

# Carbon exchange and $^{14}\text{C}$ tracer methods in a nitrogen-limited diatom, *Thalassiosira pseudonana*

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**ABSTRACT:** A simple carbon-exchange model accounted for the kinetics of  $^{14}\text{C}$  uptake and release by the diatom *Thalassiosira pseudonana* in nitrogen-limited chemostat culture. The model treats the cells as consisting of 2 pools of carbon; an exchanging pool which carries out photosynthesis, respiration and excretion, and a synthetic pool which does not exchange, but accumulates carbon from the exchanging pool. The model fitted well to observed  $^{14}\text{C}$  kinetics over a 10-fold range of growth rates and was demonstrably superior to 2 alternate models which have been prevalent in the theory and application of  $^{14}\text{C}$  methodology in primary production studies. The exchanging pool was small (4 to 15 % of cell carbon) and rapidly cycled (90 % turnover time of 1 to 12 h) in all steady-state cultures, but was larger (21 %) and more slowly cycled (15 h) in a chemostat deprived of its limiting nitrogen supply for 24 h. In all cultures, the observed kinetics indicated that usual  $^{14}\text{C}$  estimates of phytoplankton production should be close to net production rates, but that short-term  $^{14}\text{C}$  estimates of excretion should be too low for slow-growing populations.

## INTRODUCTION

In any physiological experiment using radioisotope tracers, interpretation of the results requires that the investigator adopt a schematic model of the pathways by which the tracer can move through the system under study (Sheppard, 1962). Specifically, the model is used to deduce the desired physiological fluxes from the accumulation of tracer in the various observable fractions of the system.

In phytoplankton productivity studies the  $^{14}\text{C}$  tracer method for photosynthetic rate measurement (Stemann-Nielsen, 1952; Peterson, 1980) has been in use for more than 30 yr. It has been the focus of repeated criticism, and is usually thought to be culpable when apparent discrepancies are revealed between different estimates of production (Eppley, 1980; Shulenberg and Reid, 1981; Jenkins, 1982). It is therefore all the more surprising that so little attention has been given to the choice of tracer flow model in  $^{14}\text{C}$  studies of phytoplankton production. Most practitioners of the  $^{14}\text{C}$  method are probably unaware of the tracer flow model that is implicit in the way they calculate photosynthetic rate from  $^{14}\text{C}$  uptake (Peterson, 1980; Dring and Jewson, 1982). Nevertheless, an

underlying schematic model is indispensable even if it is not given explicit expression.

Whenever a model has been made explicit in analyses of  $^{14}\text{C}$  production estimates, it has almost invariably pictured the phytoplankton as a single, well-mixed, carbon pool (Buckingham et al., 1975; Hobson et al., 1976; Li and Goldman, 1981; Marra et al., 1981; Smith, 1982). Such analyses lead to the conclusions that conventional  $^{14}\text{C}$  estimates of production should be closer to gross than net production and that conventional estimates of excretion should be strongly biased (too low).

On the other hand, ecologists generally believe that the  $^{14}\text{C}$  method gives an estimate that is closer to net production than to gross, and that excretion estimates are unbiased (Eppley, 1980; Mague et al., 1980; Peterson, 1980). We will show that this belief is equivalent to a view of the phytoplankton as a heterogeneous, rather than a well-mixed, carbon pool. Although implicit in much contemporary thinking (Williams, 1971; Shuter, 1979; Dring and Jewson, 1982), the idea of the phytoplankton as a heterogeneous carbon pool has not been the subject of quantitative analysis. It is a largely untested assumption.

Here we report application of a heterogeneous car-

bon model to studies of carbon flow in nitrogen-limited continuous cultures of *Thalassiosira pseudonana*. We show that it is able to describe successfully features of the carbon kinetics that simpler, conventional models could not accommodate.

## MATERIALS AND METHODS

### Experimental design and analysis

The strategy of our experiments was to obtain time-course measurements of  $^{14}\text{C}$  assimilation and release, i.e. release as dissolved organic carbon (DOC) which we also refer to as excretion, and then to use compartmental analysis (Sheppard, 1962) to fit simple models of carbon exchange to the observed tracer kinetics. The analysis yields estimates of functional pool sizes and turnover rates from which fluxes such as net production and excretion can be calculated (D. F. Smith, 1974). Initially we chose to work with steady-state chemostat cultures growing under continuous and invariant light and temperature, to obtain the greatest possible control over conditions influencing carbon exchange. Three different models of carbon exchange (Fig. 1) seemed particularly relevant in the context of  $^{14}\text{C}$  primary production experiments.

