIPID (X10^3)
HOURS OF IRRADIATION
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
22
23
24

Figure 15. Size-frequency distributions of the filter-retained lipid resulting from irradiation of linoleic acid for 4, 7, and 13 hours.


4 hours
FREQUENCY
15
10
5
50
100
SIZE (μ)


7 hours
FREQUENCY
15
10
5
50
100
SIZE (μ)

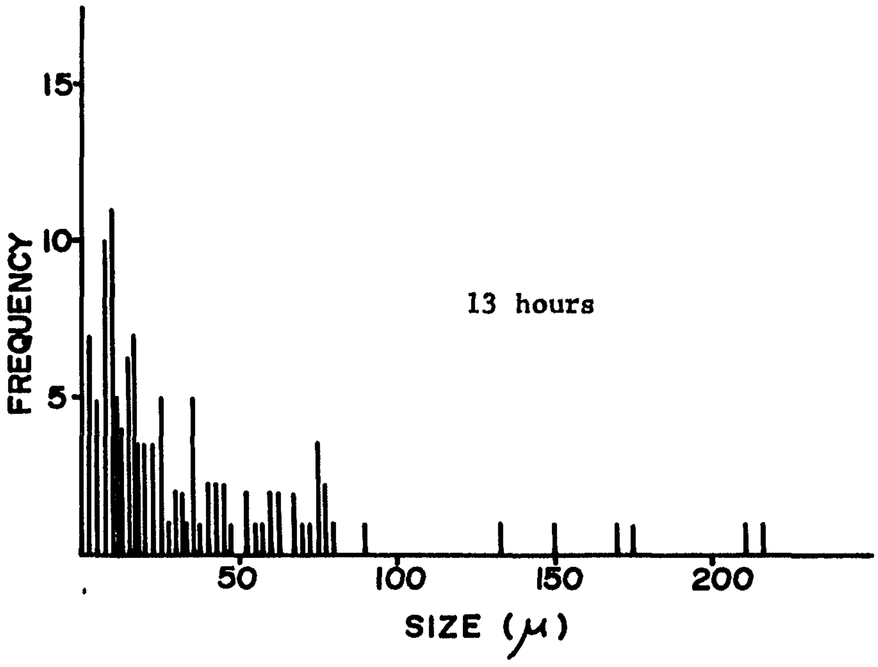

13 hours
FREQUENCY
15
10
5
50
100
150
200
SIZE (μ)

Figure 16. Absorption spectrum of oleic acid after 0 (——), 5 (----), and 15.5 (····) hours of irradiation in aerated artificial sea water.

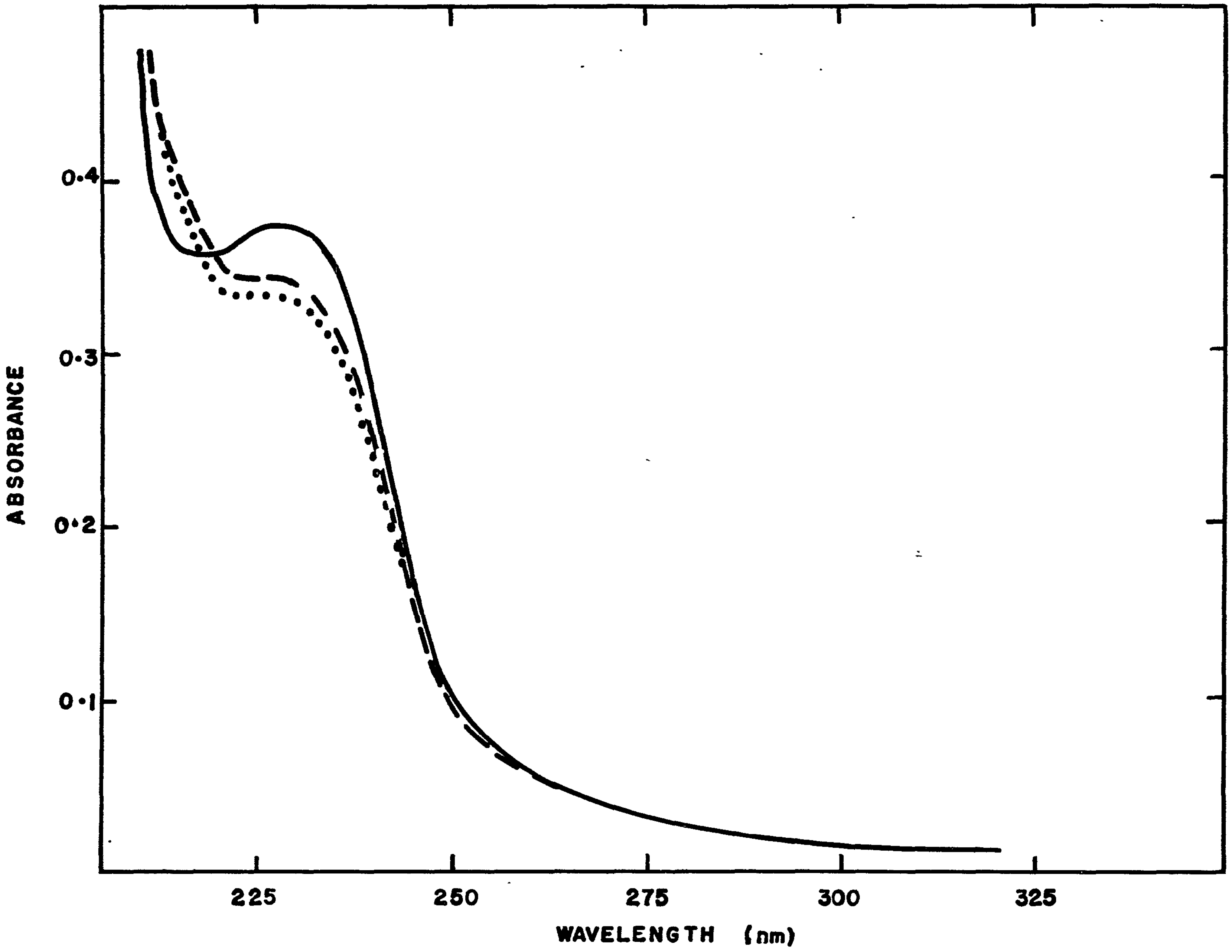

ABSORBANCE
0·4
0·3
0·2
0·1
225
250
275
300
325
WAVELENGTH (nm)

Figure 17. Absorption spectrum of linolenic acid after 0 (——), 4.5 (----), 9 (····), and 12 (·-·-·-) hours of irradiation in aerated artificial sea water.

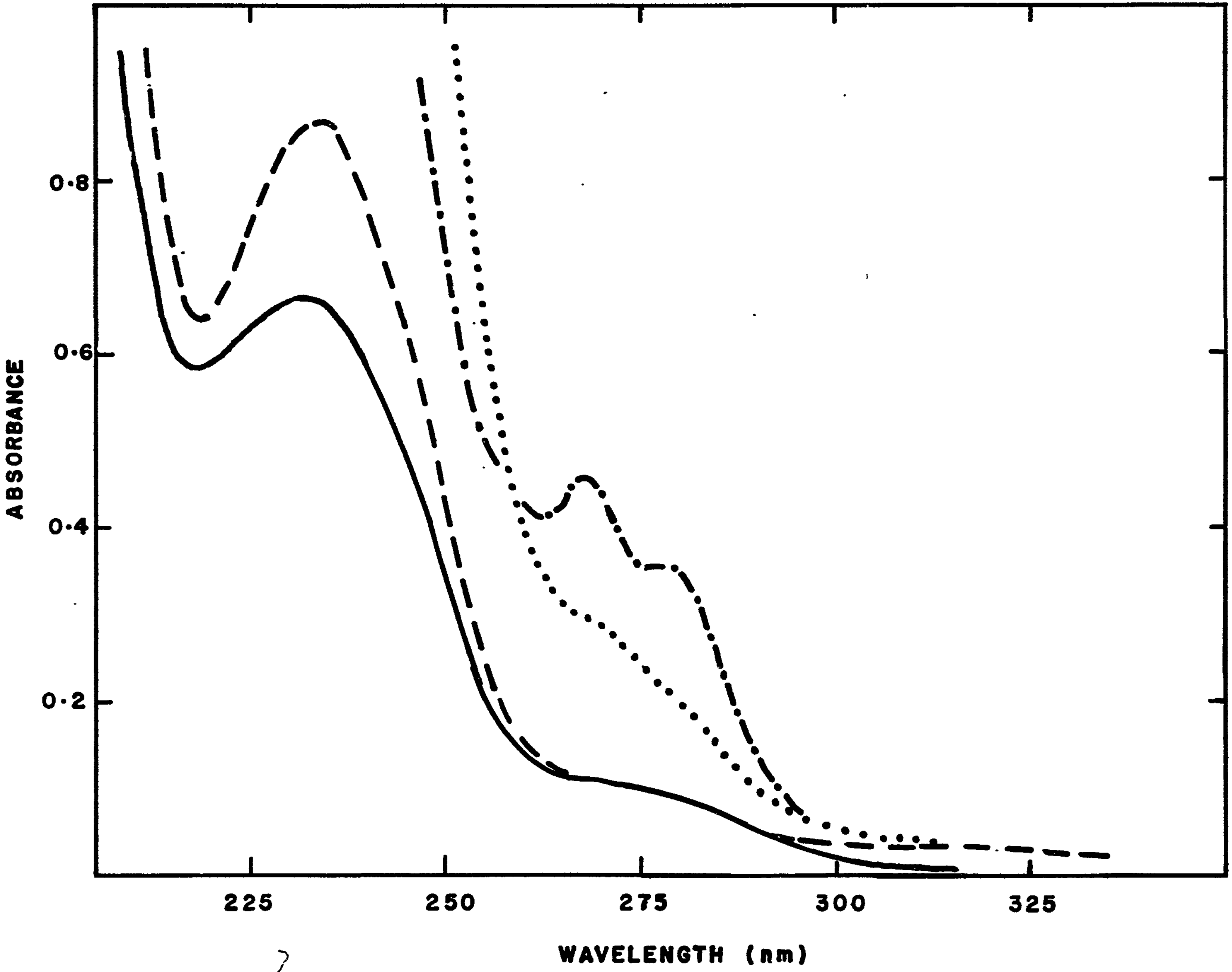

ABSORBANCE
0·8
0·6
0·4
0·2
225
250
275
300
325
WAVELENGTH (nm)

Figure 18. Filter-retained lipid (discs plus particles) resulting from irradiation of linolenic acid.

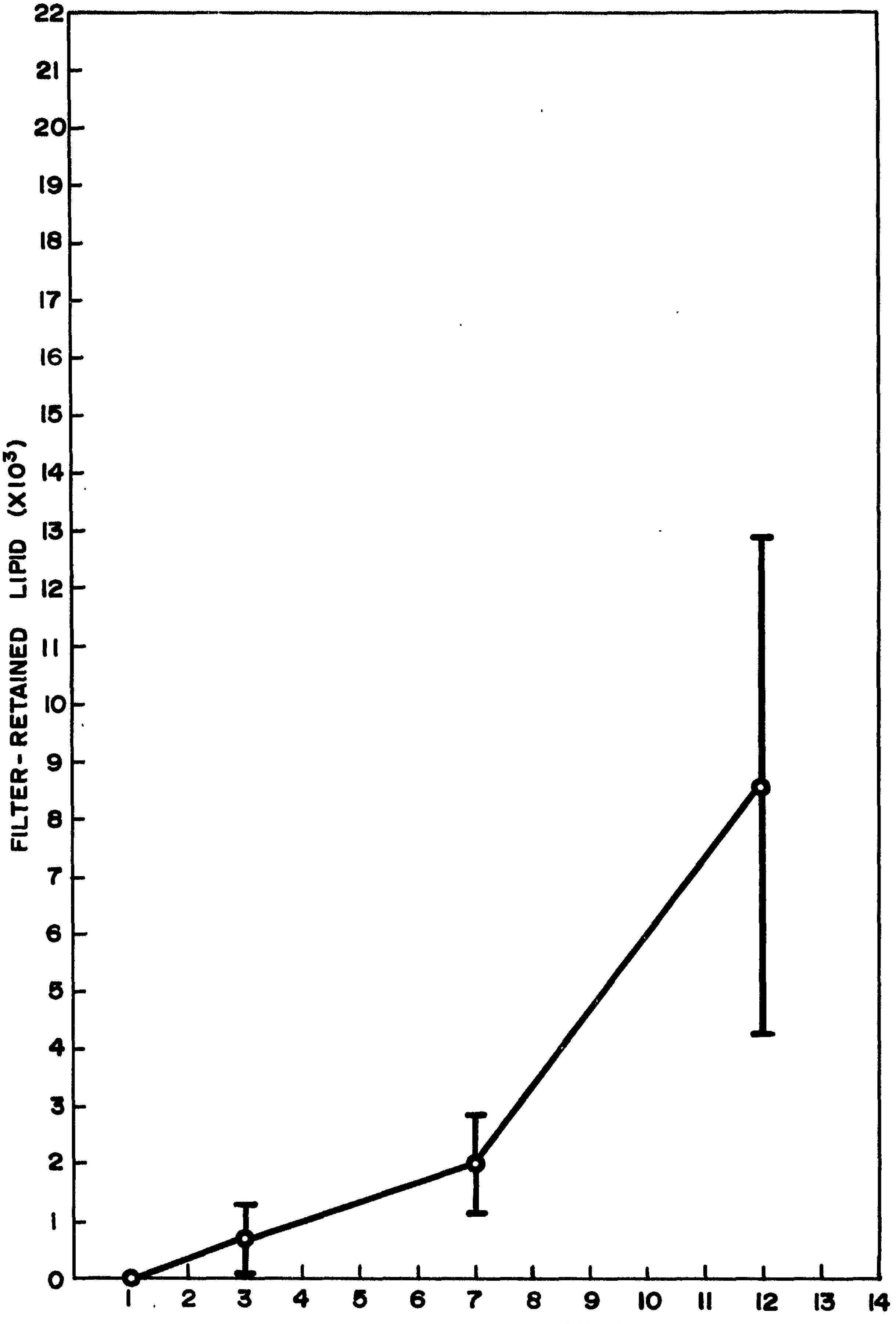
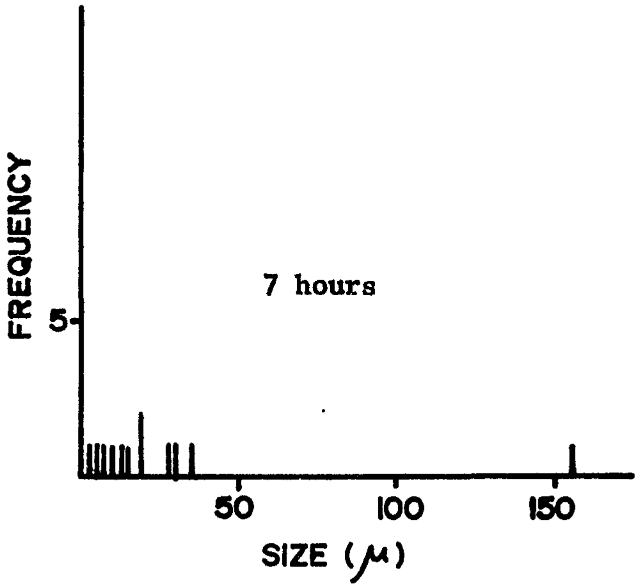

FILTER - RETAINED LIPID (X10^3)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
1
2
3
4
5
6
7
8
9
10
11
12
13
14
HOURS OF IRRADIATION

Figure 19. Size-frequency distributions of the filter-retained lipid resulting from irradiation of linolenic acid for 7 and 12 hours.


FREQUENCY
5
7 hours
50
100
150
SIZE (μ)

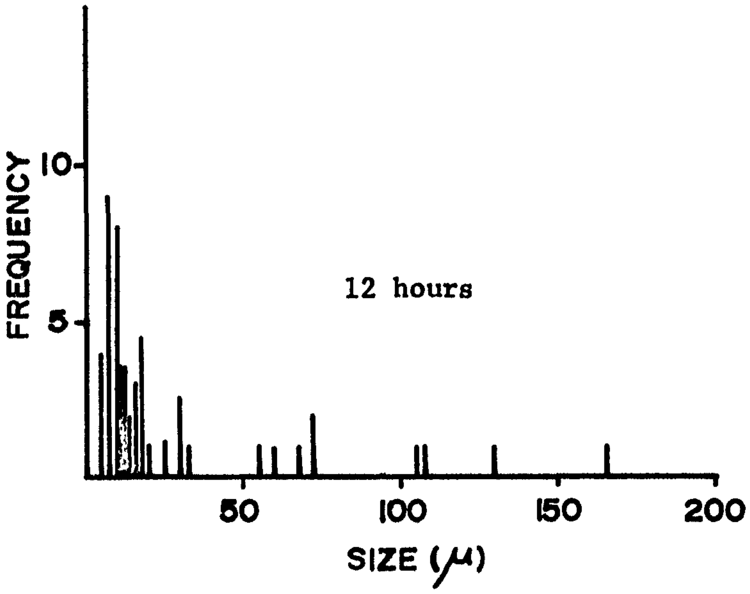

FREQUENCY
10
5
12 hours
50
100
150
200
SIZE (μ)

Figure 20. Absorption spectrum of a chloroform - methanol extract of *Thalassiosira fluviatilis*, after re-solution in cyclohexane. In addition to the maxima in the blue and red (from chlorophylls) the extract absorbs intensely in the ultraviolet.

