both sites are present, as when (−)-PB blocks excitatory responses to (+)-PB (Fig. 1, A_2).

The variable presence of the inhibitory responses on these cultured neurons contrasts with the ubiquitously present GABA responses (*11*), indicating that the inhibitory effects of PB are probably not mediated through activation of GABA receptors, as had been previously proposed (*8, 9*). The excitatory actions of (+)-PB and the inhibitory and GABA-potentiating actions of (−)-PB correlate well with the stimulant effects of (+)-PB and the depressant effects of (−)-PB observed in vivo, suggesting that these cellular actions may be important in the clinical responses to the drugs (*13*). The electrophysiological data also provide evidence for the presence of several functionally and stereospecifically distinct sites of barbiturate action and indicate that steric, in addition to hydrophobic considerations (*14*), are important in determining the pharmacologic actions of pentobarbital.

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19 September 1979

Synthesis of Lipid During Photosynthesis by Phytoplankton of the Southern Ocean

Abstract. *Assimilation of carbon-14 labeled bicarbonate into photosynthetic products was measured at four stations in the Southern Ocean. Phytoplankton populations incorporated as much as 80 percent of the fixed carbon into lipid under conditions of low temperatures (−0.2° to −1.8°C) and low light intensities. At higher temperatures (+0.3° to +0.8°C) and higher light intensities, incorporation into lipid accounted for less than 20 percent of the fixed carbon, synthesis of polysaccharide and protein being more prominent.*

Studies of primary productivity of the waters surrounding the Antarctic continent have emphasized mainly (i) the geographical distribution of productivity (*1*) and (ii) the question of temperature adaptation among the phytoplankton (*2*). To expand such studies of primary productivity in the Southern Ocean we have examined the synthesis of the major types of end products of phytoplankton photosynthesis—polysaccharide, lipid, and protein. The origin of this approach has been described (*3, 4*). In essence, this previous work—both with cultures of several marine algae and with natural phytoplankton from temperate, subtropical, and tropical waters—has suggested that environmental "stress" (limited nutrient availability or suboptimal light intensity) causes the cells to con-

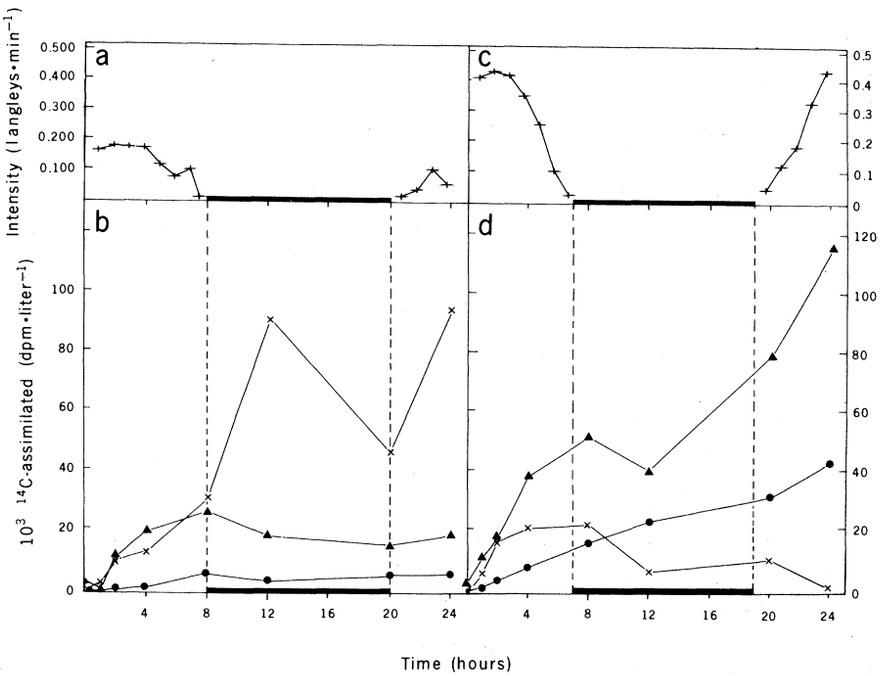

Fig. 1. Diurnal changes in the pattern of photosynthesis when 4 liters of surface water were incubated with ^{14}C-labeled bicarbonate at the ambient temperature and natural light. Data show the incorporation into lipid (crosses), protein (filled circles), and polysaccharide/metabolite pool material (filled triangles). (a) Pattern of photosynthesis observed at station 18 where the ambient temperature was − 1.0°C. (b) Pattern of photosynthesis at station 29, with an ambient temperature of 0.2°C.