The homogeneous model, which has dominated in theoretical studies, postulates that carbon fated for respiration, excretion and synthesis are all parts of a

single, well-mixed, algal pool. Accordingly, an appreciable lag will occur before respiratory and excretory products reach isotopic equilibrium with the added tracer (Smith, 1982). By contrast, the 'traditional' model is meant to represent the assumption that respired and excreted carbon equilibrate almost instantaneously with the added inorganic tracer. In compartment terms, this means that tracer accumulates in the particulate pool only at the rate of net production (i.e. tracer efflux from the cells by respiration and excretion exactly balances the tracer influx required to support these processes) and hence the model shows no losses from the particulate pool. Because the carbon entering the DOC pool is always in isotopic equilibrium with the dissolved inorganic carbon (DIC) pool, the tracer will apparently move directly from DIC to DOC, even though we know it must in fact pass via the phytoplankton. The homogeneous and traditional models represent 2 extremes in our view of algal carbon exchange and its kinetics.

The third model (4-compartment) is more complex, and assumes that the phytoplankton comprise 2 functionally different carbon pools (Fig. 1). One (the exchanging pool) exchanges carbon with the DIC by assimilation and respiration, and donates carbon both to the DOC and to the second intracellular pool (the synthetic pool). The latter pathway represents net production, or growth. The 4-compartment model can resemble either of the other models, depending on the relative sizes of the exchanging and synthetic pools. The homogeneous and traditional models are limiting cases as the exchanging pool approaches 100 or 0 % of the cell total, respectively.

Each model predicts different time courses of labelling in the DIC, phytoplankton, and DOC pools, and the models can be fitted to measurements by simultaneous non-linear regression (Berman et al., 1962). We used a program written expressly for such compartmental analysis (SAAM 27, Berman and Weiss, 1978) to compare the fit of the models to our observed kinetics. The usefulness of this approach in analyzing material flow in ecological systems has been described previously (D. F. Smith, 1974). The analyses yield non-linear least-squares estimates of the models' parameters, together with the variance of the estimates and the total remaining unexplained variance in the observed values. Owing to the non-linear techniques used, exact confidence levels cannot be attached to the measures of variance reported here without considerable further work to define the error distribution associated with each type of model (Draper and Smith, 1966). This does not interfere with the conclusions drawn, which rest mainly on the ability of the different models to reproduce the observed time courses without systematic lack of fit. Lack of fit was detected by

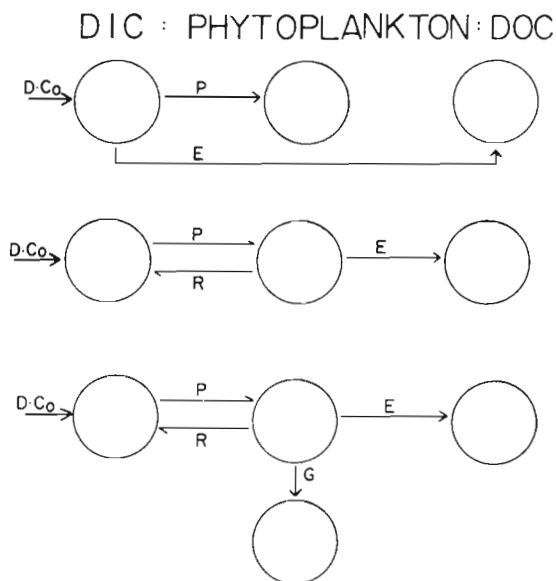


Fig. 1. Compartmental models of algal carbon exchange. From top to bottom: 'traditional', homogeneous, 4-compartment model. Material is also lost from each pool due to chemostat dilution (D) but for simplicity this is not diagrammed

examination of time series and residuals plots (Draper and Smith, 1966) for evidence of trend in the direction and magnitude of differences between observed and predicted values.

When a model has been fitted, its parameters allow calculation of steady-state rates of carbon turnover and, in the case of the four-compartment model, the relative sizes of intracellular pools. We will be concerned here with the estimates of this model, which is represented by the following set of equations for our chemostat populations:

$$\dot{A}_1 = D(a_0 \cdot C_0 - a_1 \cdot C_1) - P \cdot a_1 \cdot C_1 + R \cdot a_2 \cdot C_2 \quad (1)$$

$$\dot{A}_2 = P \cdot a_1 \cdot C_1 - (D + R + E + G) \cdot a_2 \cdot C_2 \quad (2)$$

$$\dot{A}_3 = G \cdot a_2 \cdot C_2 - D \cdot a_3 \cdot C_3 \quad (3)$$

$$\dot{A}_4 = E \cdot a_2 \cdot C_2 - D \cdot a_4 \cdot C_4 \quad (4)$$

where  $\dot{A}_i$  = time rate of change of activity (dpm ml<sup>-1</sup> d<sup>-1</sup>) in pool *i*;  $C_i$  = carbon concentration in pool *i* (g ml<sup>-1</sup>);  $a_i$  = specific activity in pool *i* (dpm g<sup>-1</sup>). The pools are: inflowing medium (*i* = 0), DIC (1), exchanging pool (2), synthetic pool (3), and DOC (4). The rate constants, all in units of d<sup>-1</sup>, are  $D$  = dilution,  $R$  = respiration,  $E$  = excretion,  $G$  = growth,  $P$  = gross assimilation.