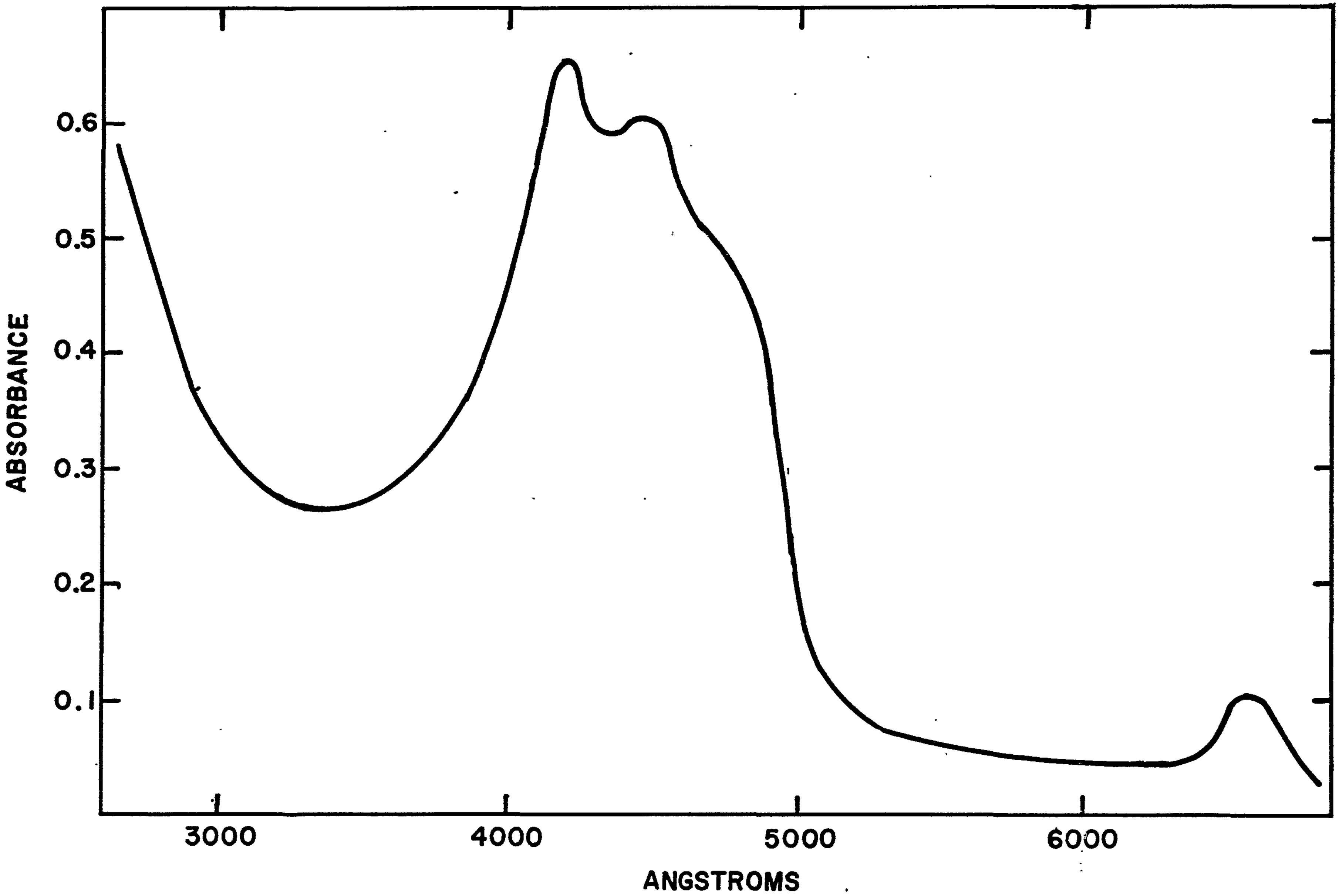
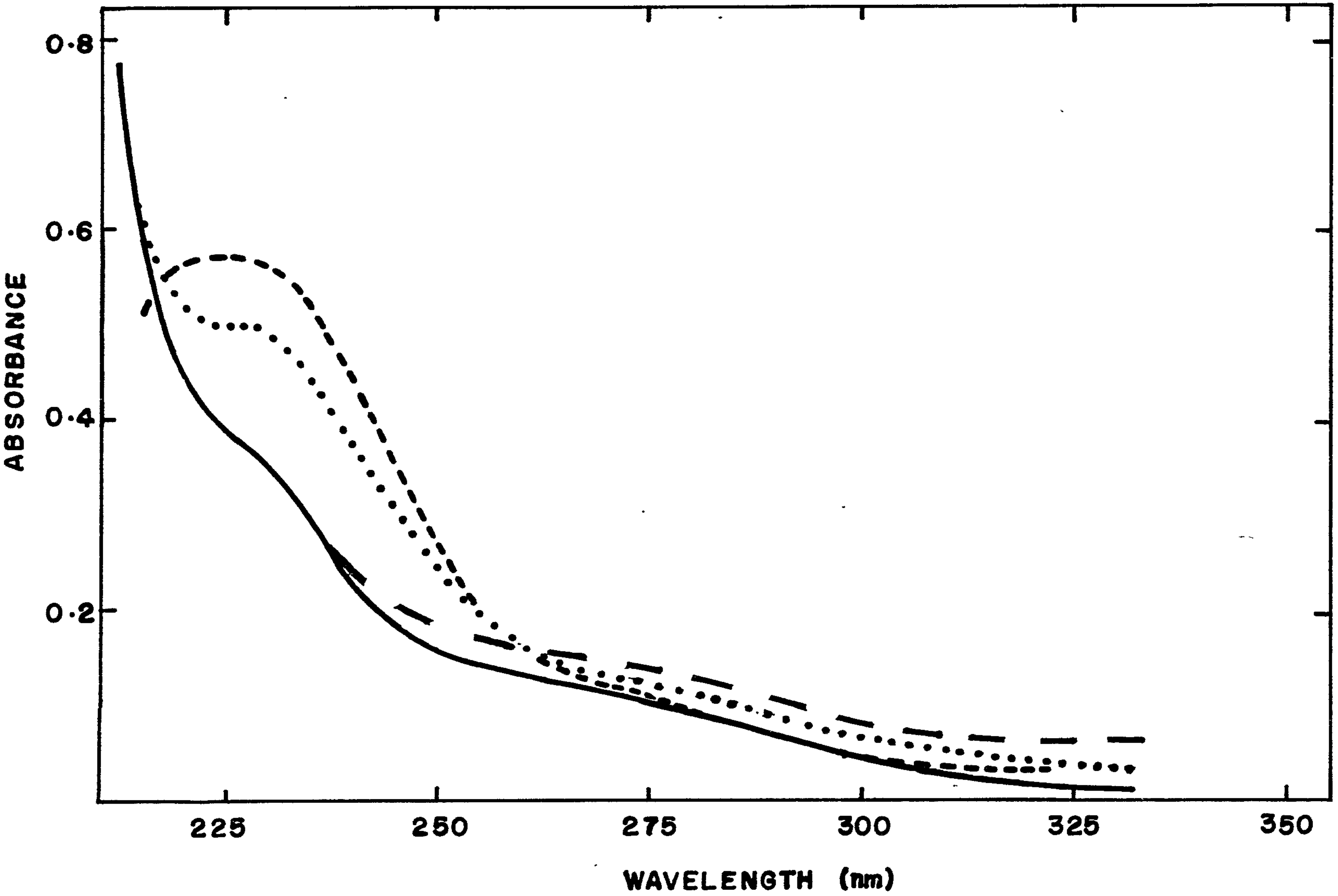

0.6
0.5
0.4
0.3
0.2
0.1
ABSORBANCE
3000
4000
5000
6000
ANGSTROMS

Figure 21. Absorption spectrum of a lipid extract of *T. fluviatilis* after 0, (——), 1.5 (— — —), 6 (····), and 15.5 (----) hours of irradiation in aerated artificial sea water.


0·8
0·6
0·4
0·2
ABSORBANCE
225
250
275
300
325
350
WAVELENGTH (nm)

Proteins

Trypsin and ovalbumen were chosen for investigation as both are very soluble in water. Some of the protein under irradiation was therefore in bulk solution.

Trypsin

Upon irradiation at 250 ug/l, particulate material was formed within 4 hours. The particles were retained on a 0.8 u pore-size filter, and stained intensely with bromphenol blue, which is positive for protein. The stained particles were evident from cursory examination of the filter with the unaided eye. The particles were predominantly of filament-like morphology (figure 22a & b); the remainder were flake-like (figure 22c & d), resembling very closely the flake-like proteinaceous particles found in sea water (Gordon, 1970). Some proteinaceous particles from bucket samples of surface Sargasso sea water are illustrated in figure 23: note especially that both the natural particles and the particles from the reaction vessel are folded upon themselves.

Trypsin subjected to ozonated sea water in 4 hour control experiments yielded no particulate material as observed above. However, some protein tended to adhere to the filter surface producing large positively staining areas around the periphery, which were possibly associated with irregularities in flow of the protein solution through the filter. These areas stained only mildly with bromphenol blue to a yellow color, while the particles produced with radiation stained intensely blue-green. The experimental filters, in addition to particulate material, also showed mildly yellow-staining areas similar in appearance to those on the control filters. When artificial sea water without added protein was irradiated for 4 hours, neither mildly yellow-staining areas nor the abundant blue-green-staining particulate material was produced.

Ovalbumen

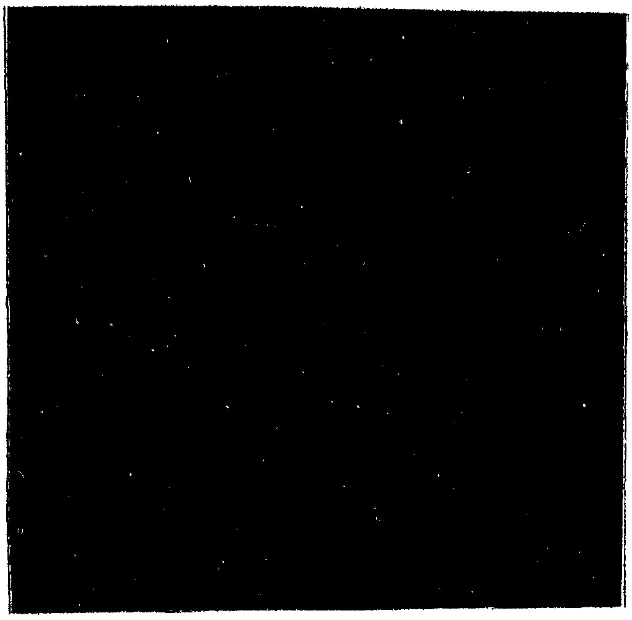
When ovalbumen at 300 ug/l was exposed to radiation for 4 hours, particulate material was produced which was indistinguishable in morphology and staining reaction from the trypsin precipitate. Similar yellow-staining areas were present upon the filters from the control (ozonation) experiments.

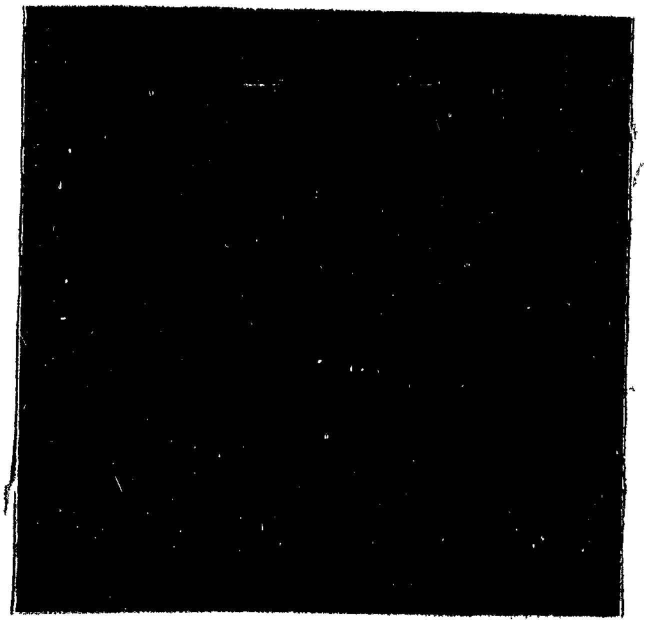
Quantitative analysis of filtered material

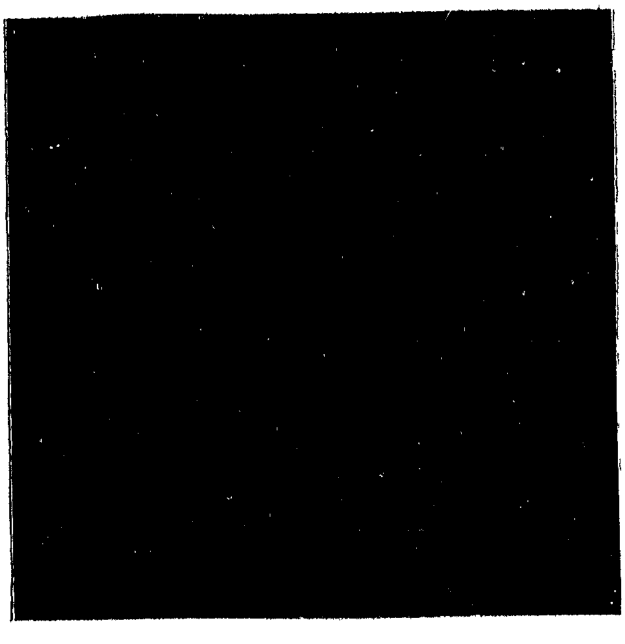
Ovalbumen at 750 ug/l was irradiated in the 500 ml vessel. Seven experiments were run: 4 of these involved exposing the protein solution to radiation for 4 hours; 3 were "zero time" experiments to determine filterable carbon present prior to irradiation (see Methods, p. 14).

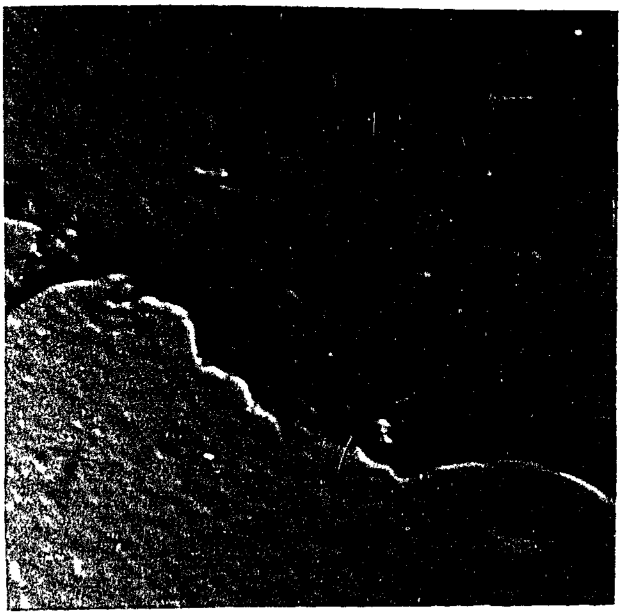
The results are illustrated in figure 24. Each "4 hour" and "zero time" value is subtended by its 95% confidence interval, which is a function of the error in determining the CHN analyzer standard curve. Nine filter blanks were determined, and their average is likewise bracketed by its 95% confidence interval. The differences between each of the 3 sets of data is significant: a "t" test for the difference between the means of the blanks and "zero time" samples yields $P < 0.001$; a "t" test for the

Figure 22. Some filament-like and flake-like particles resulting from irradiation of trypsin at 300 ug/l for 4 hours. x400









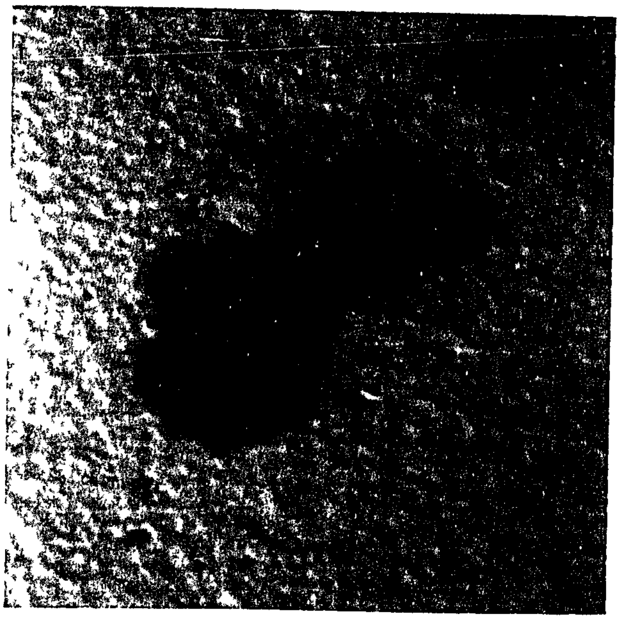
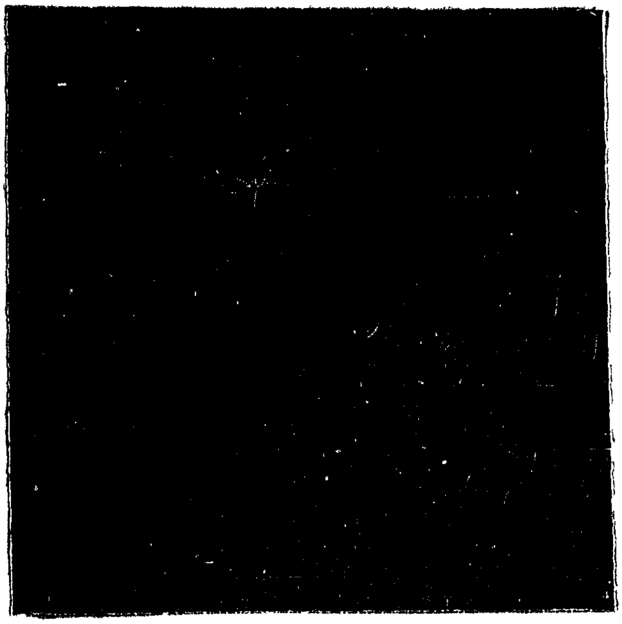
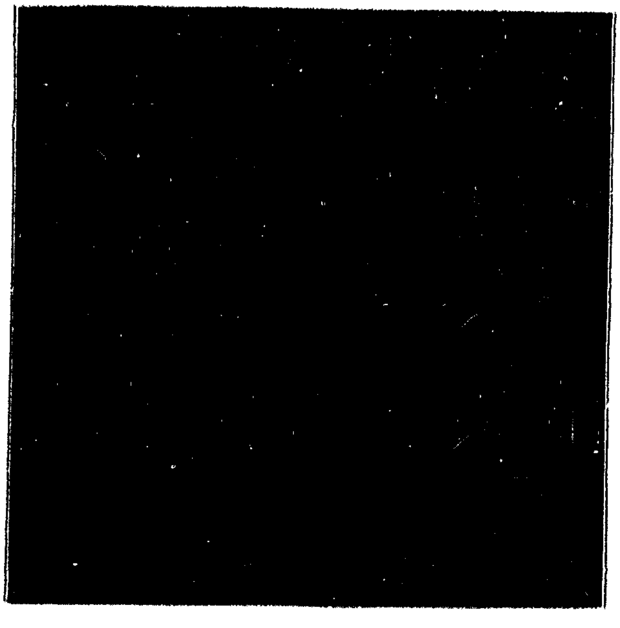
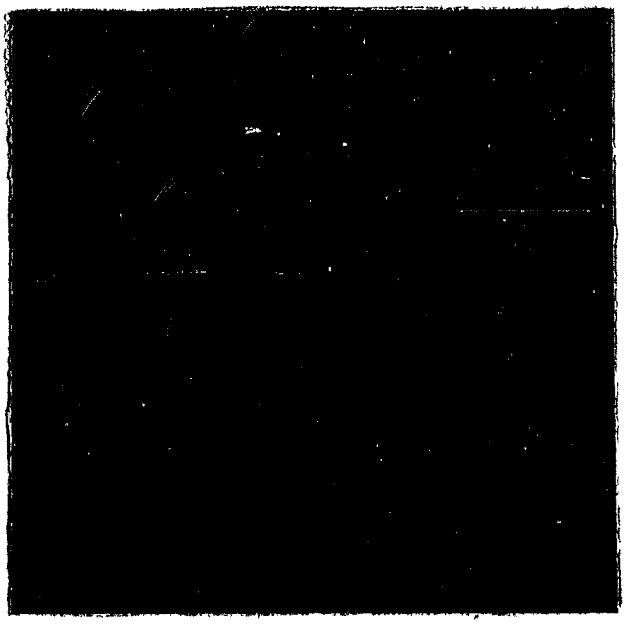
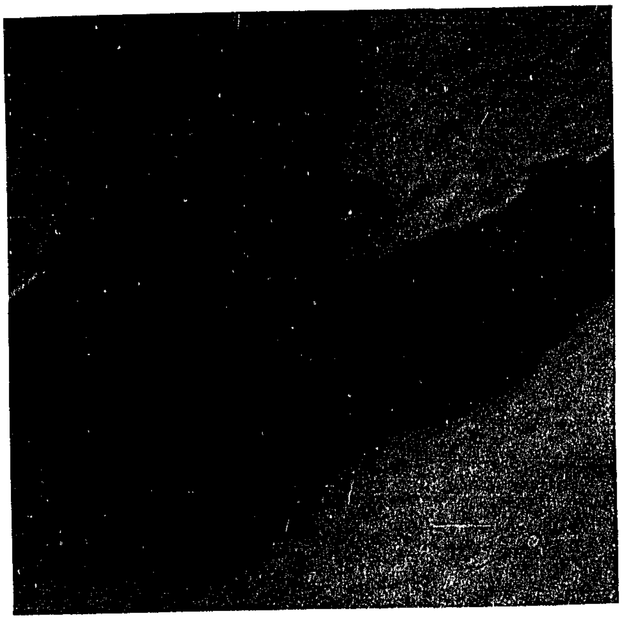