0036-8075/80/0111-0197$00.50/0

serve the synthesis of protein. This is reflected in an increased proportion of carbon-14 incorporated into protein during photosynthesis at reduced light intensities or by nutrient-limited populations. This increased relative rate of protein synthesis is generally accompanied by a reduced proportion of carbon incorporated into polysaccharide. Throughout all these studies with temperate and tropical phytoplankton, incorporation into polysaccharide accounted for 40 to 70 percent of the total carbon fixed, that into protein 10 to 40 percent, and that into lipid and small-molecular-weight compounds approximately 20 percent. We now report a difference in the pattern of photosynthesis in phytoplankton from the Southern Ocean.

Studies were conducted aboard the ARA *Islas Orcadas* during September to October 1978. Results from three stations are presented. The stations were station 18 59°41′S, 45°51′W; station 19, 59°46′S, 42°26′W; and station 29, 54°51′S, 35°51′W. Surface temperatures varied from −1.8° to 0.2°C, and the maximum light intensity varied from 0.175 to 0.438 langleys per minute. At stations 18 and 29, samples of surface populations were incubated with ^{14}C-labeled bicarbonate for 24 hours at ambient temperature under natural light. At station 19, samples of surface populations were incubated at a range of temperatures, in constant temperature water baths illuminated by reflector floodlights. The extraction procedure was a modification of that used earlier (*3*, *5*), and the lipid was extracted by an adaptation of the technique of Bligh and Dyer (*6*).

Labeled algae, harvested by filtration onto glass-fiber filters, were treated with 5 percent trichloroacetic acid, giving a soluble fraction (small-molecular-weight compounds, nucleic acids, polysaccharide, and lipid) and a residue (protein). Subsequent extraction of lipids from the fraction soluble in cold trichloroacetic acid was performed with a mixture of chloroform, methanol, and water (2:2:1.8, by volume). The layered suspension was separated giving a fraction soluble in chloroform (lipid), and another soluble in a mixture of methanol and water. The protein residue and portions of the lipid and aqueous fractions were counted with a Beckman LS100C liquid scintillation spectrometer.

In parallel extractions (*3*, *4*), an initial extraction with hot ethanol was followed by treatment with hot 5 percent trichloroacetic acid, yielding an ethanol-soluble fraction (small molecular weight compounds and lipid), a fraction soluble in tricholoroacetic acid (polysaccharide and nucleic acids), and a residue (protein). Results from the two procedures paralleled each other. That is, when there was significant radioactivity in the lipid fraction extracted by the method of Bligh and Dyer (*6*), comparable radioactivity appeared in the ethanol-soluble fraction of the other procedure. Only results from the first procedure are presented here. Throughout our presentation, the methanol-water fraction is referred to as the "polysaccharide-metabolite pool" fraction reflecting the relatively low level of radioactivity in nucleic acids after fixation of ^{14}C-labeled bicarbonate by eukaryotic algae (*5*).

The pattern of photosynthesis in the Southern Ocean and that measured in other regions of the oceans were different. Significant labeling of the lipid fraction was a characteristic of phytoplankton from the Antarctic region—an observation not duplicated in our other studies (Figs. 1 and 2). This enhanced synthesis of lipid was most marked when extremes of low temperature (< −1.0°C) and low light intensities were combined. Figure 1, for example, compares the patterns of synthesis from two stations: one where the ambient temperature was −1.0°C and the other where it was +0.2°C (temperatures in the deck incubators were higher; −1.0° to 0° and +0.2° to 5°C, respectively). In the latter the pattern resembled that observed in temperate waters with prominent synthesis of "polysaccharide-metabolite pool" material and a significant synthesis of protein that continued in the dark. At the colder station, incorporation of ^{14}C into lipids paralleled that into polysaccharide-metabolite pool material, and protein synthesis was much less significant. The direct effect of temperature (Fig. 2c) reveals a change from lipid synthesis to polysaccharide-metabolite pool synthesis with increasing temperature.