Of the pools, we measured DIC and DOC activity directly, but could measure only the sum of the exchanging and synthetic pool activities, i.e. the particulate activity. This did not prevent the resolution of the exchanging and synthetic pools and their parameters; consider the time-explicit solution for the radioactivity in a given compartment (Berman and Schoenfeld, 1956):

$$A_i(t) = \sum_{j=1}^4 K_{ij} \cdot e^{-\alpha_j t} \quad (5)$$

where  $\alpha$  = the *j*<sup>th</sup> eigenvalue of the matrix of rate constants corresponding to the Laplace transformation of Equations 1 to 4;  $K_{ij}$  = the *i*<sup>th</sup> element of the corresponding eigenvector. If we can observe only the sum of 2 compartments,  $C_x$  and  $C_y$ , we have:

$$A_x(t) + A_y(t) = \sum_{j=1}^4 [K_{xj} + K_{yj}] \cdot e^{-\alpha_j t} \quad (6)$$

Equation 6 states that the kinetics of the summed compartments will possess a number and weighting of exponential terms characteristic of the full, 4-compartment system even if only 3 different pools can be measured. There are practical upper limits to the degree of summing that can be done, imposed by the progressive loss of degrees of freedom and increasing likelihood that some similar  $\alpha_j$  will become impossible to distinguish, but Equation 6 shows that separate sub-compartments within a single measured pool can, in principle, be resolved. For a simple system like the

present one, resolution is most often possible and did not present problems in the current work.

Of the rate constants in Equations 1 to 4, the dilution rate is known *a priori*, but the remaining rate constants are estimated by the non-linear regression. All except the assimilation constant are, as fitted, specific rates normalized to the exchanging pool. The assimilation constant is normalized to the DIC pool. We report gross assimilation as the sum of respiration, excretion, and growth to provide a carbon balance uniformly normalized to the exchanging pool.

The relative size of the exchanging pool can be calculated from the steady-state form of Equation 3:

$$C_2/C_3 = D/G \quad (7)$$

We can further calculate the time course of tracer equilibration in the exchanging pool by assuming that  $a_1$  is effectively constant (a good assumption in this case):

$$a_2(t) = a_1(1 - e^{-P' t}) \quad (8)$$

where  $P'$  = gross assimilation normalized to the exchanging pool. Equation 8, yields the time required to approach any given degree of tracer equilibrium, e.g.:

$$\tau_{90} = -\ln(.1)/P' = \text{time to 90 \% of tracer equilibrium.}$$

The  $\tau_{90}$  is the time required to effectively eliminate effects of tracer disequilibrium on <sup>14</sup>C estimates of production and excretion. The specific gross assimilation rate,  $P'$  (Table 3) is equal to the fractional turnover rate of the exchanging pool.

## EXPERIMENTAL PROCEDURE

### Steady-state cultures

*Thalassiosira pseudonana* clone 3H was cultured at 18.5 °C in chemostats of either 600 or 1,500 ml volume. The larger vessels were used for the lower growth rates to minimize flow perturbation during sampling for the kinetics experiments. Continuous lighting was provided by cool-white fluorescent tubes at 350 to 410 μE m<sup>-2</sup> s<sup>-1</sup> PAR. The cultures were mixed by teflon spin bars, but were not bubbled. Axenic techniques were used, but the cultures were not bacterium-free. Bacterial counts using DAPI fluorescent stain (Coleman, 1980) showed that bacterial biomass (cell volume) was at least 3 orders of magnitude or more smaller than algal biomass. Even allowing for wide variation in metabolic rates of bacteria (Tempest and Neijssel, 1977) such a small biomass could be ignored.