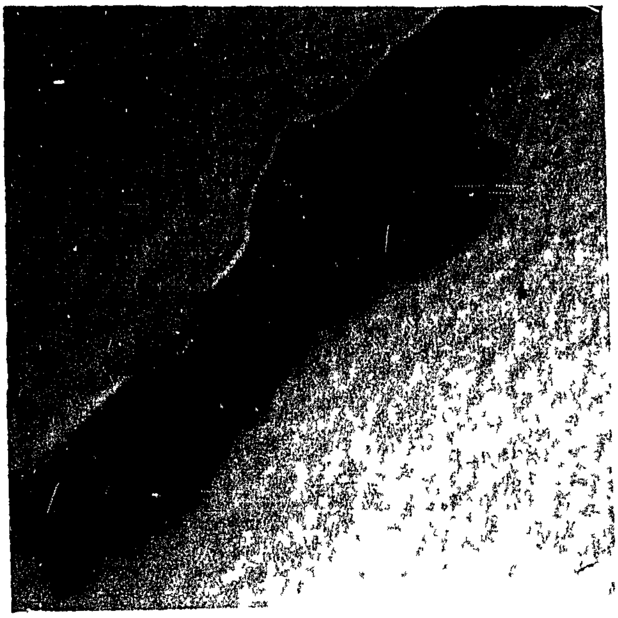
Figure 23. Some filament-like and flake-like particles filtered from bucket samples of Sargasso Sea water. x400











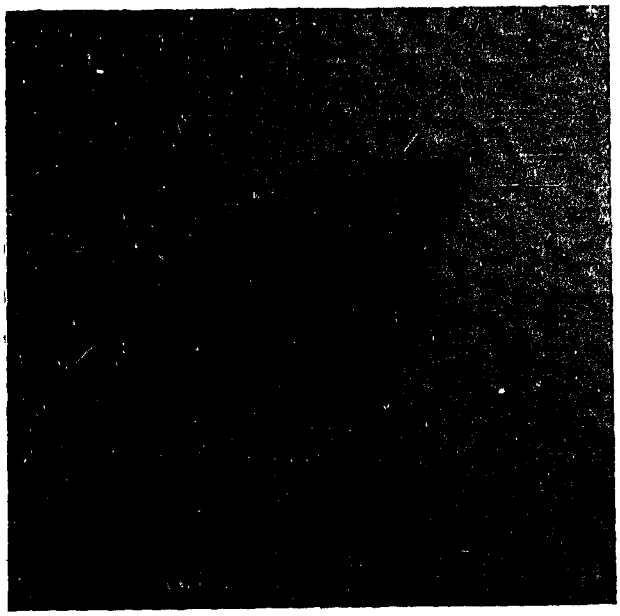
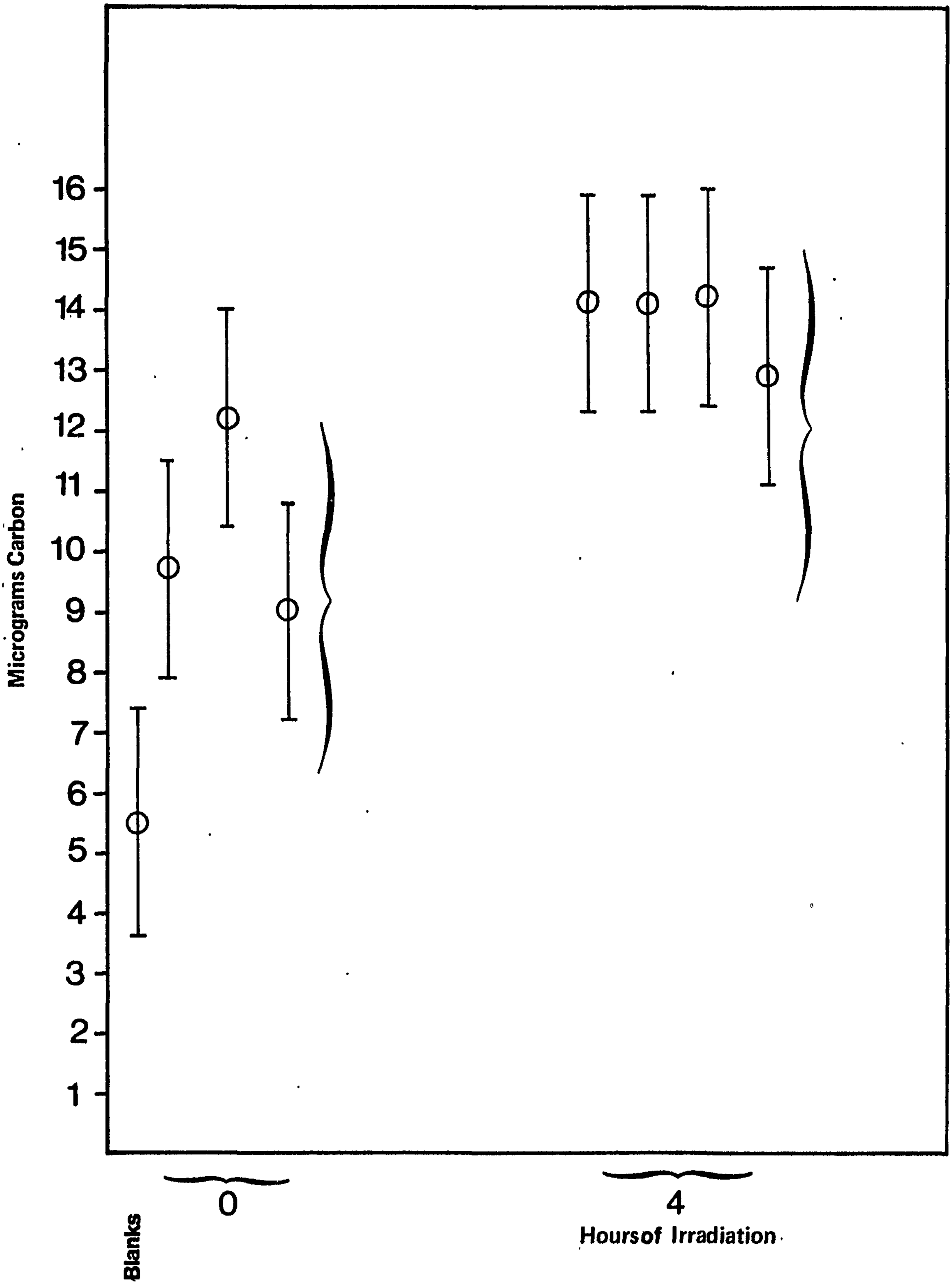


Figure 24. Ovalbumen (at 750 ug/1 in artificial sea water) retained by a 0.8u filter at "zero time" and after 4 hours of irradiation.


Micrograms Carbon
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
Blanks
0
4
Hoursof Irradiation

difference between the means of the "zero time" and "4 hour" samples yields $0.01 < P < 0.02$.

Lipid with protein

Irradiation of linoleic acid with ovalbumen (each at 1 mg/l concentration) resulted in particulates which stained both with bromphenol blue and with OsO_4. The bromphenol blue-staining material was identical in morphology to the material produced upon irradiation of ovalbumen and trypsin. The material that stained with OsO_4 appeared to be in large part adhered to the filaments and flakes; there appeared to be lesser amounts of lipid particulates similar in morphology to those produced upon irradiation of linoleic acid alone.

Rate of accumulation of surface pressure

Sea water

Filtered Sargasso Sea surface water was allowed to stand in the trough, and the rise in surface pressure was measured as a film accumulated. Two experiments were run without ultraviolet, while one incorporated the lamp. The presence of the radiation did not appear to have any effect on the rate of change of surface pressure (figure 25).

Lake water

Filtered Halifax reservoir water was investigated as was the sea water above. Three experiments were without, and two were performed with radiation. Again, the simulated solar radiation was indicated to have no affect on the rate of change of surface pressure (figure 26).

Sea water with protein

Solar levels of radiation significantly increased the rate of accumulation of pressure at the surface of a solution of ovalbumen (1.71 mg/l) in filtered surface Sargasso Sea water. In figure 27, curves A and B illustrate the results of two experiments in which surface tension was recorded as the film accumulated in the presence of radiation. Curve C illustrates the accumulation of pressure in the absence of radiation. In experiment D tension was recorded in the absence of radiation for 21 hours; at that time the lamp was turned on, and tension was noted for an additional 14 hours.

To test the significance of the apparent difference in $\Delta F/t$ between experiments A and B and experiments C and D, the data for A and B up to 6 hours, and the data for C and D up to 11 hours were combined. Straight lines were fitted to each of the two groups of data (figure 28). The "t" testing each regression was significant: $P \ll 0.001$ for the data of experiments A plus B; $0.01 < P < 0.02$ for the data of C plus D. The difference between the slopes of the two regressions was examined with a "t" test, and was found to be significantly different ($0.01 < P < 0.05$).

Film dynamics

Because radiation increased the surface pressure of sea water moderately enriched with protein, we investigated the effects of such increases of lateral pressure on film dynamics.

Lake water

Filtered lake water was allowed to form a surface film for periods ranging from 10 minutes to 104 hours; the film was then subjected to lateral pressure by compressing the film to a smaller area. The surface tension at the collapse pressure (the point at which there begins a decrease in the rate of change of ΔF) was within the narrow limits of 51.4 to 54.6 dynes/cm irrespective of film age (figure 29).

Such increases in the lateral surface pressure of lake water films (as may occur in windrows or along leeward shores, and possibly assisted by solar radiation) can be accompanied by a transformation of film organics to a state incapable of re-expanding at the interface. Filtered lake water was allowed to accumulate a film for either 14 or 18 hours, and the film was compressed and released (Methods, p. 17). Film re-expansion was measured by recording subsequent decrease in surface tension. The results are illustrated in figure 30: the film, after compression, was not capable of re-expanding at the interface to yield the same decrease in surface tension as was effected by the presence of the original film. Although the amount of organics lost to the new state was not known, that amount was capable of exerting a surface pressure of approximately 3 dynes/cm. After compression, a visible surface solid was left at the end of the trough, and was readily collected by immersing and withdrawing a filter through the interface. When shaken in low-carbon water, the surface solid was disrupted into small particles.

A portion of a slick on a Nova Scotian lake was compressed from an area of approximately 100 cm^2 to approximately 40 cm^2. A visible surface solid formed, which was readily disrupted by disturbing the surface: the resulting particles ranged from about 1 cm^2 in area to colloidal (capable of producing a tyndall effect). The particles were observed to sink through the water column. The large particles could be easily disrupted into smaller ones.

Sea water

Organic films from filtered Sargasso Sea water did not exhibit a collapse point (figure 31), nor was there evidence of the formation of a new state (incapable of re-expanding at the interface) upon lateral compression of the film (figure 32).

Protein in artificial sea water

A film on a solution of ovalbumen in artificial sea water (1.71 mg/l) was allowed to accumulate for 12 hours. Upon lateral compression as above, the film was converted to a surface solid. When a film was allowed to accumulate for 12 hours in the presence of radiation, compression yielded material which did not differ from the fibers produced in the absence of radiation. When the precipitate from each experiment was shaken in artificial sea water, particles were formed which resembled very closely the bromphenol blue-staining particles from irradiation of dilute solutions of protein in artificial sea water.

Protein in sea water

Ovalbumen was dissolved in filtered Sargasso Sea surface water (1.71 mg/1), and a film was allowed to accumulate for 19.5 hours. After compression and release as above, surface tension was followed for 9.8 hours. The results are illustrated in figure 33. As was the case with lake water, some film organics were transformed into a state incapable of re-spreading at the interface.

Considering 1) the effect of radiation on the rate of accumulation of surface pressure in water moderately enriched with protein, and 2) the phenomenon of pressure-induced transformation of film organics, it is conceivable that solar ultraviolet may at times play a part in promoting the formation of particles from interfacial substances.

Figure 25. Rate of accumulation of surface pressure in Sargasso Sea surface water in the presence of (▣) and in the absence of (◉ and ◬) simulated solar radiation.

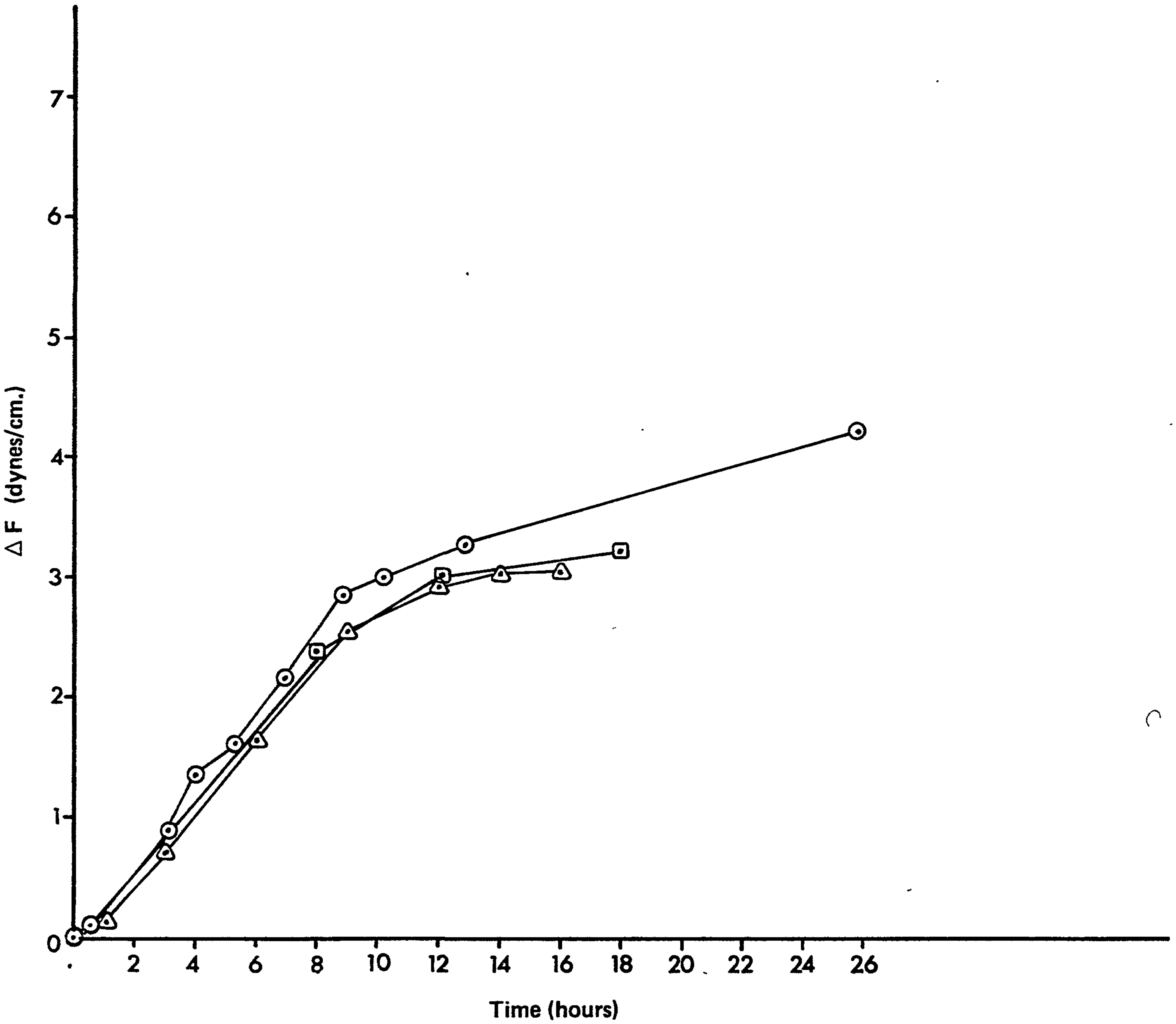

ΔF (dynes/cm.)
0
1
2
3
4
5
6
7
2
4
6
8
10
12
14
16
18
20
22
24
26
Time (hours)

Figure 26. Rate of accumulation of surface pressure in lake water in the presence of (☆ and ⊙) and in the absence of (⊡ , ◬ , and ◈) simulated solar radiation.

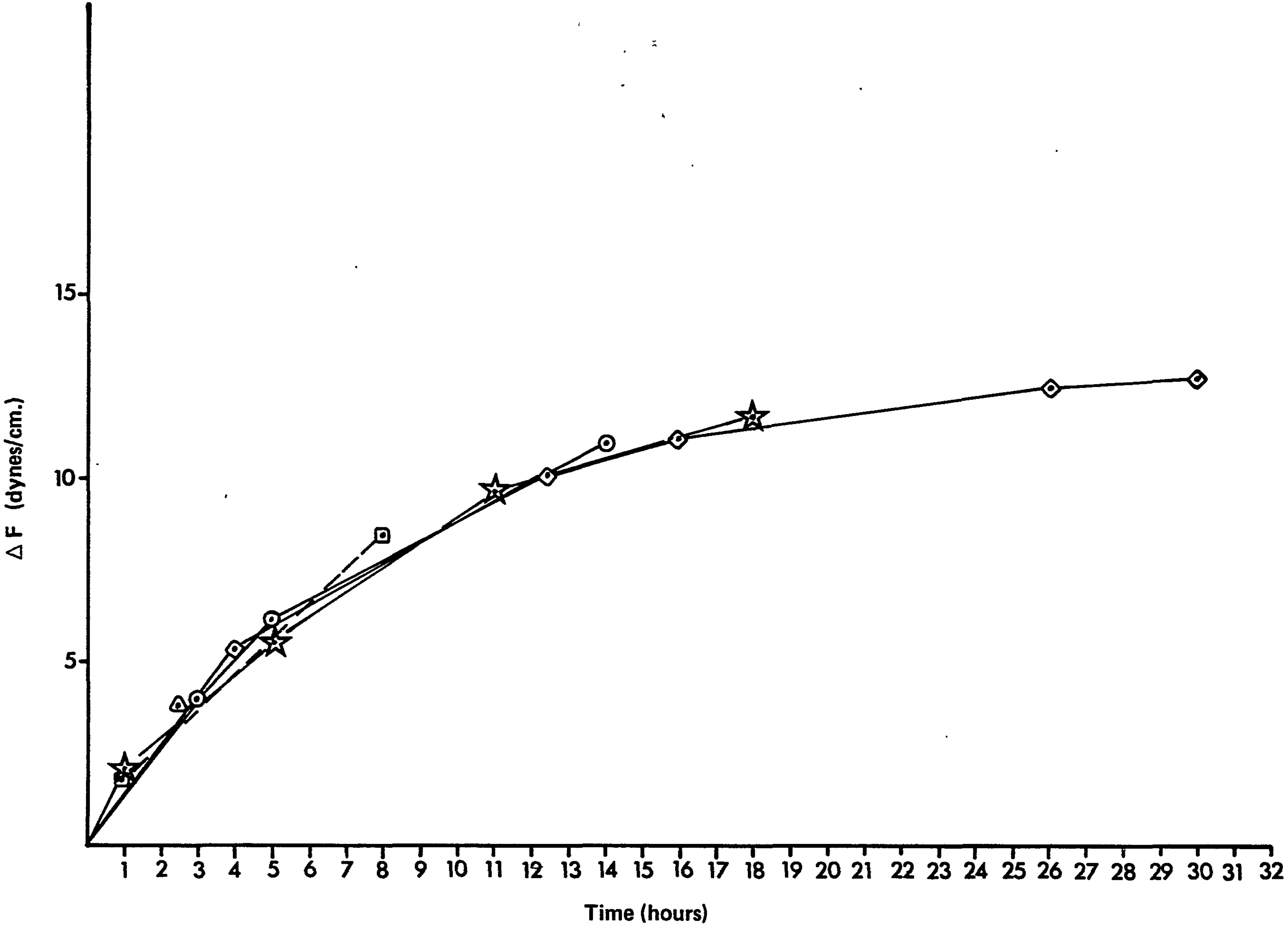

15
10
5
Δ F (dynes/cm.)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32
Time (hours)

Figure 27. Rate of accumulation of surface pressure in Sargasso Sea surface water slightly enriched with ovalbumen (1.71mg/l) in the presence of (A , B) and in the absence of (C , D) simulated solar radiation.