Temperature was not the only environmental factor affecting the pattern of synthesis. Reduced light intensity caused increased relative labeling of lipid, at the expense of incorporation into the polysaccharide-metabolite pool fraction (Fig. 2, a and b). This effect of light intensity was most marked at the lower temperature (−1.0°C, in Fig. 2a) and was less significant at the station with a water temperature of +0.2°C (Fig. 2b). The combination of low temperature and low light intensity had the most dramatic effect on lipid synthesis. Thus, with a water temperature of −1.0°C and a light intensity 10 percent of daylight, as much as 90 percent of the carbon was fixed after 8 hours in the lipid fraction.

Studies with algal cultures (*7*), sug-

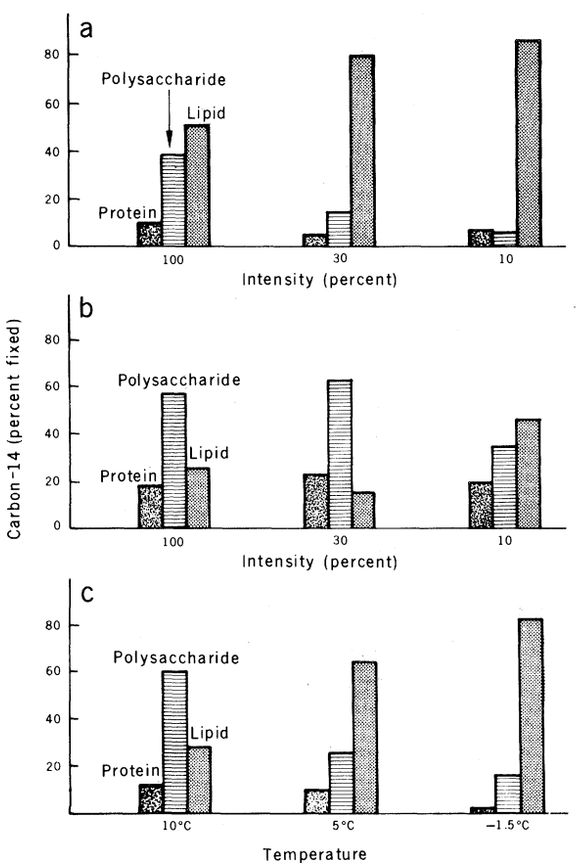

Fig. 2. Effects of temperature and light intensity on the proportions of carbon assimilated into the different fractions. (a) Pattern observed at station 18; ambient temperature −1.0°C; I_{max}, 0.175 langleys per minute. (b) Different proportions observed at station 29; ambient temperature 0.2°C; I_{max}, 0.438 langleys per minute. (c) Effect of temperature on the pattern of photosynthesis at station 19 (incubation of surface water under artificial illumination).

gested that adaptation of algae to low temperatures resulted from increased content of proteins—notably enzymes. Although this suggestion was later questioned (*8*), the possibility of increased protein synthesis being a means of responding to environmental stress remains. Although our studies of other regions (*3, 4*) have suggested such a role for protein synthesis, we have not observed such a phenomenon in the low temperatures of the Southern Ocean. Indeed, in such waters, the synthesis of lipid is the dominant physiological response of phytoplankton to stress.