The medium was prepared from filter-sterilized Bedford Basin, N. S. seawater (Irwin and Platt, 1978),

enriched to *f*/20 (Guillard and Ryther, 1962) in phosphorus ( $\text{NaH}_2\text{PO}_4$ ), silicate ( $\text{Na}_2\text{SiO}_3$ ), trace metals and vitamins, and *f*/200 in nitrogen ( $\text{KNO}_3$ ). For the first set of cultures (July, 1982, Table 1) resulting nutrient concentrations were about  $12.0 \mu\text{M NO}_3$ ,  $4.0 \mu\text{M PO}_4$  and  $40.0 \mu\text{M SiO}_3$ . For the second set (December 1982, Table 1) concentrations were about  $20.0 \mu\text{M NO}_3$  and  $6.0 \mu\text{M PO}_4$  (silicate was not measured). Ammonia was not measured in either set of cultures, but should have been  $1.0 \mu\text{M}$  or less in both sets (Irwin and Platt, 1978).  $^{14}\text{C}$ -bicarbonate (prepared from  $\text{Ba}^{14}\text{CO}_3$  by acidification and trapping in  $\text{NaOH}$ ) was added to a total of about  $1 \times 10^4 \text{ dpm ml}^{-1}$ . The relatively low nitrogen concentration of the medium was apparently successful in preventing inorganic carbon limitation, as culture pH did not exceed 8.4.

Steady-state concentrations of particulate organic carbon were estimated from the steady-state distribution of tracer (immediately before the kinetic experiment) and the carbonate alkalinity (end-point titration method; Strickland and Parsons, 1972). We had hoped to measure DOC similarly, but were unable to do so because our blanks (prepared from sterile labeled medium) were substantial compared to the very low sample activities, and were quite variable. This happened despite our precautions in tracer preparation and purging techniques (Sharp, 1977). No similar problems occurred in the kinetic experiments, which yielded larger sample activities.

Nutrient analyses followed the automated procedure described by Strickland and Parsons (1972). Cell counts used phase-contrast optics at  $250\times$  magnification, in a 0.1 ml haemocytometer. Mean concentrations during steady-state, and during kinetic experiments, were based on several successive counts of at least 250 cells each.

Each set of cultures comprised 6 chemostats operating in parallel at different dilution rates. After inoculation, the cultures were operated until cell counts indicated that population size had stabilized. The kinetic experiments were then started in 1 of 2 different ways.

### Kinetic experiments

In most experiments,  $^{14}\text{C}$  bicarbonate was added to both the reservoir and reactor to increase total activity to about  $2 \times 10^5 \text{ dpm ml}^{-1}$ , swamping the background, steady-state, label. In a few other experiments, we switched to a new reservoir of high (about  $2 \times 10^5 \text{ dpm ml}^{-1}$ ) activity and simultaneously spiked the reactor to match. In either case, flow was interrupted for only a few minutes. The intention was to maintain the established steady-state in carbon flux and distribution, and this was usually achieved (i.e. algal

biomass did not vary from its steady-state average during the kinetic experiment).

In one of the experiments involving a reservoir switch, however, cell concentration declined after the switch, indicating loss of steady-state. The new reservoir contained an order of magnitude excess of silicate over the intended, indicating an accidental shift in nutrient supply even though nitrate was near the expected concentration. The interesting results from this perturbed culture prompted a second, deliberate, perturbation experiment. The deliberate perturbation entailed switching to a new reservoir, again of high activity, containing no added nitrate. This was intended to provide a comparison of kinetics in steady-state nutrient limited populations with kinetics in a population suddenly deprived of its limiting nutrient supply.

Total, particulate, and dissolved organic  $^{14}\text{C}$  activities were measured by liquid scintillation counting at intervals over 24 h following the increase in activity. The sampling scheme varied somewhat but was generally half-hourly until 2 h elapsed time, hourly between 2 h and 6 h, and once per 2 to 4 h thereafter. Total activities were measured on 1.0 ml samples mixed with  $40 \mu\text{l NaOH}$  (an addition which was shown to maximize inorganic label retention but minimize quenching). Particulate samples (1.0 ml) were collected on glass-fibre filters (GF/F), rinsed with 3.0 ml of filtered seawater, and fumed for 15 min over  $\text{HCl}$ . The filtration pressure was  $< 100 \text{ mm Hg}$ . Dissolved organic activities ( $\text{DO}^{14}\text{C}$ ) were measured in the filtrate (including rinse); the activity recovered did not change when pressure was reduced to 50 mm Hg, but did increase when pressure was increased to 200 or 300 mm Hg. The filtrate was purged of inorganic label by 1 of 2 techniques; either acidification to about pH 1.8 followed by complete evaporation at 60 to 70 °C, or by acidification to pH 2.5 to 3.0 followed by 24 h of atmospheric equilibration. Both methods successfully eliminated inorganic activity, as tested with our stock  $^{14}\text{C}$ -bicarbonate, and gave better than 95 % recovery of an amino acid mixture resembling algal hydrolyzate (NEN Canada). The 2 techniques were also not significantly different when compared using culture filtrate.