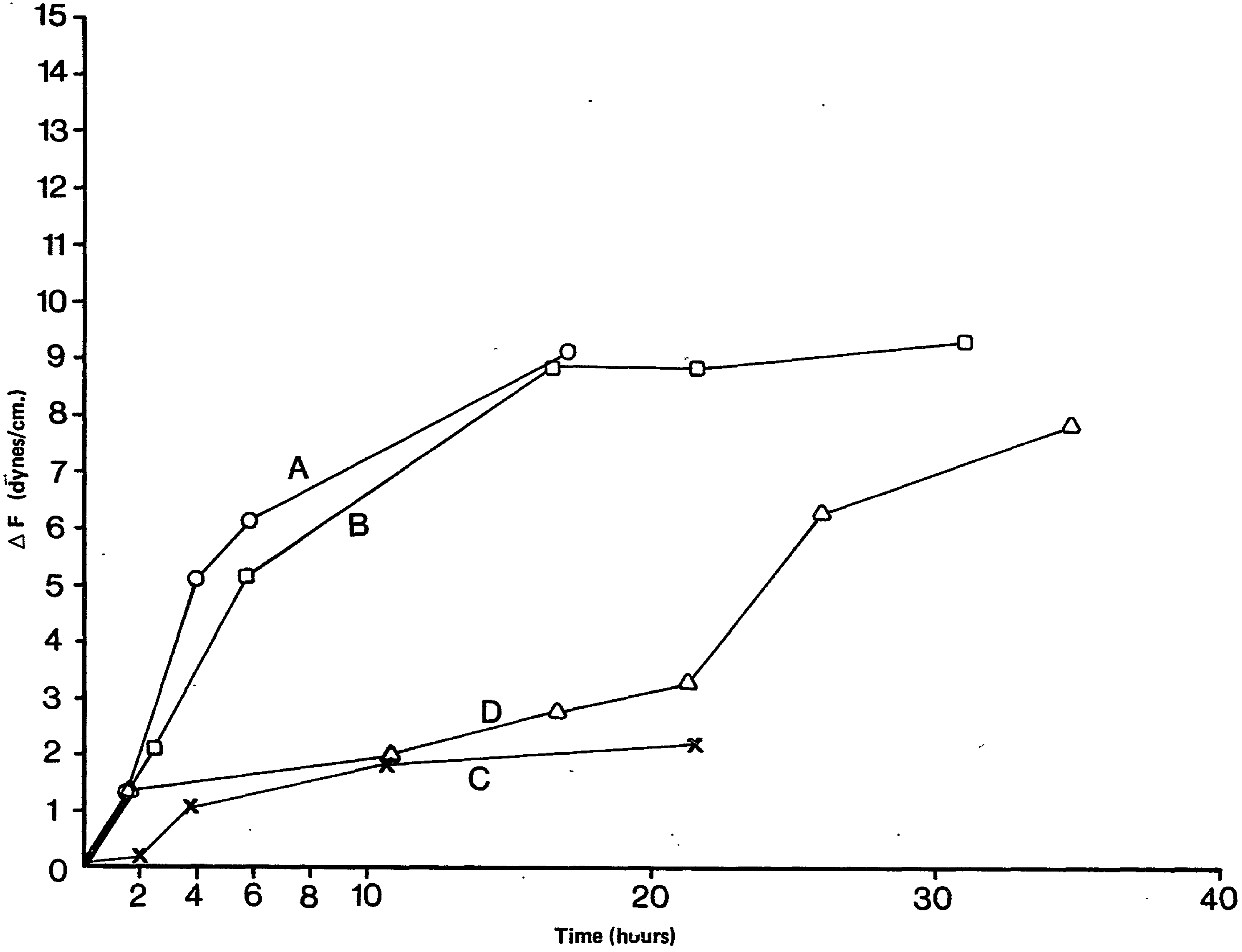

15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Δ F (dynes/cm.)
A
B
C
D
2
4
6
8
10
20
30
40
Time (hours)

Figure 28. Straight lines fitted to data of Figure 27 to test the significance of the difference in the rate of accumulation of surface pressure between experiments A & B, and experiments C & D. See p.52 for discussion.

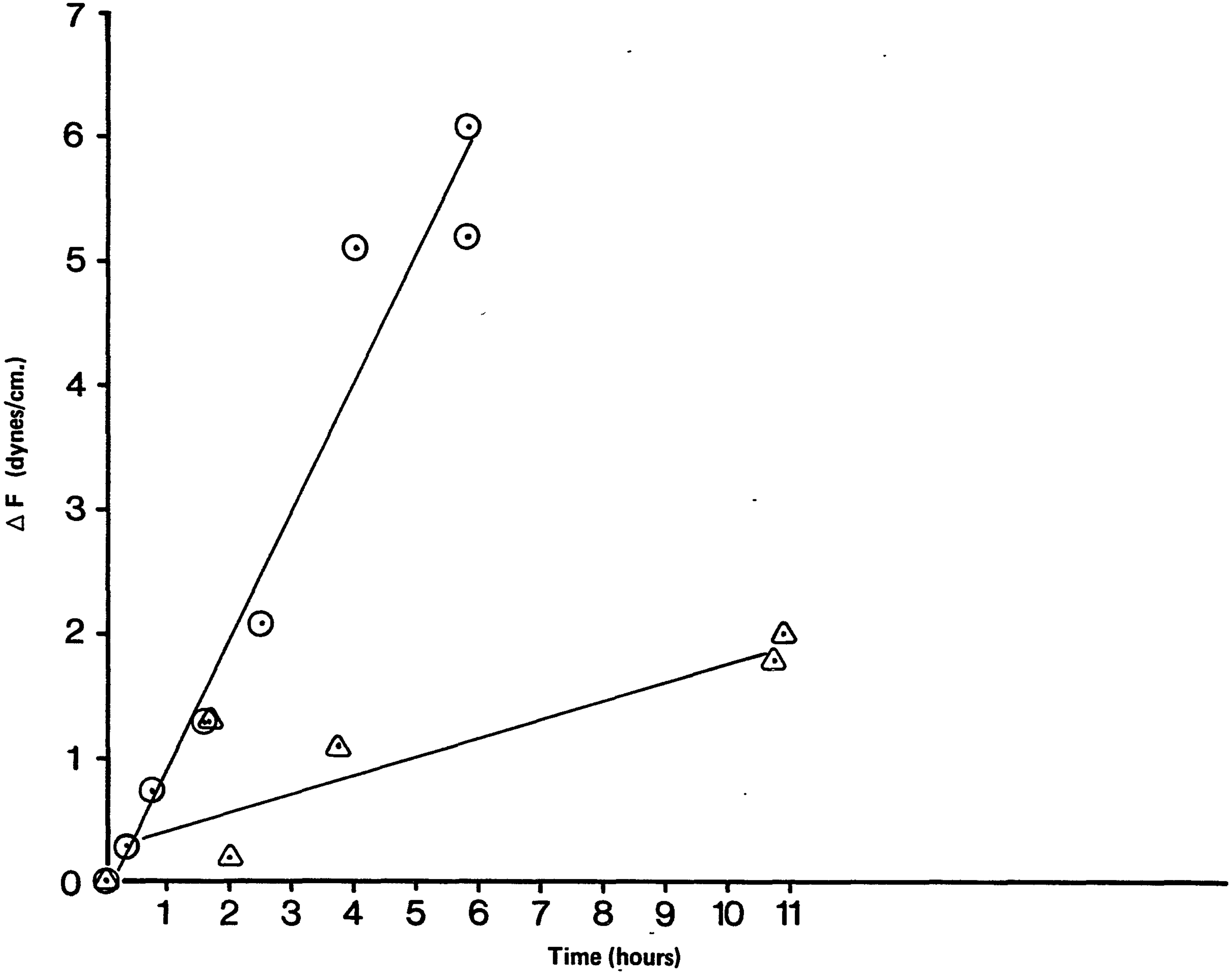

7
6
5
4
3
2
1
0
ΔF (dynes/cm.)
1
2
3
4
5
6
7
8
9
10
11
Time (hours)

Figure 29. Force-area relationships for lake water films which have accumulated for 10 minutes (———), 2.5 hours (----), and 14 hours (····). Note that the collapse points (indicated by the arrows) lie within a small range irrespective of the large variation in film age.

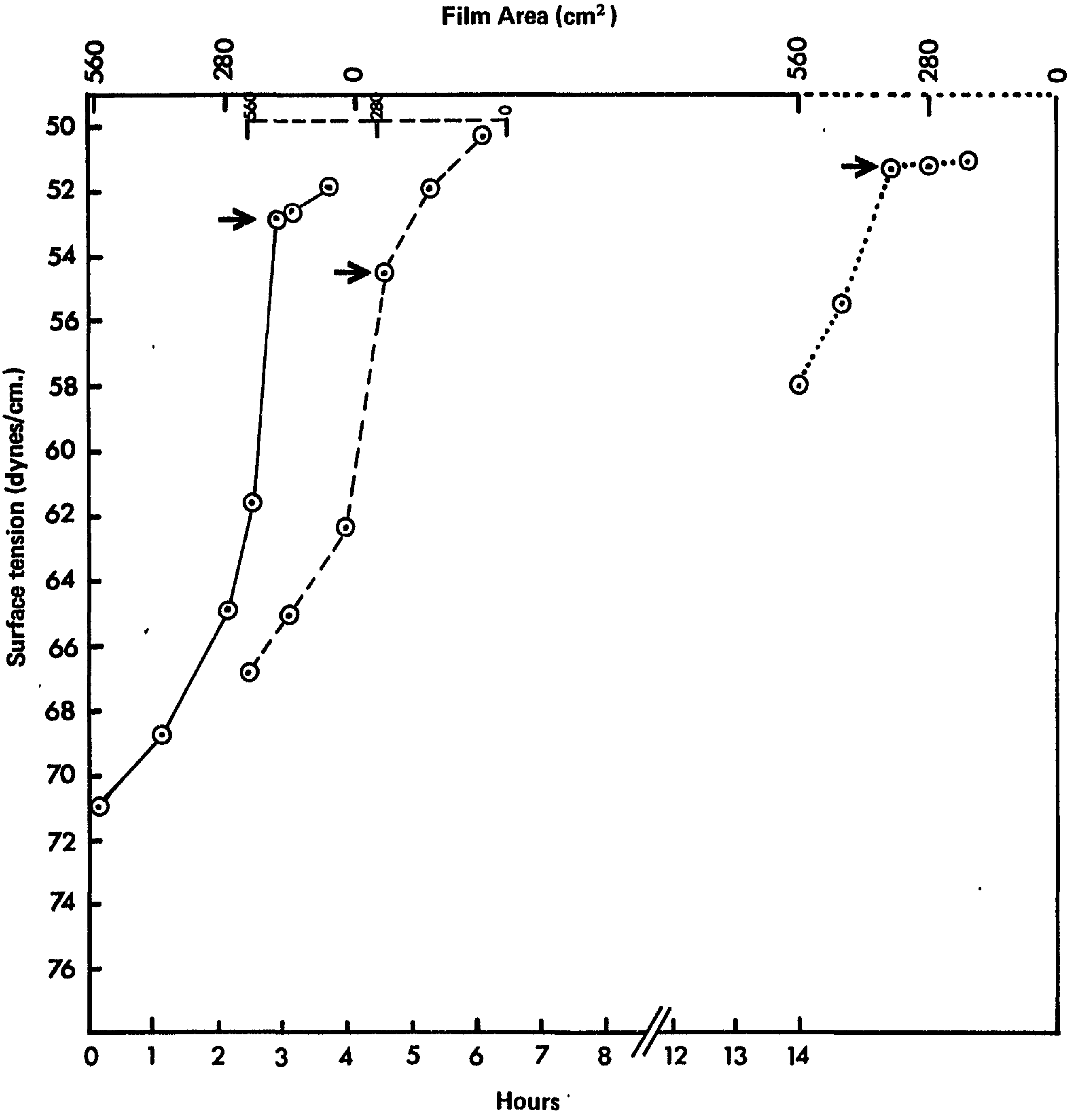

Film Area (cm^2)
560
280
0
560
280
0
560
280
0
560
280
0
Surface tension (dynes/cm.)
50
52
54
56
58
60
62
64
66
68
70
72
74
76
0
1
2
3
4
5
6
7
8
12
13
14
Hours

Figure 30. Accumulation (upper curves) and re-expansion after compression (lower curves) of films on lake water. The film, after compression, is not capable of re-expanding to yield the same decrease in surface tension as was effected by the presence of the original film.

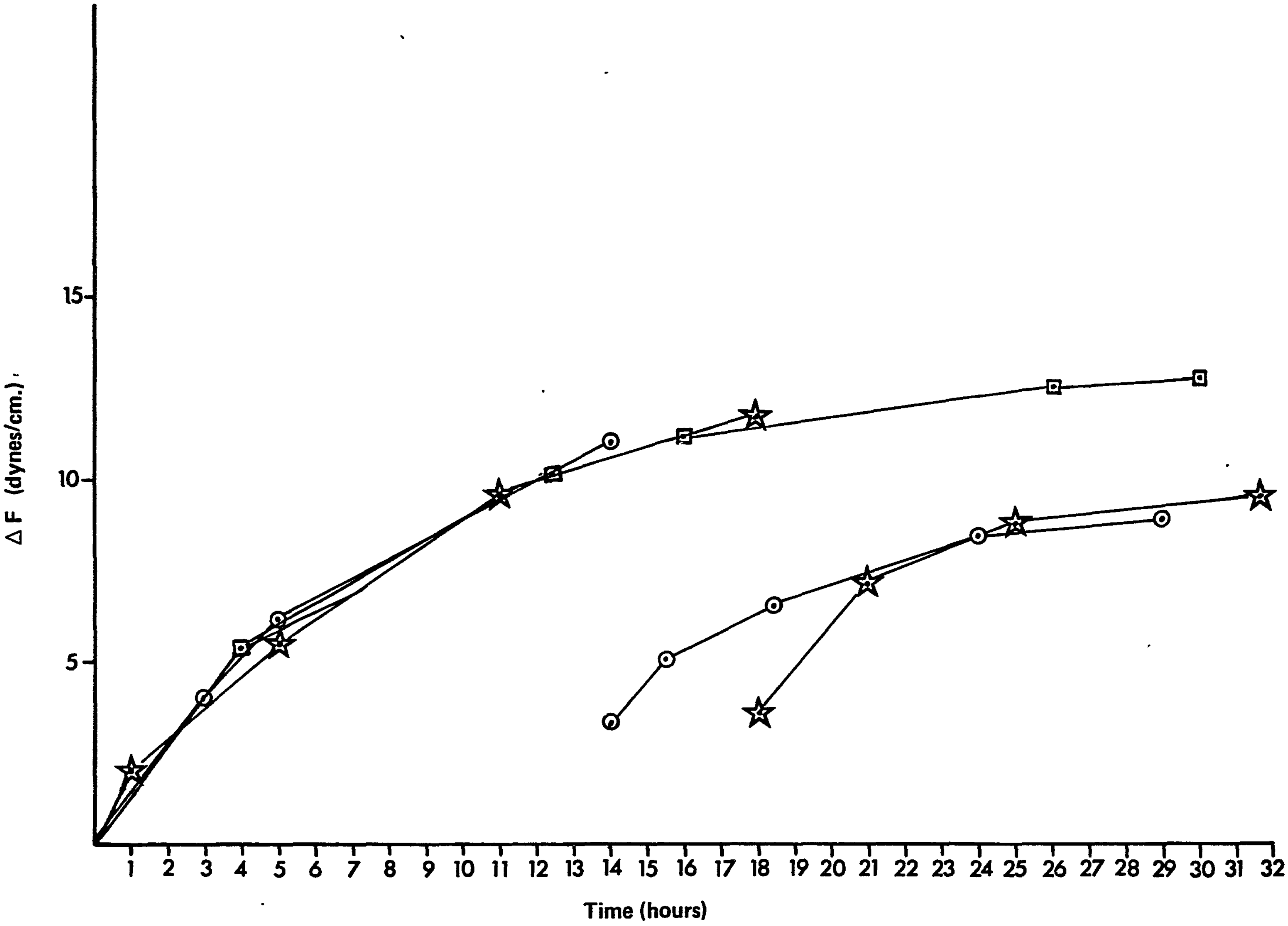

15
10
5
ΔF (dynes/cm.)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32
Time (hours)

Figure 31. Force-area relationships of two films that developed on Sargasso Sea surface water.

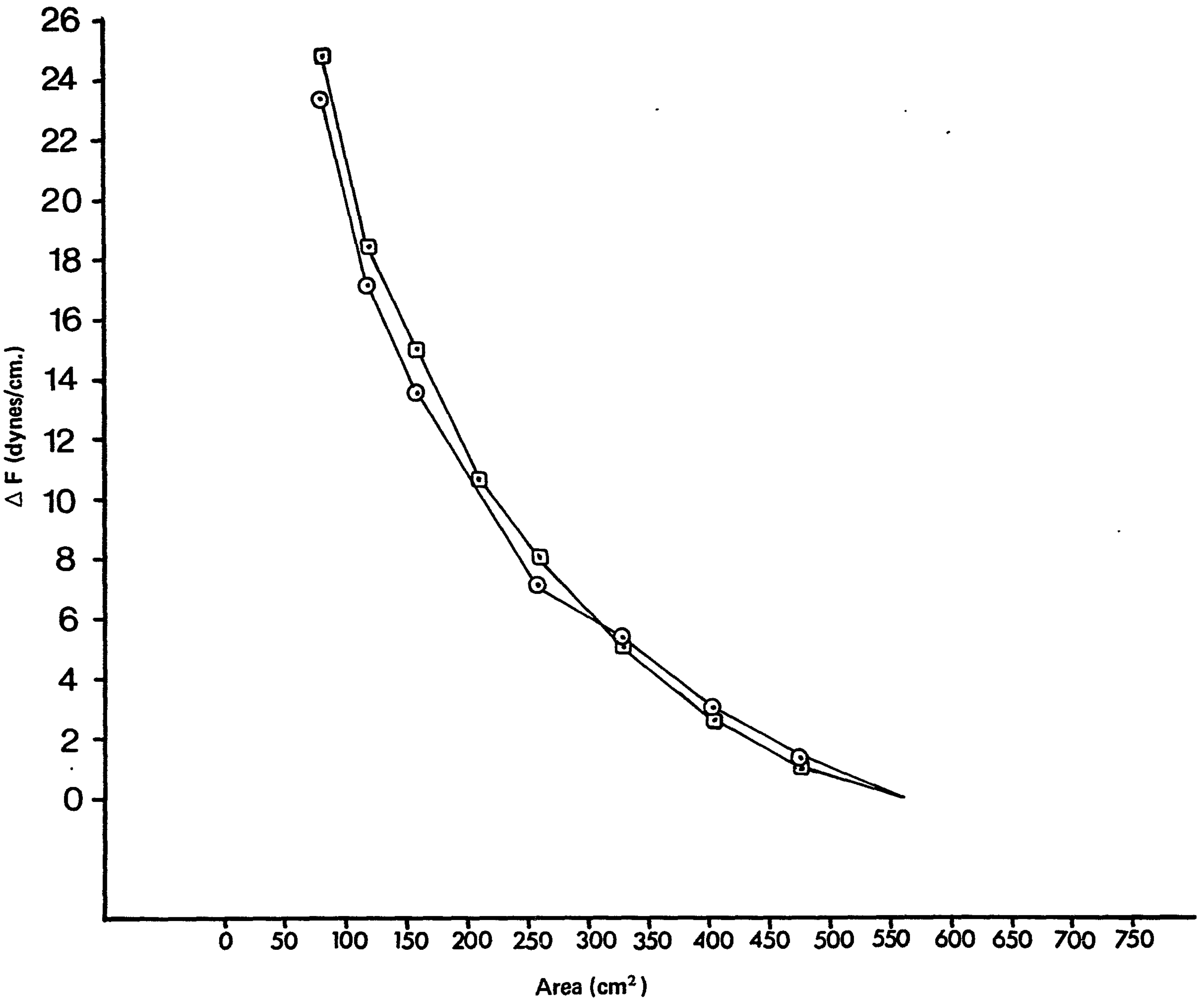

26
24
22
20
18
16
14
12
10
8
6
4
2
0
Δ F (dynes/cm.)
0
50
100
150
200
250
300
350
400
450
500
550
600
650
700
750
Area (cm²)

Figure 32. Accumulation and re-expansion after compression of three films that developed on Sargasso Sea surface water.

