Carbon Flow into the End-Products of Photosynthesis in Short and Long Incubations of a Natural Phytoplankton Population

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Abstract

During a cruise to the eastern Canadian Arctic (Northern Baffin Bay) in the summer of 1980, we took advantage of the 24-h photoperiod to conduct a 32-h time course experiment of 14C accumulation under natural solar radiation. The degree of non-linearity in the time course was judged against a time-dependent curve of radioactivity constructed by cumulatively adding the amount of 14C taken up in sequential short (2 h) incubations of plankton held in a replicate bottle but left unlabelled until removed for assay. Departure from linearity was due first to decreasing rates of 14C incorporation into polysaccharides and then into lipids. There was a close correspondence between 14C incorporation into proteins in the 32-h incubation and in the sequence of short incubations. These observations are consistent with patterns in utilization of photosynthetic end-products established from laboratory studies of unicellular algal cultures. Based on parallel or independent control experiments, it was judged that complicating factors such as diel light changes, nitrogenous nutrient exhaustion, bottle size effects or inhibitory contaminants in NaH14CO3 stock solutions would not seriously affect our interpretation that non-linearity resulted from catabolic loss of radiocarbon.

Introduction

Much of the long-standing uncertainty (reviewed by Peterson, 1980) of whether the 14C method (Steemann-Nielsen, 1952) measures gross or net photosynthesis can be traced to interpretation of data collected only at the end-points of the incubation period. On the other hand, it has been pointed out repeatedly that since the processes of 12C uptake and loss occur simultaneously, estimates of their rates (or their difference, which is net photosynthesis) based on 14C accumulation are strictly valid only with time-course measurements (Bernhard et al., 1975; Buckingham et al., 1975; Hobson et al., 1976; Dring and Jewson, 1982).

A progressive decrease in net radiocarbon accumulation rate is often observed when natural phytoplankton populations are incubated from morning to evening (Vollenweider and Nauwerck, 1961; Barnett and Hirota, 1966; Saunders, 1972). Some of this decrease is undoubtedly related to progressively diminishing solar radiation after midday. However, downward concavity in time-dependent curves of radiocarbon accumulation has been obtained even when incubations were carried out in constant light incubators (Savidge, 1978; Lancelot, 1979; Larsson and Hagström, 1979). A favoured explanation (e.g. Marra et al., 1981) is that because carbon uptake and loss are in dynamic equilibrium in the cell, the time-dependent curve of radiocarbon accumulation asymptotically approaches an upper plateau where the rates of assimilation and loss of the label are equal and opposite. The rate of accumulation is initially high and asymptotically approaches the true carbon uptake rate in the cell since little intracellular radiocarbon is available to be lost.

If, however, carbon uptake exceeds loss, as is the case for a growing population, the time-dependent curve of radioisotope accumulation possesses an inflection point (e.g. Dring and Jewson, 1982). The shape of the curve reflects the asymptotic approach towards intracellular radioisotopic equilibrium, followed by an exponential increase in radioactivity at a specific rate equal to the population growth rate. Such "non steady-state" kinetics is descriptive of the incorporation of radiolabelled glutamate (Taylor and Jannasch, 1976; Taylor, 1979; Cuhel et al., 1981), sulfate (Cuhel et al., 1981, 1982) and bicarbonate (Li, 1982) in growing cultures of marine bacteria.

Calculations of intracellular specific activity as a function of time using typical rates of phytoplankton growth indicate that at 12 h (a typical incubation time for 14C
measurements), total carbon specific activity is not yet at equilibrium (Dring and Jewson, 1982). For equilibrium to be effectively attained (e.g. 99% saturation) in 12 h, a population doubling time of about 2 to 3 h is required.

Growth rates of this magnitude have been reported for *Chlorella pyrenoidosa*, high temperature strain 7-11-05, under optimal conditions of light and temperature (39°C) (Sorokin, 1960), but are high for marine phytoplankton, even under optimal culture conditions (Eppley, 1972; Baars, 1981). Reported generation times of 3 h for microplankton determined by ATP analysis (Sheldon and Sutcliffe, 1978) require confirmation since cellular levels of ATP change on a diel basis (Weiler and Karl, 1979) and in response to environmental perturbations and nutrient limitations as might occur in bottle incubations (Karl, 1980).