### Batch incubations

Batch incubations in light and dark bottles (performed only with the second set of cultures) were initiated by filling triplicate glass B.O.D. bottles (30 ml volume) immediately following the 24 h kinetic experiment. The bottles were incubated under the same light and temperature conditions as the parent cultures for 24 h, but were not mixed. After incubation,

we measured cell density, and total, particulate, and dissolved organic activities as described above. The increase of particulate activity in the light was taken as net production, and loss of particulate activity in the dark as respiration plus excretion. The dark loss was partitioned into respiration and excretion using the measured increase in dissolved organic activity.

## RESULTS

### Steady-state characteristics

At steady state, residual nitrate was detected only in the 2 cultures of highest dilution rate ( $< 0.2 \mu\text{M}$ ) but  $2.0 \mu\text{M}$  phosphate and  $10.0 \mu\text{M}$  silicate, or more, was routinely found. The selective depletion of nitrate gives some support to our assumption that the cultures were nitrogen limited.

Cell concentrations at steady state were on the order of  $10^5 \text{ cells ml}^{-1}$ , tending to decrease at the highest dilution rates (Table 1). The higher nutrient content of

Table 1. Steady-state cell density and algal carbon, and carbon:nitrogen composition ratio, in nitrogen-limited chemostats from July 1982 (Set 1) and Dec. 1982 (Set 2). The last 2 cultures were perturbed (see text) following these measurements

Growth rate ( $\text{d}^{-1}$ )	Set	Cell density ( $\text{ml}^{-1}$ )	Algal carbon conc. ( $\mu\text{g-atm l}^{-1}$ )	C:N Ratio (molar)
2.50	1	$8.9 \times 10^4$	81.46–97.9	8.15–9.79
1.95	2	$1.9 \times 10^5$	194.2	8.56
1.18	2	$3.4 \times 10^5$	220.0	9.23
1.10	1	$2.1 \times 10^5$	87.26–111.77	9.09–11.65
0.60	1	$1.7 \times 10^5$	70.40–92.50	8.45–11.10
0.58	2	$3.3 \times 10^5$	194.2	9.57
0.29	2	$3.7 \times 10^5$	298.3	13.44
0.80	1	$2.3 \times 10^5$	122.94–154.19	10.92–14.48
0.52	2	$3.2 \times 10^5$	216.7	10.03

the medium in the second set of cultures caused higher cell densities and algal carbon concentrations. The carbon concentrations varied with cell density, ranging from about 80 to  $300 \mu\text{moles C l}^{-1}$  (Table 1). In the first set of cultures, some uncertainty in the estimates of steady-state total activity (caused by contamination of the NaOH reagent) allowed only a range to be stated for the algal carbon values. This was not a problem in the kinetic experiments, in which the much larger total activity swamped the background contamination. Algal C:N ratios (estimated using the difference in nitrate concentration between influent medium and the culture) were consistent with the assumption of nitrogen limitation, increasing from about 8.5 to 13.5 as culture growth rate varied from fastest to slowest.

### Model fit to exchange kinetics – steady-state cultures

Representative plots of tracer appearance in particulate and dissolved organic carbon during a kinetic experiment are shown in Fig. 2. The time courses comprised 36 to 42 individual observations for each of total and particulate activities, and 72 to 84 of  $\text{DO}^{14}\text{C}$ ,