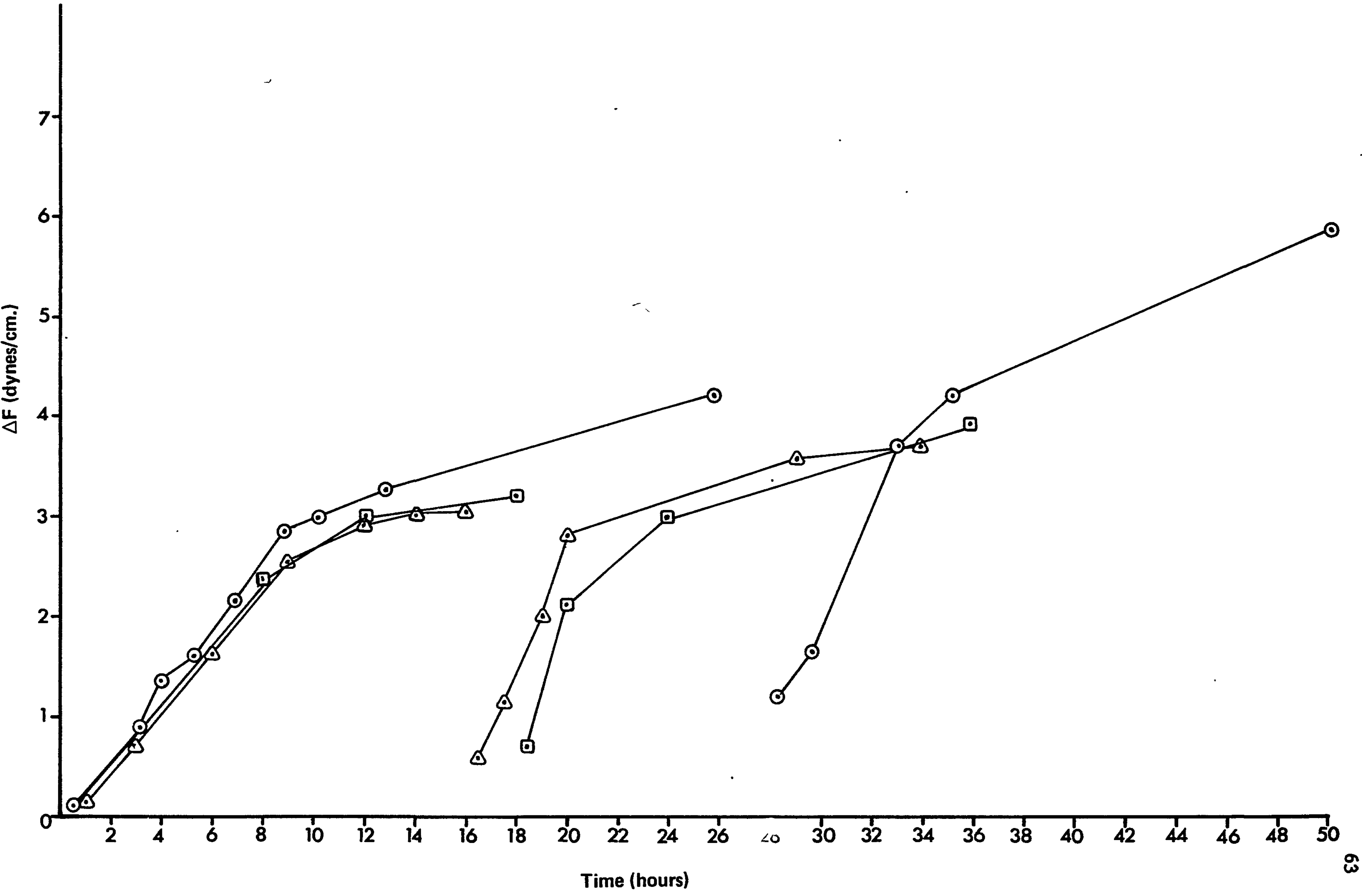
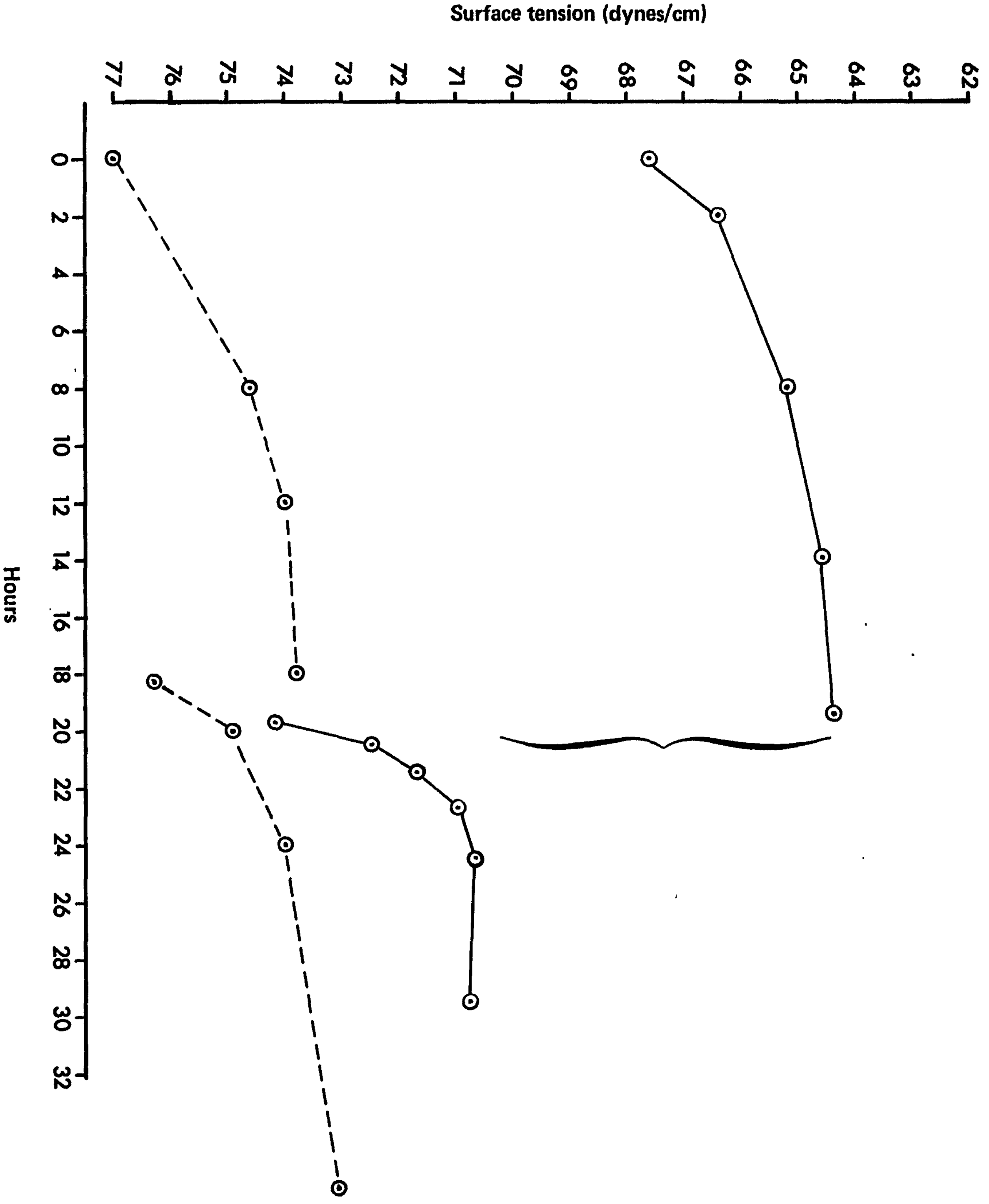

ΔF (dynes/cm.)
0
1
2
3
4
5
6
7
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
50
Time (hours)

Figure 33. Accumulation, compression, and re-expansion of 1) a film from Sargasso Sea surface water (----) compared with 2) a film from Sargasso Sea surface water with protein at 1.71 mg/1 (——). In (2) some film organics are transformed into a state (particulate) incapable of re-expanding at the interface.


Surface tension (dynes/cm)
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
Hours

DISCUSSION

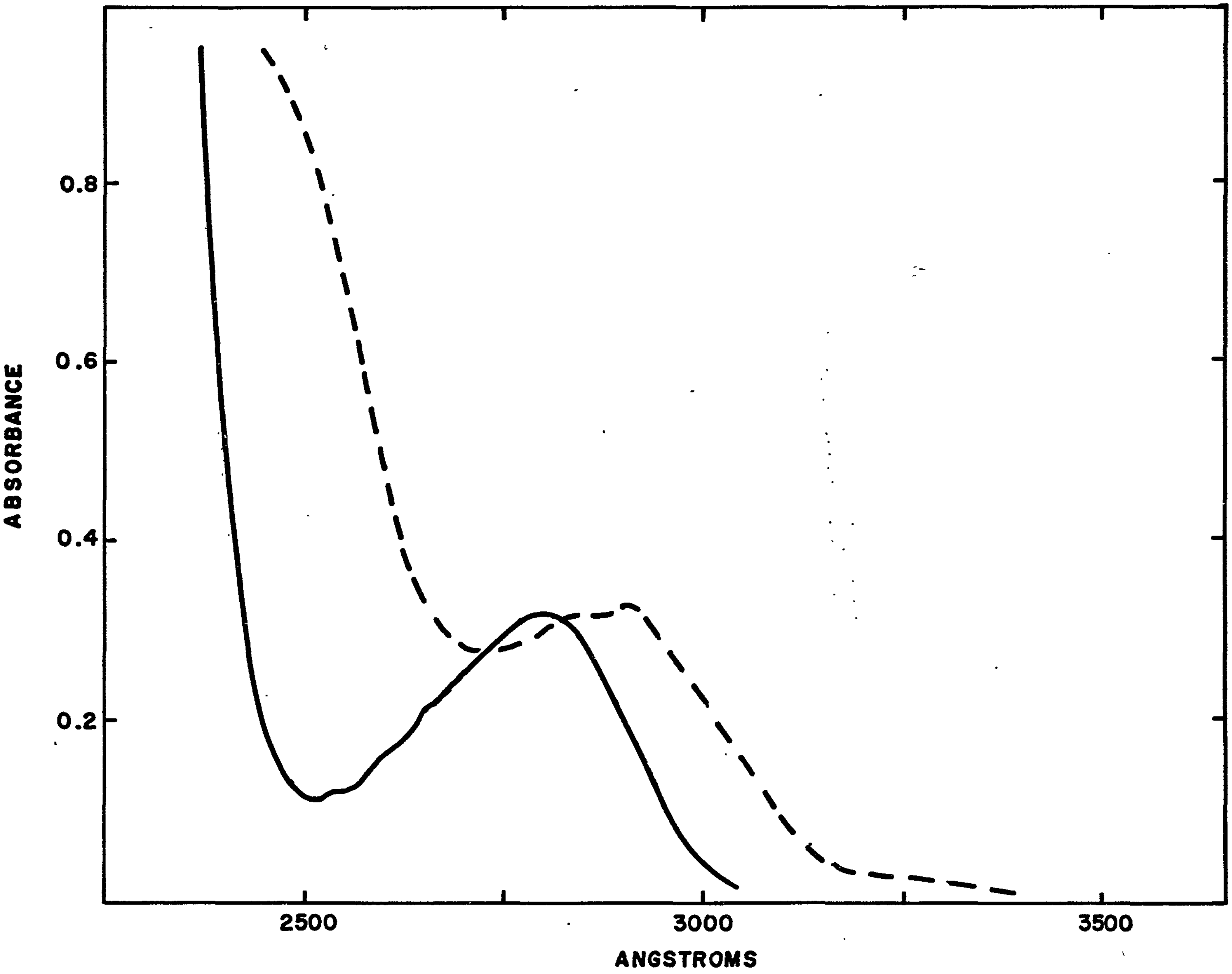
The interaction of proteins with radiation

Previous studies of the effects of ultraviolet radiation on proteins have almost invariably employed unfiltered emission of unspecified intensity from low or medium pressure mercury arcs. Such radiation consists in large part ($>$ 90% for many low pressure arcs) of energy at wavelengths less than 2600 Å. Such high-energy quanta may be responsible for primary and secondary processes that may not occur at solar wavelengths; the reactions observed in those studies may not, therefore, be indicative of the fate of proteins at the sea surface. As will be discussed below, some results obtained in the present work differ from results reported in previous studies; these differences are probably due in part to differences in the wavelengths employed.

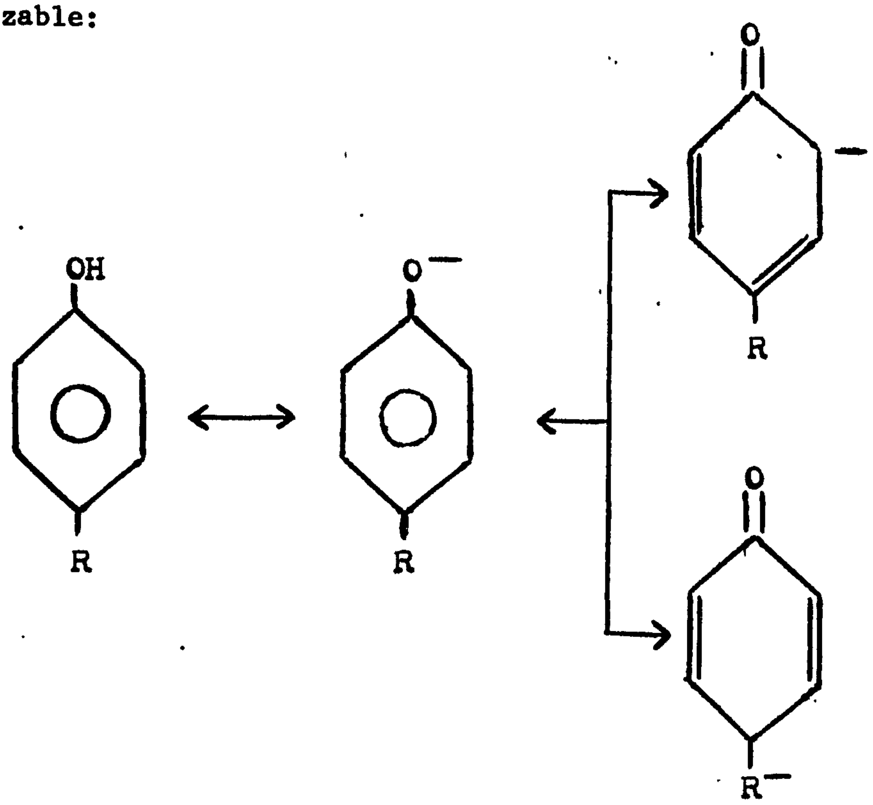
Ultraviolet absorption by proteins in sea water

The absorption spectra of ovalbumen in artificial sea water at ph 5.8 and pH 11.8 are illustrated in figure 34. The maximum in the 2800-2900 Å region is due mainly to absorption by the aromatic amino acids, with slight contributions by cystine and cysteine. The absorption maximum, its red shift with increase in pH, and its increased extinction at higher pH are characteristic of all proteins containing aromatics. The increase in extinction may be due to the greater number of possible resonance structures at higher pH for tyrosine, as the chromophore is

Figure 34. Absorption spectra of ovalbumen in artificial sea water at pH 5.8 (——) and pH 11.8 (----).


0.8
0.6
0.4
0.2
ABSORBANCE
2500
3000
3500
ANGSTROMS

ionizable:



This may result in increased stabilization of the excited state, and thus increased extinction (West, 1956).

This red shift and concomitant increase in absorption of proteins may be significant oceanographically: since the pH of organisms is lower than that of sea water, proteins upon release into sea water would tend to absorb solar ultraviolet more intensely by virtue of the change in pH. The present research illustrates that (in artificial sea water) absorption of solar levels of ultraviolet by proteins results in the formation of discrete particles. Such transformation into a state which can be readily ingested may be a significant process in the cycle of organic nitrogen in surface waters.

Absorption by the peptide bond is believed not to occur at wavelengths at 2800 Å and longer, indicating that this chromophore is responsible for little if any absorption of solar radiation. "End absorption" of low extinction extends well into the solar ultraviolet: Setlow and Doyle (1957), in an investigation of the action spectrum for the inactivation of ribonuclease, reported a high quantum yield at 3130 Å. Also, Steele and Szent-Gyorgyi (1958) reported flourescence from proteins irradiated at 3400 Å. The chromophores responsible for end absorption have not been determined.

Effects of radiation on proteins in surface films: a comparison of previous and present results

Protein at a concentration of 1.71 mg/l in artificial sea water was found to form a film which, upon compression, yielded a fibrous material. Similar observations on buffer and on distilled water have previously been reported; however, McLean and Geisa (1950) found that unfiltered radiation from a low pressure arc results in proteolysis in surface films, the protein fragments being detected in the substratum liquid. Kaplan and Frase (1958), irradiating an ovalbumen film, followed changes in area as the film was kept at a constant pressure. They detected 1) an initial increase in area (equivalent to the increase in pressure at a constant area observed in the present work), followed by 2) a prolonged decrease in area. Neurath (1936) and Dognon and Gougerot (1943) also report decreases in the surface activity of proteins upon ultraviolet irradiation. Kaplan and Fraser attribute their observed initial increase in surface activity to further unfolding brought about by light absorption and rupture of a labile bond. They believe that the well-documented prolonged decrease in area observed by them and others was due to photolysis, resulting in depletion of the film as the protein fragments dissolved in the bulk liquid. Such a decrease in surface activity (which would have been manifested in the present work as a decrease in surface pressure) was not found in these studies of protein films on ocean surface water. This indicates that solar levels of ultraviolet radiation are not measurably responsible, over 32 hours, for proteolysis in films on sea water.