It seems therefore that incubation times longer than 12 h would be necessary for a complete kinetic analysis of ^14^C uptake by natural phytoplankton. Unfortunately, in most latitudes, the diel cycle of insolation goes through its full amplitude on this time scale. During a cruise to the eastern Canadian Arctic in the summer of 1980, we took advantage of the 24-h photoperiod to conduct a 32-h time course of ^14^C uptake by phytoplankton incubated under natural sunlight. Our hypothesis was that if non-linearity was detectable in the time course of total ^14^C accumulation, and if the non-linearity could be ascribed to catabolic loss of labelled substrate, curvature should be more pronounced in the time courses of ^14^C incorporation into those photosynthetic end-products known to supply the respiratory and excretory pools.

**Material and Methods**

Experiments were performed during CSS “Hudson” cruise 80-027 (July–August, 1980) to Northern Baffin Bay and adjacent waters. On August 5, a sample of surface seawater at Station 52 (Lat. 73°52.6’N, Long. 81°47.5’W) was collected by a Niskin bottle.

Each of 2 one litre borosilicate glass bottles was filled with 11 of the water sample and placed in a deck incubator receiving full incident solar radiation (100% Io). Temperature was maintained at about 3°C by surface seawater flowing through the incubator. Five hundred microlitre of NaH^14^CO_3_ (New England Nuclear) was added to one of the samples at the start of the incubation (final activity = 0.23 #Ci #mol⁻¹). The time course of ^14^C accumulation in particles retained on glass fibre filters (Whatman GF/C) was established by sequential sampling from this bottle for 32 h. The water sample in the other 1-l bottle was incubated without radiolabel. At 2-h intervals, beginning at time zero, aliquots of 50 ml were removed and placed in 125-ml Pyrex glass bottles containing 25 #Ci NaH^14^CO_3_ (final activity = 0.23 #Ci #mol⁻¹). These subsamples were immediately returned to the deck incubator. Uptake of ^14^C was terminated after 2 h by collection of particulates on glass fibre filters.

A further 500 ml of the water sample was taken to establish the light dependence of H^14^CO_3_ uptake. One hundred-ml aliquots of the sample were placed in each of five 125-ml Pyrex glass bottles containing 25 #Ci NaH^14^CO_3_ (final activity = 0.11 #Ci #mol⁻¹). The samples were incubated for 4 h in deck boxes which received incident solar radiation attenuated to 100, 50, 25, 10 and 1%. Additionally, a 225-ml water sample was placed into a 250-ml borosilicate glass bottle containing 4.5 #Ci NaH^14^CO_3_ (final activity = 0.009 #Ci #mol⁻¹) and incubated for 4 h in the 100% Io deck incubator.

The experiments described above allowed us to compare the amounts of ^14^C taken up in 4 h in bottles of different sizes at 100% Io. However, since the added activity of ^14^C (and therefore presumably also the concentration of trace metals or other metabolic inhibitors contaminating the commercial ^14^C solution) was different, we performed the following experiment, which was more rigorously designed to test for possible bottle size effects. Surface seawater was collected on July 28 and measured into borosilicate glass bottles of various sizes (125, 250, 500 and 1000 ml) in quadruplicate. Each bottle received a volume of water equal to 80% of its nominal capacity. Each sample received NaH^14^CO_3_ to a final activity of 0.012 #Ci #mol⁻¹ and all were incubated in the 100% Io deck incubator. Two bottles of each size were removed after 4 h and 20.5 h; the amount of ^14^C in particulates was determined from aliquots of 100-ml sample.