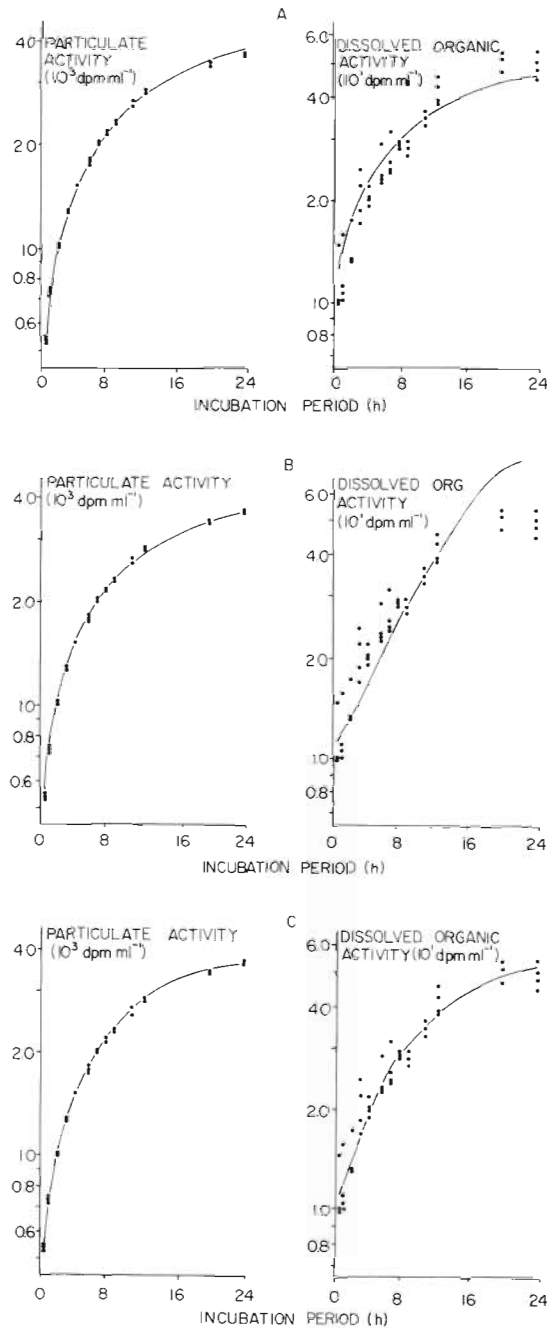


Fig. 2. Observed activity time series together with curves fitted by compartmental analysis, for the kinetic experiment on a culture growing at  $.60 \text{ d}^{-1}$ . Curves fitted using (A) traditional model; (B) homogeneous model; (C) 4-compartment model

but many points are superimposed in the plots. Replication was good for total and particulate activities (95 % C.I. generally  $\leq 6$  % of the mean for triplicates) but poor for  $\text{DO}^{14}\text{C}$  (95 % C.I. commonly  $> 20$  % of the mean of six replicates). The change of  $\text{DO}^{14}\text{C}$  over time was much greater than the apparent error variation, however, so even this pool contributed useful information for model fitting.

In the example shown (Fig. 2) all 3 models of carbon exchange fitted the particulate time series quite well. This was found to be generally true for the particulate data in all cultures. Differences were more pronounced in the DOC pool. The example (Fig. 2) shows that the homogeneous model gave systematic lack of fit to the  $\text{DO}^{14}\text{C}$  kinetics. The traditional model performed better, but the four-compartment model was better yet. A residuals plot for the latter model (Fig. 3) revealed no obvious, systematic lack of fit to the  $\text{DO}^{14}\text{C}$ . The

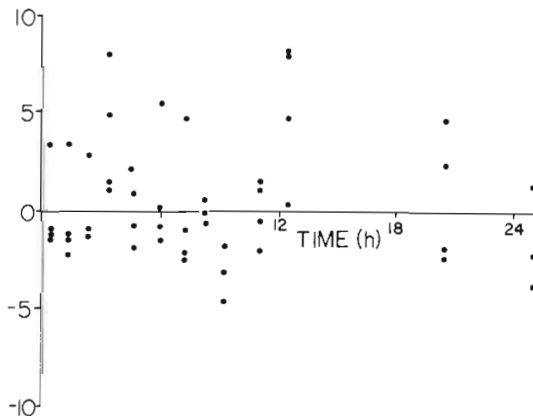


Fig. 3. Residuals (ordinate) plot for the DOC compartment, illustrating goodness of fit obtained in Fig. 2C

superiority of the 4-compartment model proved general among cultures, and subjective inspection of its fitted curves never revealed any apparent lack of fit.

The residual sum of squares (res. SS, a measure of the variance not explained by the model) offers one means of summarizing the performance of the different models. The 4-compartment model always explained as much or more variation than the others, although the difference was not often large (Table 2). Neither of the alternative models was a consistently superior fit, although each was at times effectively as good as the more complicated model. The relative applicability of the traditional and homogeneous models was apparently not simply related to growth rate *per se* (Table 2).

#### Model fit to exchange kinetics – perturbed cultures

Table 2 shows that the 2 perturbed cultures both fitted better to the homogeneous than to the traditional

Table 2. Residual sum of squares from non-linear least-squares fit of each carbon exchange model. Growth rate listed for perturbed cultures (last 2) is the pre-perturbation rate. Entries are sums of squares for POC plus DOC pools; the large contribution from DIC was removed here to better illustrate differences among models in fit to the biologically interesting pools

Culture growth rate ( $\text{d}^{-1}$ )	Traditional	Sum of squares (arbitrary units) Homogeneous	4-compt
2.50	1.37	1.58	1.27
1.95	2.75	3.54	2.00
1.18	6.28	8.08	4.73
1.10	2.33	2.00	1.56
0.60	1.29	2.00	0.86
0.58	3.79	2.60	1.46
0.29	1.79	1.95	1.67
0.80	5.59	3.44	3.22
0.52	3.25	1.13	.73

model. The 4-compartment model fitted slightly better than the homogeneous, as judged by residual plots.