Kaplan, Andrews, and Fraser (1953) found that the ability of protein films to form fibers upon compression is lost entirely after two or more hours of exposure to unfiltered radiation from a low pressure arc; they refer to this loss as a "regular and extremely reproduceable phenomenon," and attribute it to proteolysis. The wavelengths and intensities employed in the present work, however, apparently did not effect such proteolysis over 12 hours as to render the film less capable of fiber formation upon compression.

While the differences between the present results and those of previous studies (regarding both surface activity and film collapse) may be due to either 1) differences in wavelength employed, or 2) differences in intensity, or both, it is possible that differences in wavelength played a significant role. The peptide bond itself absorbs below 2500 Å (Beavon and Holiday, 1952); such wavelengths were employed in previous work. Absorption by the peptide bond may not necessarily result in proteolysis; photolysis can also result from absorption of a quantum of sufficient energy by a second chromophore (e.g. an aromatic amino acid), with the energy being transferred to a nearby peptide bond (Szent-Gyorgyi, 1958; Shore and Pardee, 1956). Although the dissociation energy of the peptide link within proteins is not known with certainty, it is questionable whether the solar levels of radiation employed possessed sufficient energy to effect proteolysis. There is no doubt, however, that proteolysis can result from absorption of frequencies employed in previous work (Bernhart, 1939; Giesa, 1950; Wheeler et al., 1970).

The differences between the present and previous results concerning the action of ultraviolet radiation on protein films is probably due in part to differences in wavelength employed. This indicates that in considerations of solar photochemistry one should view with reservation the results of experiments utilizing radiation of higher frequency than is found in sunlight.

Effects of radiation on proteins in solution

The nature of the "control" experiments in the work with trypsin and ovalbumen in dilute solution leaves little doubt that the particulates observed microscopically (figure 22), and determined analytically (figure 24) were formed due to the presence of the simulated solar radiation. The particles were sheet- or film-like, and identical in morphology to disrupted compressed protein surface film: both of these characteristics strongly indicate that the particles were formed at interfaces. The resemblence of some of the particulates to the "flakes" and "oblongs" of sea water may not be entirely coincidental: it is possible that some oceanic proteinaceous particles may also be formed by surface denaturation; the extent of solar catalysis at or near the sea surface is, however, questionable.

If film-like proteinaceous particulates do (as indicated by their morphology) form at interfaces, this would imply that interfacial forces act on the molecules prior to their incorporation into particulate material. The resulting interfacial unfolding may have the effect of increasing the protein's susceptibility to enzymatic hydrolysis, as segments of its amino acid chain would be more exposed to the action of proteolytic enzymes. In sea water, interfacial forces may thus aid in organic nitrogen and carbon being cycled back into the biosphere; exposure to ultraviolet radiation would, at least in the case of protein in artificial sea water, result in conversion into a form in which this more readily hydrolyzable protein could be ingested.

Interfaces are numerous both in surface water and in deep water, and it is conceivable that continual accumulation of protein at an interface, and concomitant increase in surface pressure, could result in particle formation from protein films in sea water. Such pressure-induced transformation of organics to the particulate state was demonstrated in this research at the interface of protein-enriched sea water. The compressed material, upon disruption, closely resembled some of Gordon's (1970) proteinaceous "flakes" and "oblongs". Bubble surfaces and the sea-air interface offer sites for the accumulation of protein in surface waters. A bubble surface, moreover, is subject to tangential forces from the flow of sea water about it as it rises through the water column; such forces would tend to increase surface pressure in adsorbed films, and may result in the aggregation of any adsorbed protein. Any organics, inorganics, organisms, etc. within the protein matrix on the bubble surface would probably be included in the resulting proteinaceous particle.

Processes resulting in the collapse of protein films on bubble surfaces or at the sea-air interface may account for some of the particulate material resulting from bubbling of sea water samples (Riley, 1963; Riley, 1970; Wangersky, 1965). Gordon (1970) found that the flakes and oblongs of sea water stained intensely with bromphenol blue, indicating a high protein content. Sharp (1972) agitated filtered sea water on a shaker, resulting in the "vigorous production of bubbles." The particles produced by this procedure had (with few exceptions) definite edges, resembling in morphology Gordon's flakes. The above work, and the present demonstrations that film-like particulates can form at the air-water interfaces of 1) protein-artificial sea water solutions, 2) protein-sea water solutions, and 3) lake water lend support to the concept that the sea-air interface, or bubble surfaces, or both may be of importance in the transformation at sea of dissolved carbon to the particulate state. While protein has herein been shown to be capable of yielding film-like particulates at the sea water-air interface, it is not known with certainty whether the presence of protein is essential for particle formation from interfacial organics. If the flakes and oblongs of sea water are indeed derived from pressure-induced transformation film organics, Gordon's finding that these particles stain predominantly and invariably with bromphenol blue supports a concept of protein playing an essential role in the formation of particulates via the lateral compression of surface films.

Unfolding of proteins is not limited to air-water interfaces, but will occur at any interface where the phase difference is favorable to a tertiary structure other than that of the dissolved state. Adsorption at an oil-water interface, for instance, results in a modification of poly-DL-leucine tertiary structure (Cheesman and Davies, 1954). The form of this peptide at the air-water interface is that of an incompletely unfolded chain, the residual folding being due to internal cohesion between the hydrophobic $-CH_2-CH-(CH_3)_2$ groups. At the oil-water interface, however, the peptide conforms to a different teriary structure: the non-polar side chains penetrate into the oil phase, reducing internal cohesion to close to zero. Thus, unfolding of (and film formation by) proteins is not limited to air-water interfaces, but may be a characteristic of proteins at interfaces in general. Unfolding and film formation may occur, for instance, at a solid-water interface provided the phase difference is conducive to a modification of tertiary structure. It is possible that deep water offers interfaces that would promote unfolding and film formation by dissolved proteins. Adsorption coupled with increases in film pressure could result in the formation of film-like particulates. It is possible that such processes account for the proteinaceous flakes and oblongs Gordon has found to be abundant in deep water.

The quantitative analysis of the particulates produced (see Figure 24) yielded an estimate of the amount of dissolved protein that was converted to the particulate state by radiation. Of the 150 ug of protein (103 ugC) within the vessel at 750 ug/l concentration, 13.8 $\pm$ 1.8 ugC (or 13.4 $\pm$ 1.8 %) was filterable after 4 hours of irradiation. However, 10.0 $\pm$ 1.8 % of the 150 ug was filterable immediately following dissolution (see the "zero time" values of Figure 24). At lower concentrations (300 ug/l and 250 ug/l) ovalbumen and trypsin, upon filtration immediately following dissolution, left only macroscopic mildly yellow-staining areas on Millipore filters; there was no evidence of particulates upon microscopic examination. These areas were interpreted as being due to protein which had adhered as the solution was being filtered. It cannot be definitely said that adhered protein was responsible for the carbon measured on the silver filters at "zero time", as 1) the concentrations involved were different between the solutions that were Millipore filtered and those that were silver filtered, and 2) the filters were of different composition. There is little doubt, however, that protein adhered to the Millipore filters.

If the "zero time" protein values illustrated in Figure 24 are indeed due to adherence of protein to the filter (as opposed to the actual trapping of particulates by the filter surface), it could indicate that some organics of ocean water may adhere to silver filters. Menzel (1966) has suggested that some dissolved carbon is adsorbed by such filters, although Riley (1970) doubts that such adsorption is of significance. Wangersky (cited in Riley, 1970) passed sea water through a dialysis membrane to remove particulates and all dissolved substances with a molecular weight in excess of 10,000. He passed the dialysate through silver filters but could not detect a significant increase in filter-retained carbon. Ovalbumen has a molecular weight of approximately 46,000; its presence (possibly in the adhered or adsorbed form) on silver filters in this work could indicate that high molecular weight organics tend to adsorb upon filtration. Good experimental work is needed to resolve the question of the extent (if any) of adsorption of sea water organics onto filters. If such adsorption occurs to a significant extent, and it is not taken into account in analyses of carbon retained on silver filters, our estimates of the standing stock of particulate carbon in the water column would be higher than is actually the case.

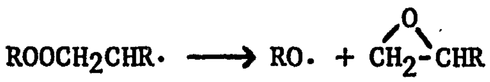
Lipids

Previous work

Previous research on the effects of ultraviolet radiation on lipids has invariably employed energies greater than that found in solar radiation reaching the earth's surface. As was indicated in the discussion of protein reactivity, the results of such work must be viewed with reserve in considerations of solar photochemistry: primary and secondary processes may differ at different wavelengths. Moreover, all previous work has utilized higher concentrations than the present study: much research has employed pure lipids (100% concentration). Concentration can influence courses of reactions: a free-radical mechanism may proceed at greater concentrations, but (due to a short half-life of the radical) may not be a significant process in dilute solution. Much previous work employed an atmosphere of pure oxygen: oxygen pressure is known to influence courses of autoxidation. For instance, in the oxidation of α-methylstyrene (Mayo, 1958) high oxygen pressure favors reaction (1) over reaction (2):

$$(1) \quad ROOCH_2CHR\cdot + O_2 \longrightarrow ROOCH_2CHROO\cdot$$

(2)



$$(2) \quad ROOCH_2CHR\cdot \longrightarrow RO\cdot + CH_2\overset{O}{-}CHR$$

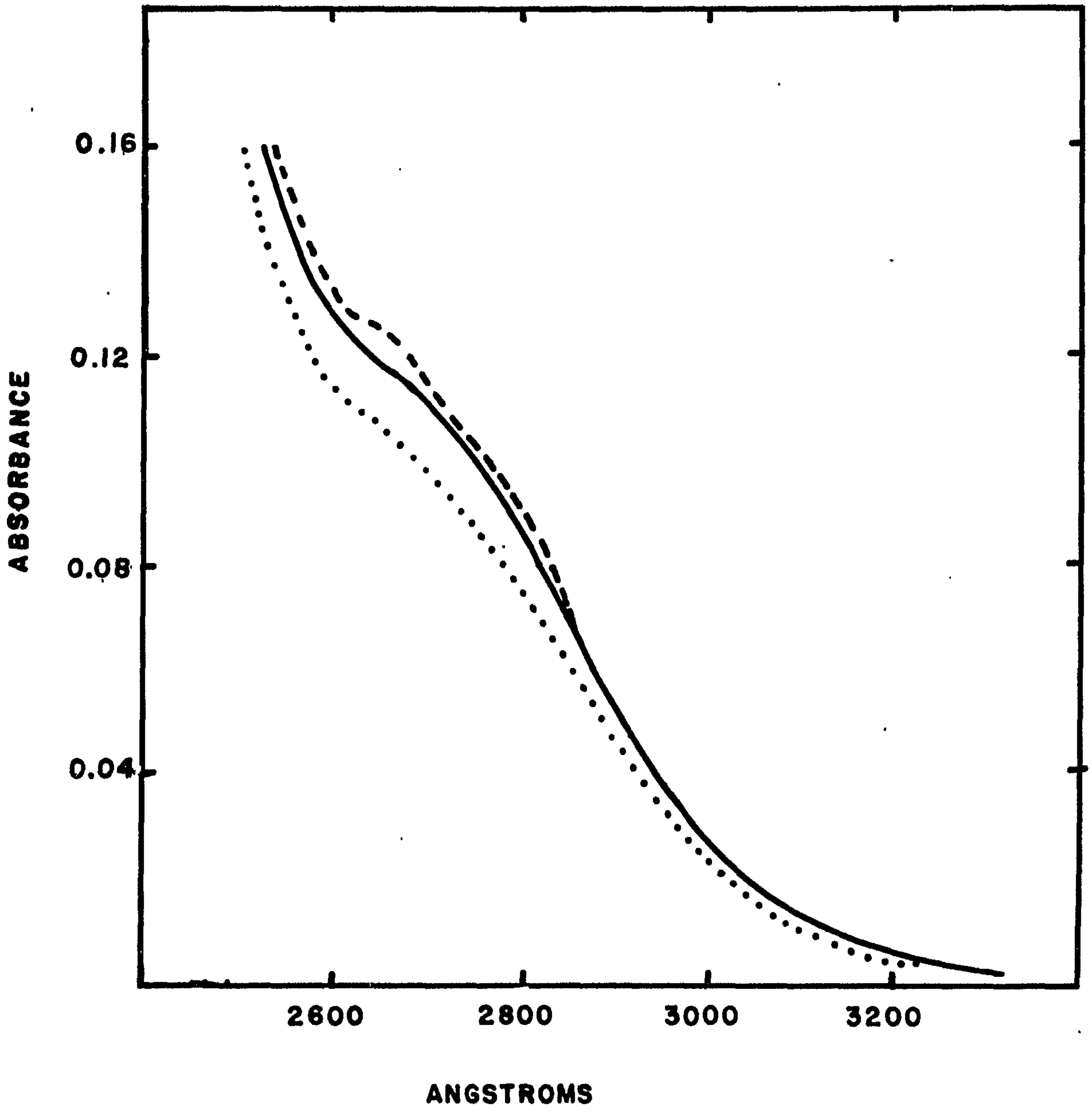
Where (1) is favored, the major product is a polymeric peroxide, while at low pressures the epoxide is stable and will predominate. In the present study, the findings of particulate (possibly polymeric) reaction products was of significance. Thus, it was essential to use an oxygen pressure representative of sea surface conditions, rather than the high pressures employed in previous work: reactions that predominate under pure oxygen (e.g. the polymerization from (1)) may not do so in air at one atmosphere.

In the present study, only solar levels of radiation were employed. The concentrations investigated were not unrealistic in terms of the concentrations of unsaturates at the sea surface; the solvent, moreover, was artificial sea water. Tank compressed air (rather than pure oxygen) was used to aerate the sample. In effect, the methods were more suitable to consider the question of what may be the fate of unsaturated lipids at the interface.

Absorption of solar radiation by unsaturated lipids in sea water

The absorption spectra in the region of 3000 Å of the three fatty acids investigated are illustrated in figure 35. The spectra are of the lipids in cyclohexane. However, absorption of solar wavelengths by these substances in sea water, or when adsorbed to a surface which is polar with respect to cyclohexane, may be greater than that indicated. The process responsible for the absorption of near ultraviolet radiation by unsaturates is a $\pi \rightarrow \pi^*$ transition (i.e. upon absorption an electron occupying a ground state π orbital is excited to occupy a vacant π orbital). Polar solvents such as sea water, and polar adsorbants such as the surfaces of mineral grains, interact more strongly with and stabilize an excited (π,π^*) state than the ground state; this is due to the excited state being more polarizable than the ground state (Nicholls and Leermakers, 1971). The energy of the excited state would therefore be

Figure 35. Absorption spectra of oleic acid (....), linoleic acid (——) and linolenic acid (----) in cyclohexane.


0.16
0.12
0.08
0.04
ABSORBANCE
2600
2800
3000
3200
ANGSTROMS

lowered more than that of the ground state (compared with the energy of these states in cyclohexane). The energy gap between the two states would thus be reduced, and hence there would be a red shift in the absorption spectrum. This red shift probably takes place upon the transfer of the lipids from cyclohexane to sea water or to artificial sea water. The absorption of solar wavelengths by unsaturates in sea water would therefore tend to be more intense than would be indicated by their absorption spectra in non-polar solvents.

Reactions

The development and intensification of absorption in the 234 mu and 269 mu regions have long been employed in following the course of oxidation of polyunsaturated lipids (Mitchell and Kraybill, 1941; Hendrickson et al., 1948; Swift et al., 1949). In methylene-interrupted olefinic acids, development of such absorption has been associated in many investigations with hydroperoxidation (Bergstrom, 1945; Khan et al., 1954; Privett et al., 1954). In the case of linoleic acid, abstraction of a hydrogen atom in the presence of oxygen would result in the formation of a resonance hybrid, which would be preferentially peroxidized at C-9 or C-13 to yield conjugated hydroperoxides.

That oxygen is essential for the development of conjugation is indicated by the finding that no significant change in the absorption spectrum of linoleic acid developed when it was subjected to artificial sea water without oxygen; in the aerated medium, however, there was a significant increase in conjugated diene absorption. The oxidation processes deduced in earlier studies would appear, therefore, to offer a plausible explanation of the observed reactions.

The increased extinction that developed in the near ultraviolet upon irradiation of linoleic acid, linolenic acid, and the diatom lipid extract, was due to absorption by the reaction products. Both the linoleic and linolenic acid conjugated hydroperoxides and their decomposition products (conjugated ketones) absorb solar wavelengths more intensely than do the parent lipids. As the wavelengths are fixed in an area of weak extinction by the substrate, the reaction products are soon responsible for most absorption. The O-O bond dissociation energy of the peroxide is exceeded by the energies of much of the near ultraviolet quanta, and thus absorption of solar ultraviolet by the reaction products may lead to peroxide decomposition. The resulting free radicals would tend to cause further hydrogen abstraction from the substrate. This chain reaction would tend to be autocatalytic at solar wavelengths. However, it has been indicated that singlet molecular oxygen (produced in a photosensitized reaction from triplet oxygen) may be involved in some peroxidations (Foote and Wexler, 1964; Wilson, 1966). Also, Gollnick and Schenck (1965) consider that an excited oxygen-sensitizer adduct may operate in photosensitized oxidations. It is not certain which of the above mechanisms were operating within the reaction vessels: each would be expected to result in the observed spectral changes, and in peroxidation.

At the sea surface the abundance of unsaturated lipids and the presence of peroxide (Van Baalen and Marler, 1966) and high-energy radiation would appear to favor hydroperoxidation. Polyunsaturated lipids can undergo hydroperoxidative polymerization (O'Neill, 1954):

such polymerization may account for the presence of particulate reaction products. It is possible, however, that the particulates were the result of a phase change, possibly brought about by photochemical decarboxylation and separation of the resulting hydrocarbons. But the latter would be soluble in hexane and would tend to be washed through the filter during the hexane rinse. A macromolecular space network afforded by a polymer may offer a better explanation of the filter-retained particulates.

The reactivity of the diatom lipid extract was examined in an attempt to approach, in the reaction vessel, the lipid complexity of the sea surface. Ackman et al. (1968) have indicated that <u>Thalassiosira fluviatilis</u> contains relatively minor amounts of oleic, linoleic, and linolenic acids; the predominant unsaturates are 16:1, 16:0, 20:5, 14:0, and 16:3 (27%, 14%, 5%, 5%, and 4% of the total lipid, respectively). The concentrations of the polyunsaturates was less in this study than in the studies investigating the pure lipids. As was the case with oleic acid, 16:1 would not be expected to develop a diene chromophore upon oxidation, as there is no second double bond available with which it can become conjugated. In the case of monoenes, migration of the bond into conjugation with the carbonyl group is improbable. The development of both positively-staining disc areas and diene conjugation upon plankton oil irradiation (see figure 21) is evidence that processes were occurring which were similar to those that took place when pure lipids were irradiated. Considering that linoleic and linolenic acids constitute only about 0.2% of the total lipids of this diatom (Ackman et al., 1968), it is questionable whether the reactions of these two lipids alone could have accounted for the reactivity of the diatom extract. It is probable that similar reactions of other polyunsaturates (e.g. 20:5 and 16:3) contributed to the observed spectral changes and to disc formation. Considering 1) the findings of previous workers regarding the susceptibility of unsaturated systems to peroxidation, conjugation isomerization, and polymerization; 2) the present findings of simulated solar catalysis of what appear to be similar processes for linoleic acid, linolenic acid, and a diatom lipid extract at trace concentrations in artificial sea water; and 3) the abundance of unsaturates and the presence of peroxide at the sea surface, it appears that some unsaturated lipids at or near the interface may be photochemically reactive in the presence of sunlight.