To assess depletion of nitrogenous nutrients as a factor contributing to non-linear ^14^C uptake (Goldman et al., 1981a, b), a 30-h time course experiment was performed in which we monitored the uptake of ^15^NO_3_-, ^15^NH_4_+, ^15^N-urea and H^14^CO_3_. Water was collected from 25 m at Station 46 (Lat. 74°57.0’N, Long. 78°8.2’W) on August 4. Nutrient uptake experiments were initiated by adding K^15^NO_3_ (95.5 at % ^15^N), (^15^NH_4)_SO_4_ (99.0 at % ^15^N) and ^15^N-urea (96.5 at % ^15^N) to samples in 500-ml borosilicate glass bottles at concentrations of 0.1 μM N. Particulates were collected on glass fibre filters for ^15^N uptake measurements every 6 h for 30 h. Incubations for ^14^C uptake were performed in 250-ml borosilicate glass bottles and ^14^C specific activity was 0.009 μCi μmol⁻¹. The sampling frequency was the same as that for the three separate ^15^N incubations.

All analytical procedures have been previously described (Harrison et al., 1982; Li and Platt, 1982).

**Results**

At Station 52, the rate of C uptake during the first 4 h was 0.66 ± 0.09 μgC l⁻¹ h⁻¹ (linear regression coefficient ± 95% confidence interval) (Fig. 1). Further accumulation of ^14^C proceeded at changing rates, resulting in a non-linear time-dependent radioactivity curve (Figs. 1, 2 B). In comparison, the cumulative uptake of carbon based on sequential 2-h ^14^C incubations proceeded at a constant rate (0.68 ± 0.02 μgC l⁻¹ h⁻¹) for 22 h (Figs. 1, 2 B).
The time-dependent radioactivity curves of isotope incorporation into photosynthetic end-products are shown in Fig. 3. In the case of protein (Fig. 3 A), the curve based on continuous accumulation coincided with that based on cumulative sequential 2 h incubations up to 20 h. In the continuous accumulation curve, there was a slight decrease in protein 14C from 20 to 24 h (the sample at 22 h was lost). This decrease might have been artifactual since the 2 curves progressed in parallel from 24 h onwards.

In the case of polysaccharide (Fig. 3 B), the time course of continuous accumulation showed a definite non-linearity. The deviation of this curve from that based on cumulative sequential incubations occurred as early as 4 h.

In the case of lipid (Fig. 3 C), the departure from linearity in the time course of continuous accumulation did not occur until after about 10 h.

The light dependence of 14C uptake in a 4-h incubation (Station 52, 1430–1830 hrs) is shown in Fig. 4. By the end of the incubation, photosynthetically active solar radiation (PAR) had decreased to 63% of its initial value, from 246 to 155 Wm⁻² (Fig. 2 A). However, the rate of 14C uptake during the 4-h incubation remained constant (Fig. 1). This was because the range of PAR for light-saturation of 14C
uptake extended from 100 to at least 25% \( \text{I}_0 \) (Fig. 4). By visual inspection, the light-saturated rate was reduced by 50% at approximately 2% \( \text{I}_0 \) (\( \approx 5 \) to \( \approx 3 \text{ Wm}^{-2} \)). This compares with the lowest level of \( \text{PAR} \) (7 \text{ Wm}^{-2}) recorded during the experiment, from 0100 to 0300 hrs, August 6 (Fig. 2A). Thus, it is not surprising that \( ^{14}\text{C} \) uptake continued throughout the 32 h of observation (Fig. 2B).

Figure 4 also shows a comparison of 4-h \( ^{14}\text{C} \) uptake in bottles of various sizes incubated at 100% \( \text{I}_0 \). There was no systematic trend in the variation of \( ^{14}\text{C} \) uptake with bottle size. Since the specific activity of \( \text{H}^{14}\text{CO}_3^- \) was different in the various bottles (see Material and Methods), we could not rule out the possibility of compensatory confounding effects due to inhibitory contaminants in the \( \text{NaH}^{14}\text{CO}_3^- \) stock solutions. However, a separate "bottle-size experiment" with constant \( ^{14}\text{C} \) specific activity showed no effect of bottle size (125 to 1000 ml), either in 4-h or 20.5-h incubations (Fig. 5, Table 1). Because of this, we can probably also rule out any systematic effect of possible contaminants in the former experiment (Fig. 4). Significantly, the mean rate of \( ^{14}\text{C} \) uptake in the 20.5 h incubations (Fig. 5) was 0.17 \( \mu \text{gC} \text{l}^{-1} \text{h}^{-1} \). This value is less than the mean rate in the 4-h incubations (0.21 \( \mu \text{gC} \text{l}^{-1} \text{h}^{-1} \)) and thus indicates non-linearity in the time course of \( ^{14}\text{C} \) accumulation.