The first perturbation experiment ( $\mu = 0.8$ , Table 2) represented stress response presumably related to the aberrant nutrient content of the medium supplied during the kinetic experiment. Cell density declined steadily to about 65 % of its initial value over 3 d following the start of the kinetic experiment. The actual change in cell density over the first 24 h period used in the compartmental analysis was only 10 %, however, and so should not have significantly invalidated our steady-state analysis. Rather, a metabolic change seemed to be involved.

The second perturbation experiment was a deliberate nutrient stress applied by cutting off the input of limiting nitrogen while maintaining flow. The cells, grown to a steady state at an influent concentration of about  $22.0 \mu\text{M}$  available nitrogen, were forced to adjust to an influent concentration of only about  $10.0 \mu\text{M}$ . Such a downshift in limiting nutrient supply must force a downward adjustment, for some time, in growth rate, and ultimately population size. In fact, no significant decline in cell density was observed over 24 h, implying a progressive dilution in cell nitrogen content (the batch incubation described below showed that growth was eventually impaired). Although less dramatic in effect than the first perturbation experiment, the deliberate perturbation also produced kinetics more consistent with the homogeneous than the traditional model.

#### Carbon exchange rates of steady-state cultures

Among unperturbed cultures, carbon loss as respiration and excretion was apparently very small

Table 3. Carbon exchange rates and pool sizes from 4-compartment model. Figures in brackets are twice the % standard error ( $n \geq 100$  throughout); for calculations see 'Materials and Methods'. Last 2 cultures again are the perturbed

Growth rate ( $d^{-1}$ )	Exchange rates, normalized to exchanging pool ( $d^{-1}$ )				$\tau_{90}$ , h	Exchanging pool size (% of cell total)
	Growth	Respir.	Excret.	Gross assim.		
2.50	71.21 (98)	1.76 (435)	0.854 (85)	73.82 (99)	0.75	3.5
1.95	13.29 (29)	0 ( $\leq .24$ )*	0.350 (24)	13.64 (29)	4.05	14.7
1.18	14.47 (19)	0 ( $\leq .18$ )*	0.245 (14)	14.71 (19)	3.76	8.1
1.10	6.33 (16)	0 ( $\leq .63$ )*	0.112 (10)	6.44 (16)	8.58	17.4
0.60	10.86 (20)	0.62 (48)	0.16 (14)	11.64 (22)	4.74	5.6
0.58	4.55 (19)	0.031 (129)	0.127 (12)	4.71 (19)	11.74	12.9
0.29	7.60 (18)	0 ( $\leq .063$ )*	0.178 (13)	7.78 (18)	7.10	3.8
0.80	1.43 (34)	2.05 (17)	0.090 (10)	3.57 (33)	15.48	56.0
0.52	2.45 (21)	0.61 (17)	0.075 (11)	3.13 (21)	17.63	21.1

\*  $\leq 2 \times$  standard error

(Tables 3, 4). Gross assimilation was little larger than net assimilation over a range of growth rates from .29 to  $2.50 d^{-1}$ , and the difference was not obviously related to growth rate. Excretion never amounted to more than a few percent of assimilation rate, and respiration was apparently zero in many cases. The unperturbed cultures were also characterized by a very small and rapidly cycling pool of exchanging carbon (Table 3). This pool varied in size from 3 to 17 % of total cell carbon, but its relative size was not systematically related to growth rate. The exchanging pool reached 90 % of tracer equilibrium in .75 to 11.7 h ( $\tau_{90}$ , Table 3). Slower-growing populations tended to have longer  $\tau_{90}$ 's, although still much shorter than comparable values for the total cell carbon pool.

Table 4. Carbon allocation (as % of gross assimilation), calculated from Table 3, for nitrogen-limited chemostats

Growth rate ( $d^{-1}$ )	Growth	Respiration	Excretion
2.50	96.5	2.4	1.2
1.95	97.4	0	2.6
1.18	98.4	0	1.6
1.10	98.3	0	1.7
0.60	93.3	5.3	1.4
0.58	96.6	0.7	2.7
0.29	97.7	0	2.3
0.80	40.1	57.4	2.5
0.52	78.3	19.5	2.4

The error in estimates of assimilation and excretion was generally moderate, with approximate 95 % confidence ranges of about 20 % of the mean. One experiment ( $\mu = 2.5 d^{-1}$ ) yielded very poor precision, probably because that experiment, alone, spanned only 12 h. Errors were much worse in respiration estimates (Table 3). This was somewhat to be expected, because direct observation of respired  $^{14}CO_2$ , unlike  $PO^{14}C$  and  $DO^{14}C$ , was impossible. Respiration therefore had to be inferred solely from its influence on  $PO^{14}C$  and  $DI^{14}C$  kinetics, which is small when respiration rates are so low. Precision was much better in the perturbed cultures, which had larger respiration rates (below).