Geochemistry of unsaturated lipids

General

The greatest concentrations of unsaturated geolipids are found within organisms and as part of the dissolved organic matter of natural waters. Traces of oleic acid have also been detected in soil (Khainskii, 1916), and in the D'Arcy oil (Ramsay, 1966), its presence in crude oil perhaps attesting to the stability of this particular lipid over geologic time. C18:1 has also been detected in shales (Leo, 1966).

Unsaturated lipids have been found in the surface layers of algal mats (Parker and Leo, 1965), and in some sediments. Leo (1966) detected 16:1 and 18:1 in recent sediments in the Gulf of Mexico. Peterson (1967) found the only unsaturated acids in slope sediments to be 16:1 and 18:1, while in shelf surface sediments he detected some polyunsaturates (e.g. 20:5) in addition to these two monoenes. Rosenfeld (1948) reported a decrease in the iodine number with depth in sediments, indicating decreasing concentrations of unsaturates. This sparcity of unsaturates in sediments is in contrast to their abundance in marine organisms and in sea water (see pp. 4-5).

The decrease in unsaturates with depth in sediments is accompanied by a decrease in the total fatty acids: soon after deposition the fatty acid content falls to values approximating those of ancient sediments (Parker, 1967). However, fatty acids are very stable, being resistant to both abiotic and biotic fragmentation. In view of 1) this resistance of lipids to fragmentation, 2) the susceptibility of the oxidation products of unsaturated lipids to polymerization, and 3) the findings of this research that unsaturates may be subject to rapid oxidation and polymerization upon release into sea water, it is conceivable that some of the abundant unsaturates of marine waters are (rather than fragmented) transformed into a state which is not extractable from the sediment -- ie. kerogen. The decrease in unsaturates and total fatty acid with depth may be in part a reflection of the transformation of lipids to a new "particulate" (or "inextractable") state, rather than their fragmentation. It is possible that settled polymeric lipid, or the oxidation and precipitation of unsaturates within the sediment, may contribute to the framework of kerogen.

Unsaturated lipids at the sea surface and in the water column

The transformation of lipids to the particulate state may occur in enclosed bodies of water where they can become concentrated by intense biological activity. Stadnikoff (1930) suggested that Coorongite and Balkshashite result from lipid polymerization in oxygenated waters. Coorongite is a material of rubber-like consistency found on the shores of a lagoon in southern Australia; it has been considered to be derived from the blue-green alga <u>Elaeophyton coorongians</u>. Balkshashite, also described as "rubber-like", is found in Lake Balkshash in Siberia, and appears to be a product of the oil-rich green alga <u>Botryococcus braunni K</u>. Stadnikoff believes that both these materials are derived mainly from the lipids of the algae with which they are associated. Although he believes the process to be one of "polymerization" of unsaturates, there is no evidence that the substances are truly polymeric. However, the finding of the present work that particulate material (possibly polymeric) is produced when low concentrations of unsaturated lipids are exposed to simulated solar radiation, lends some support to the suggestion that these substances may be products of unsaturated algal lipids.

Breger (1969) applies Stadnikoff's suggestion of polymerization to sea surface processes and proposes that polymerization of unsaturates occurs at the sea-air interface. He suggests that the process may contribute to suspended particulate carbon. However, extensive polymerization into discrete particles may not be possible at the ocean surface due to the presence at the interface of high concentration of non-reactive organics (e.g. saturated lipids). Relatively high concentrations of non-reactive substances could be expected to inhibit polymerization of activated unsaturates physically. In such a situation peroxidation and conjugation may occur, but peroxide radicals could conceivably be prevented from interacting on a large scale in the bimolecular reactions necessary for the production of polymers of high molecular weight. Such a situation is perhaps seen in the irradiation of linoleic acid and protein together: it was apparent that the presence of the protein inhibited to some extent the formation of particulates with the morphology characteristic of those from irradiation of linoleic acid alone. Reaction of the lipid may have taken place, however, on the surface of the sheet-like protein particles (where most of the lipid appeared to be adhered), and a type of membrane formation is a possibility.

The particulate products of lipid irradiation stained intensely with OsO_4; however, when non-living particulate matter from the sea surface and various depths is subjected to this stain, very little positive reaction occurs. Parsons and Strickland (1962) examined the biochemical composition of non-living oceanic particles, and concluded that the lipid content was less than 1%. The above field evidence does not support the concept of extensive lipid polymerization into discrete particles at the ocean surface. However, Lisitsyn et al. (1965) present evidence that lipids constitute a significant fraction of suspended particulate matter. In their analysis of suspended "bituminous substances" (filterable material whose origin they believe to be the lipids of marine organisms), sea water was passed through membrane and powdered glass filters; the filters were subsequently extracted with organic solvents. They report that extractable lipids (determined by the difference: organic matter − [proteins + nucleic acids + carbohydrates]) in particulate matter at four stations in the tropical Pacific varies from 2.16-48.29 ug/l. Their data indicate 1) a correlation of concentration with geographical location, and 2) a slight tendency of suspended lipids to decrease with depth in the water column. These investigators report higher concentrations of resins and asphaltenes in the non-living as compared to the living particulate fractions: they believe that gradual transformation of biochemically-resistant lipids from surface water organisms results in precursors of these and other cyclic petroleum hydrocarbons. Meinschein (1969), however, doubts that the polyunsaturates of organisms, in their methylene-interrupted configuration, can undergo such cyclization over geologic time. The rapid oxidations and isomerizations that are indicated, by the present research, to occur upon release of lipids into sea water render these substances much more susceptible to cyclization. It is thus possible that the unsaturated cyclic hydrocarbons indicated in the Russian work (and also some benzenes and naphthalenes of petroleum) may have their origin in the oxidation products of the abundant unsaturates of surface waters. Although this is only a possibility and there is no direct evidence in support of such cyclization within the water column, the indications of rapid conjugation isomerization upon release of lipids into sea water provide a link in the hypothetical transition of marine lipid → petroleum hydrocarbon.

Organic films on natural bodies of water

Organics at the sea-air interface are derived from atmospheric deposition, and via accumulation from the water column. Some may be derived in situ from the neuston. They are subject to 1) ejection into the atmosphere to become a part of the marine aerosol, 2) adsorption onto particulates and subsequent sinking (Bader et al., 1960), and 3) (as illustrated in this research) possible solar photochemical reactions, and transformation into organic particles.

Jarvis (1967) and Jarvis et al. (1967) have indicated that sea surface water contains a spectrum of organics with varying tendencies to adsorb at the interface. As a surface film ages, the less surface active material appears to be pushed up or down out of the interface due to the gradual accumulation of the more surface active molecules. When the interface is swept with a barrier, a relatively clean water surface is left that permits accumulation of more film material. Presumably, if this process of surface film accumulation and sweeping were continued, the water sample would eventually become depleted of surfactants.

The present work has illustrated that films that accumulate from filtered lake water, and from filtered Sargasso Sea water containing protein in very dilute solution, are subject to collapse upon application of lateral pressure. Collapse is accompanied by the transformation of some film organics to a state which is not capable of re-spreading; this new state (capable of being disrupted into small particles) was formed from organics previously dissolved in the bulk liquid. In view of 1) the demonstration by Jarvis that dissolved organics will accumulate at the interface if the previous film is swept away, and

2) the present demonstration of the susceptibility of films to collapse and subsequent disruption into discrete particles that tend to sink -- it is conceivable that such film collapse phenomena (within windrows, for instance) may be a part of a continual cycle that tends to remove dissolved organics from the bulk water and precipitate them as particles back into the water column.

Simulated solar radiation was found to increase significantly the surface pressure of protein-enriched sea water, probably via the photolysis of intramolecular bonds responsible for maintaining tertiary structure. Such increases in pressure effectively decrease the area to which a film must be compressed for collapse to occur, and therefore solar ultraviolet may aid in collapse and particle formation. Thus, at sea, in addition to possibly being responsible for the transformation of dissolved to particulate protein and lipid (see results with ovalbumen, trypsin, linoleic acid, and linolenic acid solutions), solar ultraviolet may act indirectly in the dissolved ⟶ particulate transition by decreasing the area to which a film must be compressed for pressure-induced particle formation to occur.

The demonstrations of particle formation from interfacial organics lend support to the proposal of Sutcliffe et al. (1963) that the increase in light scattering they observed beneath windrows may have been due to "monomolecular films which may be aggregated into insoluble organic particles." Garret (1969), however, expresses doubt that films within windrows are compressed to the collapse pressures of protein or lipid monolayers. It may not be entirely valid, however, to assume that the physical chemistry of the organically-complex sea surface film is entirely analogous to that of simple monolayers: the collapse pressures, and the reactions of complex natural films to lateral pressure, may differ from what would be expected from consideration of simple monomolecular systems. As an example, the particulates from lake water films were observed to form only within 1 cm in front of the moving barrier, and at no other location in the trough. This suggests that the natural film, unlike simple lipid or protein monolayers, is slow to equilibrate upon application of lateral pressure. Regions of high pressure effecting collapse and particle formation may thus develop locally (as one developed in front of the barrier in this case), while perhaps at the same time there would be less of an increase in pressure in adjacent regions of the same film. While simple monolayers afford a convenient and useful tool for the study of the physics of surface films, the study of the dynamics of natural films themselves contributes more directly to our understanding of processes occurring at the sea surface.

Surface films, proteins, and lipids as sources of particulate food

It appears from the present research that one of the photochemical effects of solar radiation at the sea surface may be to aid in the transformation of dissolved carbon to the particulate state. As the non-living particulate fraction of sea water has been indicated to be a source of nutriment at the base of the food web in the sea (Baylor and Sutcliffe, 1963; Riley, 1963; Riley, 1970), it is indicated that solar radiation may play a part in supplying such nutriment to the water column. This abiotic transformation of dissolved organics is in contrast to the biotic photochemical organic synthesis within organisms containing chlorophyll a.

Surface films

The nutrient value of collapsed surface film remains to be examined, but is possibly high. As proteins are highly surface active it is probable that they occur, perhaps invariably, in natural films. As this unfolded protein would be included in (or perhaps form an integral part of) pressure-induced particulates, it is indicated that collapsed films have at least some value as a food source. Bacteria are known to be scavenged from bulk water by bubbles, and to be deposited at the interface (Blanchard and Syzdek, 1972); it is probable that other organisms are likewise scavenged and deposited, along with a wide spectrum of organics. Much of this material would be incorporated into collapsed film, and some would certainly have nutrient value.

Proteins

It is probable that 1) a variety of (if not all) herbivores contain proteolytic enzymes, and 2) in many organisms the activities of these enzymes are responsible for the bulk of the animal's supply of organic nitrogen. It is known that proteins at air-water interfaces undergo unfolding, and it has been indicated in this research that solar levels of radiation are responsible for further unfolding at the sea water-air interface (see Figure 27). As discussed above, this unfolding and the subsequent formation of protein particulates would appear to provide a source of ingestible and readily hydrolysable nitrogen and carbon to the water column.

Lipids

Lipids exist in sea water adsorbed at interfaces (solid-water, or water-air); some may be present in solution. It was evident from the present research that lipids in artificial sea water readily adhered to protein particulates; moreover, this adsorption appeared to inhibit the formation of lipid particulates as observed from irradiation of linoleic and linolenic acids alone. Lipids are ingested by filter feeders, as these substances would tend to adsorb to some extent on the standing stock of particulates in the real ocean; the extent of lipid utilization by herbivores, however, has not been studied. It is uncertain whether these organisms can utilize any ingested triglycerides. Mammals are capable of absorbing free fatty acids, monoglycerides, and diglycerides through the gut wall; however, triglycerides must first be degraded to at least a diglyceride and a free fatty acid by the enzyme lipase before absorption can occur.

It would be worthwhile to investigate the capacity of herbivores and marine bacteria to utilize the lipids of sea water. The results of such an investigation would aid in our understanding of the fate of this abundant surfactant.

Abiotic transformation of dissolved carbon to the particulate state

While Barber (1966) has indicated that bacteria may be of significance in the production of the non-living particulate organic fraction of sea water, the present work indicates that the presence of bacteria may not be essential for the transformation of dissolved carbon to the particulate state. The production of lipid and protein particles in clean, sterile artificial sea water does not imply that such solar photochemical processes occur at the organically-complex sea surface; however, the reaction vessel experiments do indicate that the phenomena observed are energetically possible. Although the surface films investigated were not sterile, the observed processes of film collapse and fragmentation were probably largely physico-chemical rather than having been dependent on any previous activity of bacteria on the film organics. Bacteria may play a significant role in the sea's cycling of organic carbon, but they are herein indicated to be not essential for the production of particles from dissolved organics.

Limitations of results

Experimental results are qualified not only by statistical limits placed on the data, but also by the fact that only certain variables are being investigated. In the experiments with reaction vessels, the formation of particulate matter and changes in absorption spectra were under consideration; it is possible that other processes, such as proteolysis or lipolysis, may have been occurring to a significant extent. In the work with protein surface films, however, proteolysis was not detected. More thorough understanding of the effects of solar levels of radiation on organics in natural waters awaits more exhaustive studies of other organics and their reaction products. Such studies should utilize simple, thoroughly characterized systems as those employed in some studies herein. But this work is best coupled with corollary studies of the effects of radiation on the composite organics of sea water itself. While investigations of the simplified systems may lead to an understanding of the photochemical reactivity and reaction products of individual substances, the more complex and natural systems will give us insight into the gross manifestations of the reactions observed. In the present study a spectrum of substrate complexities was chosen, employing what appeared to be the more valid organics. It proved possible to discuss many of the present findings in relation to previous work on particulate and dissolved carbon, and in some cases to relate the reactions observed in the elementary systems to the reactions and gross effects observed at greater substrate complexity.

BIBLIOGRAPHY

Ackman, R.G., C.S. Tocher and J. McLachlan, Marine photoplankter fatty acids, J. Fish. Res. Bd. Canada, 25, 1603, 1968.

Barber, R.T. Interaction of bubbles and bacteria in the formation of organic aggregates in seawater, Nature, 211, 257, 1966.

Barker, R.E. The availability of solar radiation below 290 nm and its importance in photomodification of polymers, Photochem. Photobiol., 7, 275, 1969.

Baylor, E.R. and W.H. Sutcliffe, Jr. Dissolved organic matter in seawater as a source of particulate food, Limnol. Oceanogr., 8, 369, 1963.

Beavon, G.H. and E.R. Holiday. Ultraviolet absorption spectra of proteins and amino acids, in Adv. Protein Chem., edited by M.L. Anson, K. Baily and J.T. Edsall, 7, 275, 1969.

Bergstrom, S. Autoxidation of linoleic acid, Nature, 156, 717, 1945.

Bernhart, F.W. Denaturation and molecular splitting of egg albumin by ultraviolet radiant energy, J. Biol. Chem., 128, 289, 1939.

Blanchard, D.C. and L. Syzdek. Bubbles and the water-to-air transfer of bacteria, a paper delivered at the Working Symposium on Sea-Air Chemistry, Ft. Lauderdale, Florida, Jan. 31-Feb. 3, 1972.

Bowen, E.J. The Chemical Aspects of Light, Clarendon, Oxford, 2nd edition, 300pp., 1946.

Breger, I.A. What you don't know can hurt you: organic colloids and natural waters, in Organic Matter in Natural Waters, edited by D.W. Hood, p. 563, Institute of Marine Science Occasional Publication No. 1, 1970.

Burt, W.V. Selective transmission of light in tropical Pacific waters, Deep Sea Res., 5, 51, 1958.

Cheesman, D.F. and J.T. Davies. Physiochemical and biological aspects of proteins at interfaces, in Adv. Protein Chem., edited by M.L. Anson, K. Baily and J.T. Edsall, 9, 439, 1954.

Coblentz, W. and R. Stair. A daily record of ultraviolet solar and sky radiation in Washington, 1941-1943, J. Res. Nat. Bur. Stds., 33, 21, 1944.

Dognon, A. and L. Gougerot. Action of Ultraviolet rays on protein surface films, Bull. Soc. Chem. Biol., 25, 183, 1943.

Duce, R.A., S.R. Piotrowicz, B.J. Ray, and G.L. Hoffman. Trace metal enrichment in the sea surface microlayer, a paper delivered at the Working Symposium on Sea-Air Chemistry, Ft. Lauderdale, Florida, Jan. 31-Feb. 3, 1972.

Foote, C.S., and S. Wexler. Olefin oxidations with excited singlet molecular oxygen, J. Am. Chem. Soc., 86, 3879, 1964.

Garrett, W.D. The organic chemical composition of the ocean surface, Deep Sea Res., 14, 221, 1967.

Garret, W.D. Organic chemistry of natural seasurface films, in Organic Matter in Natural Waters, edited by D.W. Hood, p. 469, Institute of Marine Science Occasional Publication Number 1, 1970.

Giesa, A.C. Action of ultraviolet radiation on protoplasm, Physiol. Reviews, 30, 431, 1950.

Goldberg, E.D. Minor elements in sea water, in Chemical Oceanography, edited by J.P. Riley and G. Skirrow, pp. 163-196, Academic Press, London, 1965.

Gordon, D.C., Jr. A microscopic study of organic particles in the North Atlantic ocean, Deep Sea Res., 17, 175, 1970.

Hendrickson, M.J., R.P. Cox, and F.C. Konen. Some applications of ultraviolet spechophotometry in drying oil research, J. Am. Oil Chemists's Soc., 25, 73, 1948.

Ivanoff, A., N. Jerlov, and T.H. Waterman. A comparative study of irradiance, beam transmittance and scattering in the sea near Bermuda, Linmol. Oceanogr., 6, 129, 1961.

Jarvis, N.L. Adsorption of surface active material at the sea-air interface, Limnol. Oceanogr., 12, 213, 1967.

Jarvis, N.L., W.D. Garrett, M.A. Scheiman, and C.O. Timmons, Surface chemical characterization of surface-active material in sea water, Limnol. Oceanogr., 12, 88, 1967.

Jeffrey, L.M., B.F. Pasby, B. Stevenson, and D.W. Hood, Lipids of ocean water, in Adv. Org. Geochem., edited by U. Colombo and G.D. Hobson, Monograph no. 15, 175, 1964.

Jerlov, N. Optical studies of ocean water, Rept. Swedish Deep Sea Expdn., 3, 1, 1951.

Jerlov, N. Factors influencing the transparency of Baltic waters, Medd. Oceanog. Inst. Göteborg, 25, 19, 1955, as cited in Jerlov, N., Optical Oceanography, Elsevier, Amsterdam, 1968.

Jerlov, N. Optical classification of ocean water, in Physical Aspects of Light in the Sea, Univ. Hawaii Press, Honolulu, 1964.

Kates, M. and B.E. Volcani. Lipid components of diatoms, Biochim. Biophys. Acta, 116, 264, 1966.

Kaplan, J.G., D.H. Andrews, and M.J. Fraser. Ultraviolet irradiation of dilute solutions of ovalbumen, J. Colloid Sci., 9, 203, 1953.

Kaplan, J.G. and M.J. Fraser. The effect of ultraviolet radiation on monomolecular films of egg albumen, Biochim. Biophys. Acta, 9, 585, 1952.

Khainskii, A. Organic substances in the soil humus, Pochrovedenic, 18, 49, 1916 [in Russian], as cited in R.I. Morrison, Soil lipids, in Organic Geochemistry, edited by G. Eglinton and M.T.J. Murphy, pp. 558-575, Springer-Verlag, New York, 1969.