Depletion of nitrogenous nutrients did not appear to be a problem in extended bottle incubations during our study. In a 30-h experiment (Station 46), the uptake of \( ^{15}\text{NH}_4^+ \), \( ^{15}\text{NO}_3^- \), \( ^{15}\text{N}-\text{urea} \) and \( \text{H}^{14}\text{CO}_3^- \) all proceeded at fairly constant rates (Fig. 6). By the end of 30 h, only 39, 38 and 36% of the initial \( \text{NH}_4^+ \), \( \text{NO}_3^- \) and urea respectively had been consumed. Similar results were obtained at other stations occupied in this area (Harrison, unpublished results).

![Figure 5](image.png)

**Fig. 5.** Relationship between the amount of \( ^{14}\text{C} \) taken up in 4 h (\( \bullet \)) or 20.5 h (\( \circ \)) and the size of the incubating bottle, July 28, 1980. Bottles were filled with sample to 80% of nominal capacity.

![Figure 6](image.png)

**Fig. 6.** (A) Variation of photosynthetically active radiation from 1200 hrs, August 4, 1980 to 1800 hrs, August 5, 1980. (B) Time course of uptake for \( \text{H}^{14}\text{CO}_3^- \) (\( \bullet \)), \( ^{15}\text{NH}_4^+ \) (\( \circ \)), \( ^{15}\text{NO}_3^- \) (\( \circ \)) and \( ^{15}\text{N}-\text{urea} \) (\( \circ \))

**Table 1.** Two-way analysis of variance of \( ^{14}\text{C} \) uptake, testing the effects of incubation time (4 and 20.5 h), size of bottle (125, 250, 500 and 1000 ml) and their interaction

<table>
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<th>df</th>
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<td>Error</td>
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**Discussion**

In studies of natural phytoplankton populations, the experimental protocol of comparing \( ^{14}\text{C} \) accumulation after a long incubation to the amount accumulated from a series of short incubations performed sequentially over the same period is motivated by two different goals. In one case, the major aim is to test for possible effects of bottle confinement (Rodhe, 1958; Vollenweider and Nauwerck, 1961; Gieskes et al., 1979; Redalje and Laws, 1981). It is assumed that in short incubations, effects such as cumulative photoinhibition, enhanced algal mortality and nutrient exhaustion are minimized. Therefore, the series of short incubations serves the purpose of a control against which the single long incubation may be compared. In this type of experiment, the phytoplankton assayed in the short incubations are, by necessity, sampled from the water body each time such an incubation is begun.

In the other type of experiment, which utilizes a similar protocol, the major aim is to study the dynamics of
radiotracer accumulation in an attempt to understand rates of substrate uptake and loss (Nalewajko et al., 1976; Savidge, 1978). It is assumed that the short incubations reflect mainly the uptake process while the long incubation reflects both uptake and loss processes. In this type of experiment, the phytoplankton assayed in the short incubations must experience exactly the same conditions as those in the long incubation, including confinement in a bottle. Therefore, phytoplankton used in the short incubations are from an aliquot of the originally sampled water but kept in a bottle until ready for assay at repeated intervals throughout the day.

The present study is of the second type. To discuss our results in the context of C uptake and loss processes, it is necessary to have some assurance that the departure from linearity of the time course of $^{14}$C accumulation in the long incubation (Fig. 2B) was not due primarily to other causes (Carpenter and Lively, 1980). Our main experiment was not designed primarily to investigate these problems. However, parallel or subsequent control experiments allow the following comments to be made.