The significance of this dramatic synthesis of lipid in the waters around Antarctica is unknown. At all stations, the populations were dominated by diatoms. Although Fogg (*9*) observed measured lipid synthesis when cultures of the diatom *Navicula* were nitrogen starved, there appears to be no evidence for nitrogen deficiency at the stations we studied since significant concentrations of nitrate were always observed (*10*). Mayzand and Martin (*11*) have proposed that organisms living in colder water with irregular or low food supply (for example, low light intensities for phytoplankton) might be expected to have larger lipid storage. The higher caloric content of lipid suggests that storage of high-energy products available for survival in dark periods could be of significance in the polar region; however, this is only one hypothesis. For example, Lee *et al.* (*12*) observed that phospholipids and galactolipids accounted for as much as 57 percent of the total lipid in diatoms. Such material can be important structural components [see the observations of Benson *et al.* (*13*) on chloroplast lipids]. Thus, the roles of lipids can be more than the simple one of storage products.

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2 April 1979; revised 4 September 1979

Time: A New Parameter for Kinetic Measurements in Flow Cytometry

Abstract. *The measure of time was used as an additional parameter on an existing flow cytometer to study the kinetics of enzyme activities and cell-stain interactions. By correlating all fluorescent signals from single cells with time, the dynamics of a reaction can be followed for several minutes. This advanced application of flow cytometry is easily implemented and can be incorporated into any flow cytometer that has two-parameter analysis capability.*

Quantitative multiparameter flow cytometry is becoming widely used in basic research and clinical medicine (*1*). Flow cytometers presently in use range from the elegantly simple (*2*) to the highly complex (*3*), and several types of multiparameter flow instruments are commercially available (*4*). The growing number of applications of flow cytometry result from both instrumental innovations and new methodological approaches (*5*). Generally, flow cytometric measurements are made in an equilibrium state where all dynamic changes have gone to completion. Analysis of fluorescence from a fluorochrome such as mithramycin, which binds stoichiometrically to DNA, is carried out after the dye-binding reaction has reached equilibrium (*6*). Any fluctuation in the fluorescence intensity during such an analysis increases the coefficient of variation of the pulse height distribution and confounds the interpretation of the data (*7*). Several groups have recently made kinetic measurements, using flow systems (*8*). The increases in cellular fluorescence due to enzymatic conversion of a nonfluorescent substrate to a fluorescent product have been measured by sequential analyses at discrete time intervals after the addition of substrate. This technique provides quantifiable measurements of enzyme kinetics in whole, intact, and in certain cases viable cells. Because the fluorescence from large numbers of individual cells is measured, this method also can be used to detect population kinetic inhomogeneity within a single sample.

There are several important disadvantages associated with multiple discrete measurements in kinetic studies: (i) fast kinetic reactions will not be resolved because the rapidly changing fluorescent intensity will tend to smear the distribution, even over brief sampling time periods; (ii) heterogeneous kinetic populations will not be resolved unless the reactions are slow, and each subpopulation is quite distinct; and (iii) much of the statistical precision obtained by flow cytometric analysis is lost if data are collected for only a small fraction of the total time period.

We have incorporated time as one of the parameters recorded in a multiparameter flow cytometer. It is thus possible to analyze kinetic reactions continuously with each fluorescent event correlated to a specific time. Several examples are presented which illustrate the usefulness of this technique.

We used the Los Alamos Scientific Laboratory computer-based multiparameter cell sorter system (*9*) for all measurements. Time was measured with a linear voltage ramp generator. The ramp generator (Berkeley Nucleonics Corporation model LG-1) was modified to operate for long time periods by the addition of more capacitance (33 μF) to the timing circuit. This allows a sweep of 10 V (256 channels) in approximately 7 minutes. The output of the ramp generator was calibrated as a function of time and has an excellent linear behavior. For these studies, two analog signals are processed along with the time ramp. One of these is the fluorescence pulse whose time behavior is being measured, and the other is a light scatter signal. When a cell passes through the laser beam, the light scatter signal initiates a signal processor to peak sense and hold the light scatter and fluorescence signals as well as the voltage level present from the ramp generator. By using the light scattered by a cell to trigger data acquisition, every cell passing through the flow chamber is analyzed, even those with zero or very low fluorescence. The signals are digitized and transferred to a minicomputer (PDP-11/20), where they are stored on a magnetic disk as correlated raw event files (*9*). The initial start-up time delay in sample analysis is usually between 30 and 40 seconds. This delay could be re-

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