Khan, N.A., W.E. Tolberg, D.H. Wheeler, and W.O. Lundberg, Photochemical oxidation of fatty acid esters with and without chlorophyll, J. Am. Oil Chemist's Soc., 31, 460, 1954.

Lenoble, J. Sur le rôle des princepaux sels dans l'absorption ultraviolette de l'eau de mer, Compt. Rend., 242, 806, 1956.

Leo, R.F. The geochemistry of fatty acids in recent marine sediments, M.A. Thesis, Univ. of Texas, 1966.

Lisitsyn, A.P., Y.A. Bogdanov, L.I. Potapova, and A.N. Maksimov. Bituminous substances in oceanic suspended material, Oceanology, 5, 43, 1965.

Lovern, J.A. Fat metabolism in fishes. VI The fats of some plankton crustacea. Biochem. J., 29, 847, 1935.

Lovern, J.A. The lipids of marine organisms, in Oceanogr. Mar. Biol. Ann. Rev., edited by H. Barnes, 2, 169, 1964.

McLean, D.J. and A.C. Geisa. Absorption spectra of proteins and amino acids after ultraviolet irradiation, J. Biol. Chem., 187, 543, 1950.

Markley, K.S. Nomenclature, classification, and description of individual acids, in Fatty Acids, 2nd edition, edited by K.S. Markley, pp. 23-249, Wiley-Interscience, New York, 1960.

Meinschein, W.G. Hydrocarbons-saturated, unsaturated, and aromatic, in Organic Geochemistry, edited by G. Eglinton and M.T.J. Murphy, pp. 330-356, Springer-Verlag, New York, 1969.

Menzel, D.W. Particulate organic carbon in the deep sea, Deep Sea Res., 14, 229, 1966.

Menzel, D.W. and J.H. Ryther. Organic carbon and the oxygen minimum in the South Atlantic Ocean, Deep Sea Res., 15, 327, 1968.

Mitchell, J.H. and H.R. Kraybill. Ultraviolet absorption spectra of linseed oil, Ind. Eng. Chem., Anal. Ed., 13, 765, 1941.

Neurath, H. The influence of denaturation on the spreading of proteins on a water surface, J. Phys. Chem., 40, 361, 1936.

Nicholls, C.H. and P. Leermakers. Photochemical and spectroscopic properties of organic molecules in adsorbed and other perturbing polar environments, in Adv. Photochem., edited by J. Pitts, G. Hammond, and W. Noyes, 8, 315, 1971.

O'Neill, L.A. Some recent studies on the autoxidation of drying oils, Chem. Ind., p.384, 1954.

Parker, P.L. Fatty acids in recent sediment, Univ. Tex. Contr. Mar. Sci., 12, 135, 1967.

Parker, P.L. and R.F. Leo. Fatty acids in blue-green agal mat communities, Science, 148, 373, 1965.

Parsons, T.R. and J.D.H. Strickland. Oceanic detrius, Science, 136, 313, 1962.

Peterson, D.H. Fatty acid composition of certain shallow-water marine sediments, Ph.D. Thesis, Univ. of Washington, 1967.

Pettit, E. Measurements of ultraviolet solar radiation, Astrophys. J., 75, 217, 1932.

Privett, O.S., C. Nickell, W.E. Tolberg, R.E. Paschke, D.H. Wheeler, and W.O. Lundberg. Evidence for hydroperoxide formation in the autoxidation of methyl linolenate, J. Am. Oil Chemist's Soc., 31, 23, 1954.

Ramsay, J.N. Organic geochemistry of fatty acids, M.S. Thesis, Univ. of Glasgow, 1966.

Riley, G.A. Organic aggregates in seawater and the dynamics of their formation and utilization, Linmol. Oceanog 8, 372, 1963.

Riley, G.A. Particulate organic matter in sea water, in Adv. Mar. Biol., edited by F.S. Russel and M. Yonge, 8, 1, 1970.

Roburn, J. Effect of sunlight and ultraviolet radiation on chlorinated pesticide residues, Chem. Ind., 38, 1555, 1963.

Rosen, J.D. Photodecomposition of organic pesticides, in Organic Compounds in Aquatic Environments, edited by S.D. Faust and J.V. Hunter, pp. 425-438, Dekker, New York, 1971.

Rosenfeld, W.D. Fatty acid transformations by anaerolic bacteria, Arch. Biochem. Biophys., 16, 263, 1948.

Setlow, R. and B. Doyle. The action of monochromatic ultraviolet light on proteins, Biochim. Biophys. Acta, 24, 27, 1957.

Sharp, J.H. The formation of particulate organic matter in sea water, Ph.D. Thesis, Dalhousie University, 1972.

Shore, V.G. and A. Pardee. Energy transfer in conjugated proteins and nucleic acids, Arch. Biochem. Biophys., 62, 355, 1956.

Sokal, R.R. and F.J. Rohlf. Biometry, W.H. Freeman and Co., San Francisco, 776pp., 1969.

Stadnikoff, G. Die Entstehung von Kohle und Erdol, Enke, Stuttgart, 1930.

Steele, R.H. and A. Szent-Gyorgyi. Studies on the excited states of proteins, Proc. Nat. Acad. Sci., 44, 540, 1958.

Sullivan, S.A. Experimental study of the absorption in distilled water, and heavy water in the visible region of the spectrum, J. Opt. Soc. Am., 53, 962, 1963.

Sutcliffe, W.H., Jr., E.R. Baylor and D.W. Menzel. Sea surface chemistry and Langmuir circulation, Deep Sea Res., 10, 233, 1963.

Swift, C.E., R.T. O'Connor, L.E. Brown, and F.G. Dollear. The aldehydes produced during the autoxidation of cottonseed oil, J. Am. Oil Chemist's Soc., 26, 297, 1949.

Szent-Gyorgyi, A. Energy transfer in biological systems, J. Chim. Phys., 55, 916, 1958.

Van Baalen, C. and J.E. Marler. Occurrence of hydrogen peroxide in sea water, Nature, 211, 951, 1966.

Wangersky, P.J., The organic chemistry of sea water, Am. Scientist, 53, 358, 1965.

West, W. Flourescence and phosphorescence, in Chemical Applications of Spectroscopy, edited by A. Weissberger, vol. 9, chpt.6, Interscience, New York, 1949.

Wheeler, O.H., D.A. Julian and R.A. Ribot, Photolysis and radiolysis of phenylalanylglycine, Photochem. Photobiol., 12, 505, 1970.

Williams, P.M. Sea surface chemistry: organic carbon and organic and inorganic nitrogen and phosphorus in surface films and subsurface waters, Deep Sea Res., 14, 791, 1967.

Wilson, Thérèse. Excited singlet molecular oxygen in photooxidation, J. Am. Chem. Soc., 88, 13, 1966.

Appendix I

The data from the actinometric procedure yielded a rate of oxalate decomposition of 1.12 ml/12 hours. This value was incorporated into the formula for uranyl oxalate actinometry (Bowen, 1946):

$$\text{Nhv} = \frac{\text{ml titrant}}{(e)\ (a)\ (2 \cdot 10^4)}$$

where Nhv = the number of einsteins of radiation; ml titrant = ml of $KMnO_4$ equivalent to the oxalate loss (see text p. 17); e = the quantum efficiency of oxalate decomposition; and a = the fraction of radiation absorbed by 1 cm of the actinometric solution.

The resulting value of $6.91 \cdot 10^{-7}$ Nhv/second is equivalent to $2.16 \cdot 10^5$ ergs/second, or $2.16 \cdot 10^4$ uwatts. It was determined from the solid geometry of the system that the maximum intensity of oxalate-sensitive radiation (on the surface of the solution just below the tip of the lamp) was approximately 1040 uwatts/cm^2. Of this latter value, 84.4% (or 878 uwatts/cm^2) was of energy at wavelengths less than 4000 Å. Pettit (1932) has determined that, at 32° North latitude, the intensity of direct mid-summer solar radiation of wavelengths less than 4000 Å is 2320 uwatts/cm^2. Thus, the intensity of ultraviolet radiation used in this study was

$$\frac{878}{2320} \cdot 100$$

or approximately 38% of solar ultraviolet radiation as measured by Pettit.

Appendix II

The Hewlett-Packard F&M model 185 CHN analyzer is designed for measuring the carbon, hydrogen and nitrogen content of organic material. After conversion of the elements to CO_2, H_2O, and N_2 at high temperature, the gasses are separated on a chromatographic column, and subsequently enter a thermal conductivity detector. The generated electrical signal is fed to a potentiometric recorder, resulting in a chromatogram. The instrument was calibrated for carbon in the range used in this work (Figure 36); the standard was cyclohexanone-2,4-dinitrophenylhydrazone.

The data from the quantitative analysis of particulate protein (see Figure 24) are tabulated below.

Filter blanks (peak height)	Zero time (peak height)	4 hours of irradiation (peak height)
2.1	5.6	8.8
3.4	5.1	7.9
3.0	7.4	7.9
2.8		8.7
2.3		
2.8		
1.6		
3.0		
3.2		

For the peak heights resulting from the combustion of each "zero time" and each "4 hour" sample, and for the average peak height resulting from combustion of the filter blanks, the corresponding value of ugC was estimated. The 95% confidence interval for the estimate is a function of the error in the determination of the standard curve, and was calculated as in Sokal and Rohlfe (1969):

$$95\% \text{ confidence interval} = \bar{X} + \frac{b_{y\cdot x}\ (Y_i - \bar{Y})}{D} \pm H$$

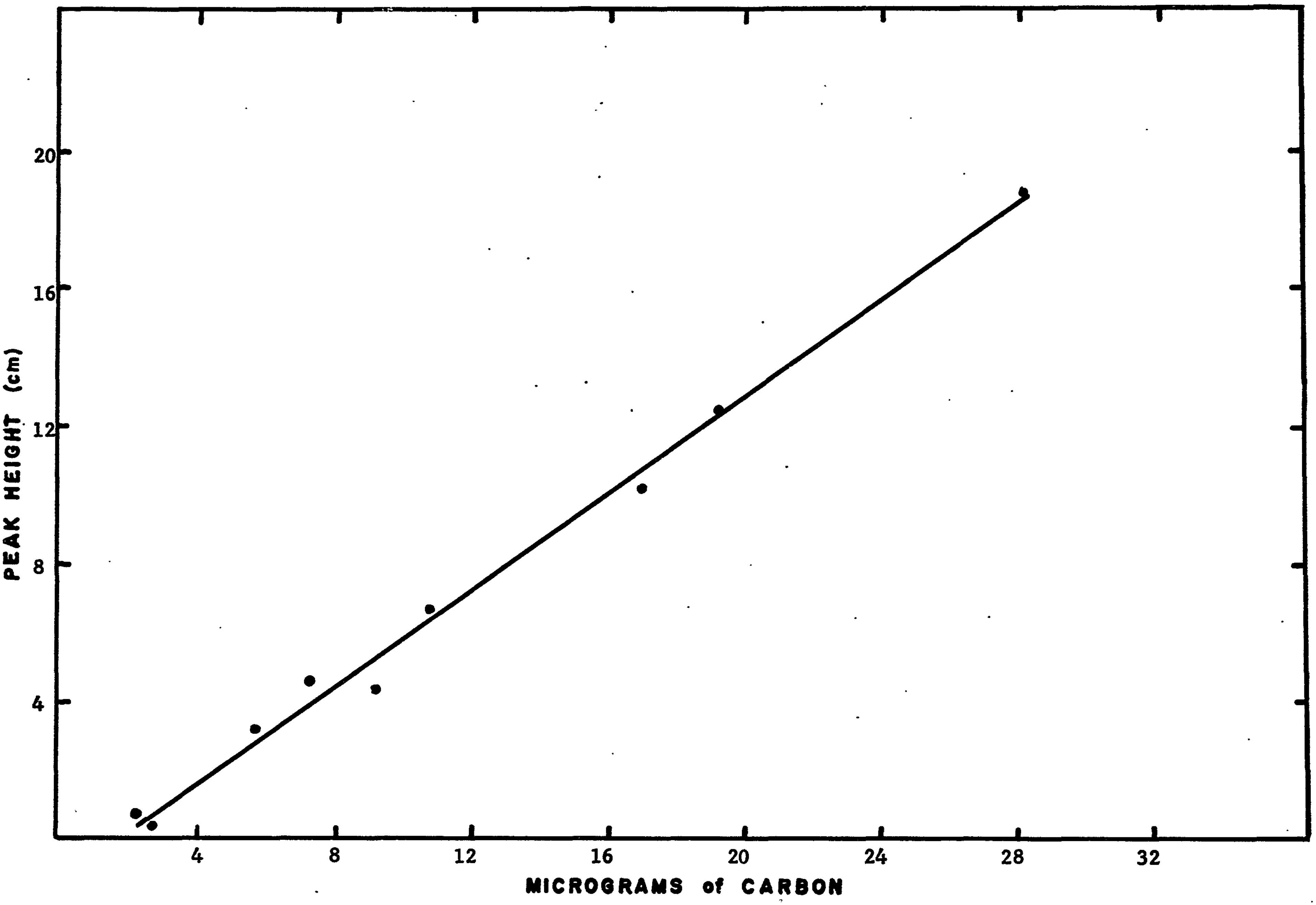
where

$$D = b^2_{y\cdot x} - t^2_{.05(n-2)}\ s^2_b$$

and

$$H = \frac{t_{.05(n-2)}}{D}\sqrt{\frac{\Sigma(Y - \hat{Y})^2}{n-2}\left[D\left(1 + \frac{1}{n}\right) + \frac{(Y_i - \bar{Y})^2}{\Sigma x^2}\right]}$$

Figure 36. Standard curve for carbon, as determined on the Hewlett-Packard CHN analyzer.


PEAK HEIGHT (cm)
20
16
12
8
4
4
8
12
16
20
24
28
32
MICROGRAMS of CARBON

Appendix III

In the cases of 1) each of the five filters resulting from linoleic acid irradiation, and 2) each of the four filters from linolenic acid irradiation (see pp. 32 and 34 , respectively) 31 microscopic fields were examined at x400 magnification. From the number of discs plus particles that were counted the total number on the filter was estimated as

$$\hat{Y} = N\ (\bar{y})$$

where $\hat{Y}$ = the estimate of the total amount of filter-retained lipid, N = the number of fields at x400 comprising the entire filter surface, and y = the mean number of discs plus particles in one field at x400.

The 95% confidence interval for Y is

$$\hat{Y} \pm 2\sqrt{\text{var}\ \hat{Y}} \ = \ \hat{Y} \pm 2\sqrt{\frac{N^2}{n}\ (\text{var}\ y)}$$

where n = 31.

The following tables contain the data from microscopic examination of filter-retained lipid; this data is the basis of figures 14, 15, 18, and 19.

Linoleic acid

1 hour of irradiation: total number of particles + discs in 31 fields = 4

size (u)	size (u)
1.9	13.4
1.9	3.8

continued

2 hours of irradiation: total number of discs plus particles observed in 31 fields = 11

size (u)

1.9	1.9	3.8	3.8
1.9	3.8	1.9	5.8
5.8	5.8	1.9	3.8

4 hours of irradiation: total number of discs plus particles observed in 31 fields = 20

size (u)

9.6	30.7	23.0	5.8
59.2	15.4	55.7	15.4
5.8	21.1	28.8	38.4
17.3	21.1	32.6	9.6
21.1	21.1	3.8	28.8

7 hours of irradiation: total number of discs plus particles observed in 31 fields = 100

5.8	28.8	15.4	55.7	9.6
86.4	3.4	86.4	92.2	3.8
15.4	13.4	3.8	99.8	7.7
51.8	17.3	15.8	1.9	61.4
5.8	7.7	42.2	5.8	49.9
7.7	13.4	51.8	7.7	13.4
23.0	11.5	9.6	11.5	15.4

continued

3.8	19.2	78.7	53.8	113.3
11.5	30.7	21.1	17.3	61.4
9.6	7.7	3.8	11.5	9.6
7.7	7.7	5.8	9.6	30.7
3.8	9.6	3.8	13.4	23.0
42.2	11.5	11.5	9.6	73.0
13.4	111.4	121.0	13.4	15.4
13.4	105.5	9.6	94.1	57.6
9.6	119.1	76.8	5.8	11.5
21.1	7.7	21.1	21.1	7.7
7.7	17.3	3.8	11.5	11.5
21.1	23.0	3.8	9.6	9.6
28.8	3.8	5.8	7.7	13.4

13 hours of irradiation: total number of discs plus particles observed in 31 fields = 126

size (u)

5.8	5.8	3.8	57.6	42.2
19.2	9.6	15.4	9.6	5.8
3.8	3.8	21.1	15.4	11.5
53.8	30.7	57.6	28.8	11.5
38.8	21.1	42.2	94.1	3.8
3.8	5.8	69.1	38.4	9.6
7.7	7.7	9.6	13.4	7.7
5.8	88.3	74.9	13.4	9.6

continued

38.4	174.7	74.9	36.5	59.5
17.3	5.8	13.4	26.9	69.1
96.0	44.2	7.7	5.8	57.6
5.8	19.2	15.4	40.3	74.9
25.0	36.5	38.4	32.6	7.7
7.7	9.6	7.7	9.6	46.1
215.0	24.9	44.1	32.6	9.6
5.8	9.6	9.6	15.4	71.0
78.7	28.8	25.0	38.4	32.6
11.5	151.7	19.2	209.3	97.9
7.7	7.7	57.6	11.5	71.0
23.0	42.2	23.0	69.1	32.6
40.3	25.0	53.8	25.0	7.8
9.6	23.0	11.5	13.4	15.4
61.4	5.8	21.1	171.0	15.4
128.6				

Linolenic Acid

1 hour of irradiation: total number of discs plus particles observed in 31 fields = 0

2 hours of irradiation: total number of discs plus particles observed in 31 fields = 5

size (u)

30.7	48.0	13.4	23.0	46.1

continued

7 hours of irradiation: total number of discs plus particles observed in 31 fields = 14

size(u)

9.6	5.8	7.7	3.8	36.5
30.7	13.4	19.2	1.9	19.2
153.6	17.3	15.4	28.8	

12 hours of irradiation: total number of discs plus particles observed in 31 fields = 60

size (u)

9.6	105.6	23.0	19.2	69.1
3.8	13.4	73.0	11.5	25.0
5.8	15.4	19.2	169.0	17.3
5.8	9.6	7.7	7.7	109.4
36.5	26.9	57.6	5.8	13.4
30.7	61.4	7.7	73.0	28.8
42.2	17.3	5.8	7.7	7.7
32.6	19.2	26.9	17.3	3.8
7.7	5.8	5.8	42.2	15.4
5.8	9.6	7.7	19.2	130.6
25.0	11.5	21.1	5.7	7.7
3.8	11.5	5.8	3.8	26.9

Appendix IV

Manufacturers or suppliers of equipment and reagents cited in the text are listed below.

Applied Science Laboratories, Inc., State College, Pennsylvania, U.S.A.

Fisher Scientific Co. Ltd., 8505 Devonshire Rd., Montreal, Quebec, Canada

Hewlett-Packard Canada Ltd., 275 Hymus Blvd., Pt. Claire, Quebec, Canada

Millipore Corporation, Bedford, Massachusetts, U.S.A.

Selas Flotronics, Inc., Spring House, Pennsylvania, U.S.A.

Sigma Chemical Co., 3500 DeKalb St., St. Louis, Missouri, U.S.A.

Ultraviolet Products, Inc., San Gabriel, California, U.S.A.