First, results from the experiment on the light-dependence of $^{14}$C uptake (Fig. 4) indicate that the decrease in rate observed in the long incubation was not due to decreases in light intensity. This is borne out by the short incubations performed during periods of reduced light which showed the same rate of $^{14}$C uptake as during the earlier periods with greater light intensity (Fig. 2B). The present study appears to be the first in which a time course of $^{14}$C uptake has been established under natural solar radiation for a period greater than one diel cycle but during which time PAR was almost always at a level sufficient to saturate the rate of $^{14}$C uptake. The P-I relationship we established in this experiment was based on only 5 data points and is presented merely to illustrate conditions on the day (Fig. 4). However, earlier we observed similarly low PAR required for saturation and lack of significant photo-inhibition in surface populations in the same area, based on more precise P-I curves generated from 19 data points (Li and Platt, 1982) and 50 data points (Platt et al., 1982).

Second, we can rule out the possibility that the different size of bottles used in the long incubation (1 000 ml) and in the short incubations (125 ml) led to a significant difference in production rates (Figs. 1, 4, 5, Table 1). This finding agrees with those of Qasim et al. (1972), Lewis (1974) and Sharp et al. (1980), but not with those of Sheldon et al. (1973) or Gieskes et al. (1979).

Third, it seems that the decrease in rate of $^{14}$C accumulation after 4 h (Figs. 1, 2B) is probably not due to inhibition by contaminants in the NaH$^{14}$CO$_3$ stock solutions (Fig. 4). Results from several studies suggest that commercially available $^{14}$C stock solutions may contain trace metals at concentrations inhibitory to $^{14}$C uptake (Steeman-Nielsen and Wium-Anderson, 1970; Carpenter and Lively, 1980; Fitzwater et al., 1982). We supposed that if this were true, there would exist a negative correlation between the amount of $^{14}$C taken up in 4-h incubations and the specific activity of $^{14}$C in the seawater. Our results do not support this contention (Fig. 4). However, it is possible that the manifestation of toxicity is apparent only after a lengthier exposure. Harrison et al. (1977) found that inhibition due to Cu$^{2+}$ was detectable in 4-h $^{14}$C incubations only after a 24-h exposure period to the metal. Although there exists considerable literature on the effects of heavy metals on phytoplankton (Davies, 1978), there is a need for more work to clarify the time-dependence of inhibition.

There are several other problems associated with confinement of samples in bottles that might render artifactual the results of $^{14}$C time course experiments. These include plankton mortality (Venrick et al., 1977), chlorophyll loss (Gieskes et al., 1979) and nutrient depletion (Goldman et al., 1981a,b). Although these problems were not investigated in our experiment, we believe that they were not important in our 32-h experiment (Fig. 2) insofar as they might be manifested in alterations to the $^{14}$C uptake process. The reason is that when we assayed short-term (2 h) $^{14}$C uptake rates of unlabelled phytoplankton kept in one of the two 1-l bottles, we obtained rates that were not less than the initial rate, even when the assays were performed after 24 h (Fig. 2B). A 30-h time course experiment initiated just one day prior to the main experiment indicated that depletion of phytoplankton nitrogenous nutrients would not be of great importance (Fig. 6). We therefore conclude that the departure from linearity of the time-changing curve for continuous accumulation of radiotracer (Figs. 1, 2B) is mainly due to progressive importance of radiolabel loss.

An important assumption implicit in arriving at this conclusion is that each of the consecutive short-term (2 h) incubations provides a measure that approaches gross carbon uptake. Tracer kinetic analysis of algal $^{14}$C uptake is usually formulated according to a system of two internally homogeneous compartments: a dissolved inorganic carbon pool and a particulate (algal) organic carbon pool (Bernhard et al., 1975; Buckingham et al., 1975; Hobson et al., 1976; Li and Goldman, 1981; Dring and Jewson, 1982). Analysis of such a system leads to the conclusion that in a time-dependent plot of algal $^{14}$C activity, "the initial slope of the curve is a measure of gross production rate, while the decrease in slope toward equilibrium gives information about respiration rate" (Buckingham et al., 1975). Our measurements of $^{14}$C activity result in a curve which is linear from zero time up to almost 4 h (Fig. 1). We suggest that in our experiment, measurements of $^{14}$C activity after a 2-h incubation were closely indicative of apparent gross photosynthesis (cf. Dring and Jewson, 1982).

It is necessary to stress that such measurements of gross photosynthesis are "apparent" because the assumption of only two compartments in the system neglects the possibility of reassimilation of respiratory CO$_2$ within the cell (Steeman-Nielsen, 1955). As Harris (1978, 1980) has pointed out, there still remains uncertainty as to the degree of CO$_2$ reassimilation in algae. However, the analysis by
Andersen and Sand-Jensen (1980) shows that even if a proportion as large as 0.6 of respiratory C is refixed, the discrepancy between apparent and true gross photosynthesis is quantitatively important only when light intensity is low (rate-limiting). Since PAR during the course of our experiment (Fig. 2A) was almost always at a level sufficient to saturate $^{14}$C uptake (Fig. 4), it may be that the rates of C uptake we measured in 2-h incubations approached the rates of true gross photosynthesis.

From studies of unicellular algae in culture, it is well established that catabolic requirements are first met by the utilization of carbohydrates, and subsequently by that of lipids (Cook, 1963, 1966; Ricketts, 1966; Handa, 1969; Cohen and Parnas, 1976; Foy and Smith, 1980; Hitchcock, 1980). The utilization of proteins appears to occur only under circumstances of extreme energy limitation (Cook, 1963; Handa, 1969). In studies of natural phytoplankton populations, Morris and co-workers have generally observed continuing incorporation of $^{14}$C into proteins during darkness; conversely, the amount of $^{14}$C in polysaccharides and lipids decrease during this time (Morris, 1981). It is also known that polysaccharides represent a considerable fraction of carbon fated for extracellular release (Hellebust, 1974; Mague et al., 1980). The results from the present experiment (Fig. 3) in fact indicate that continued accumulation of $^{14}$C into photosynthetic end-products is slowed down in polysaccharides first, and then in lipids and finally, if at all, in proteins. These observations strengthen the interpretation of non-linearity in total $^{14}$C uptake (Fig. 2B) as being due to metabolic loss of $^{14}$C-labelled algal material.

Let us now consider the error involved in estimating carbon production at Station 52 (Figs. 1, 2B) based on a measurement made at the endpoint of typical oceano- graphic “productivity” experiments, say 24 h. From Fig. 2B (cross symbols), it can be seen that after a 24-h incubation, the conventional estimate of carbon production would be 11.2 $\mu$g C L$^{-1}$. This estimate is neither one of gross production nor of net production. The series of sequential 2-h incubations (Fig. 2B, square symbols) yields an estimate of 18.1 $\mu$g C L$^{-1}$ for gross production in 24 h. Thus, the conventional estimate is only 62% of gross production.

At the same time, since the conventional estimation assumes immediate intracellular radioisotopic equilibration, net production is overestimated to the extent that net radioisotope flux does not reflect net mass flux. Explicit expressions required for the calculation of gross carbon uptake and loss (net production is simply obtained as their difference) from $^{14}$C measurements have been given by Dring and Jewson (1982) for the compartmental model of Hobson et al. (1976). Using the algorithm of Dring and Jewson (1982) to obtain best-fit estimates of the specific rates of gross carbon uptake (P) and loss (R), the following values were calculated for our experiment (Fig. 2B): $P = 0.25$ h$^{-1}$, $R = 0.20$ h$^{-1}$. In addition, a third parameter was estimated: the initial carbon content of cells in unit volume of seawater, $^{14}$C$_{0} = 3.49$ $\mu$g C L$^{-1}$. The fit of the data was extremely good: the ratio of variation explained by the model to that left unexplained was $F_{(1,19)} = 926$ (P << 0.001). Therefore, according to this model, net carbon production in 24 h ($t$) was:

$$^{14}$C$_{t} - ^{14}$C$_{0} = ^{14}$C$_{0} e^{(P - R)t} - ^{14}$C$_{0} = 8.1$ $\mu$g C L$^{-1}$.

Thus the conventional estimate of carbon production (11.2 $\mu$g C L$^{-1}$) is 138% of net carbon production as given by the model of Hobson et al. (1976).

However, as Dring and Jewson (1982) pointed out, some of the assumptions underlying the model of Hobson et al. (1976) are probably unrealistic. One questionable outcome arising from this model when applied to experimental data of Dring and Jewson (1982) was the very high value of R/P (> 0.6). Similarly, the estimates of R and P for our experiment yielded a high value of R/P (0.8). In a natural water sample such as the one considered in this experiment, radiolabel is lost not only from phytoplankton, but also from heterotrophic microzooplankton and bacteria which consume radiolabelled cells or exudes. Thus, the high value of R/P may not be unrealistic. Alternatively, it is statistically acceptable (contrasted to realistic, vide infra) to fit a straight line to the results for the experiment in which phytoplankton were radiolabelled for 32 h. In fact, a very good fit was obtained from a linear regression model: the ratio of variation explained to that left unexplained was $F_{(1,19)} = 3313$ (P << 0.001). However, the slope of this line (0.48 ± 0.02 $\mu$g C L$^{-1}$ h$^{-1}$) based on data from the entire 32-h experiment was statistically very different from the slope of the line estimated for just the initial 4 h (0.66 ± 0.09 $\mu$g C L$^{-1}$ h$^{-1}$) (Fig. 1). Moreover, the ordinate intercept (0.41 ± 0.27 $\mu$g C L$^{-1}$) was significantly (P < 0.01) greater than zero. These imply the existence of systematic (non-random) residual errors in the linear model. Specifically, the experimental measurements were systematically underestimated during the first hour but systematically underestimated over the next 7 h; residual errors appeared to be randomly distributed about the linear regression after the eighth hour. On the other hand, the model of Hobson et al. (1976) was more “realistic”: residual errors appeared to be randomly distributed about the fitted curve (not shown). Whether this curvilinear model is truly realistic is not known. However, the conclusion remains that the single measurement of $^{14}$C activity made after a long incubation, say 24 h, estimates neither gross nor net production.

In summary, although others have also observed non-linearity in time courses of $^{14}$C uptake by natural plankton samples (e.g. Barnett and Hirota, 1966; Savidge, 1978) and invoked the explanation, based on theoretical considerations that ascribe non-linearity to metabolic loss of radiolabel (Bernhard et al., 1975; Buckingham et al., 1975; Hobson et al., 1976; Marra et al., 1981), the present study appears to be the first in providing experimental evidence in support of the explanation. Furthermore, it seems likely that artifacts such as those discussed by Carpenter and Lively (1980) would not have invalidated this conclusion. Unfortunately, this does not necessarily imply that the rate
of 14C uptake by phytoplankton in the bottles is the same as that of phytoplankton left undisturbed in the natural environment (Li and Goldman, 1981). Thus, although the effect of sample confinement is not known, it was sufficient for the purposes of the present study that the duration of confinement had little effect on the phytoplankton.

An interesting implication of the close correspondence between protein-14C incorporation in a long incubation and in the sequence of short incubations (Fig. 3A) is that measurements of protein-14C may provide a better indication of phytoplankton growth than measurements of total 14C uptake. This idea has been advocated by Morris (1980, 1981, Morris and Skea, 1978). The reason is that growth and division are more closely dependent upon synthesis of protein rather than upon synthesis of storage products such as polysaccharides and lipids. Others have independently investigated the use of 32S-SO4 to estimate rates of protein synthesis and growth in marine phytoplankton (Bates, 1981) and marine bacteria (Cuhel et al., 1982).

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Literature Cited