### Carbon exchange rates of perturbed cultures

The perturbed cultures differed from the unperturbed both in the magnitude of their loss rates and the size and cycling rate of the exchanging pool (Tables 3, 4). Respiration in the first perturbed culture was actually greater than growth, and the exchanging pool comprised over half the cell total. This large exchanging pool had a relatively long  $\tau_{90}$  of 15 h. The second perturbation had less dramatic, but similar, results. In neither perturbation did excretion become a major part of carbon metabolism, remaining less than 3 % of gross assimilation.

### Carbon exchange during batch incubations

Batch incubations, performed immediately following 4 of the 5 kinetic experiments in the second set of cultures, were intended primarily to test further the apparent difference in respiration rate between steady-state and perturbed cultures. The dark loss of carbon in these incubations confirmed that the second perturbed culture had an elevated respiration rate relative to steady-state cultures (Table 5). Respiration

Table 5. Cell-division and carbon-exchange rates from light and dark bottle experiments, with standard deviations for triplicates. Last culture is the second perturbation experiment (see text)

Cell division rate (d <sup>-1</sup> )		Respiration (d <sup>-1</sup> )	Net production (d <sup>-1</sup> )
Steady state	Batch (light)		
1.95	1.01 ± .24	0.24 ± .07	1.01 ± .12
1.18	0.50 ± .08	0.22 ± .02	0.85 ± .05
0.58	0.40 ± .08	0.25 ± .05	0.58 ± .06
0.52	0.07 ± .013	0.43 ± .07	0.29 ± .04

was also larger in all the batch incubations than in the corresponding chemostats, ranging from 25 to 40 % of net assimilation among samples from steady-state cultures, and actually exceeding net assimilation in the perturbed sample. Aside from questions of containment and nutrient depletion effects, the bottle estimates of assimilation and excretion rates should be reasonably close to true because the prior 24 h kinetic incubation should have brought respiratory and excretory pools to complete tracer equilibrium even in our most extremely perturbed culture (Table 5).

The batch incubations also confirmed that our deliberate perturbation had indeed stressed the cells. Cell division was slowed, relative to the parent chemostats, in all cases but was by far most strongly inhibited in the perturbed sample (Table 5). This observation is additional evidence that the cultures were nitrogen limited. Net carbon assimilation was reduced from its steady-state rate in batch incubations of the perturbed and the fastest growing culture, but was little affected in medium growth-rate samples. Net assimilation was reduced less than cell division in the perturbed sample, resulting in an apparent increase of cell carbon content, but this effect was relatively small or absent in other samples.

Excretion, unlike respiration, was little affected either by perturbation or the transfer to batch conditions. Excretion in the light bottles was only 0.6 to 3.5 % of net assimilation, and in the dark bottles was only 2.4 to 14.4 % of total dark loss. As in the chemo-

stats, excretion remained a minor part of the carbon balance.

### DISCUSSION

Compartment models have frequently been used in examining our interpretations of conventional <sup>14</sup>C production estimates (Buckingham et al., 1975; Hobson et al., 1976; Li and Goldman, 1981; Marra et al., 1981; Dring and Jewson, 1982; Smith, 1982) but relatively little has been done to test critically the merits of alternative models. Critical appraisals have usually focussed on one or a few aspects of the kinetics of assimilation into POC (Li and Goldman, 1981; Dring and Jewson, 1982). Our compartmental analysis allowed us to use information from the kinetics of DIC and DOC labelling as well. This seems essential in comparing models, because the particulate labelling time course alone is not very sensitive to the choice of model when loss rates are small (e.g. Fig. 2) and gross and net production are very similar. The scope for error in estimating excretion rates, however, can be large even when total loss rates are small. Further, elucidation of a suitable model for such conditions gives additional insight when combined with observations on populations with much larger loss rates.

The present study appears to be the first to show that the 2 models that have dominated in primary production work (the homogeneous and traditional) have different, and incomplete, domains of applicability over a range of growth conditions. Like some previous researchers (Li and Goldman, 1981; Dring and Jewson, 1982) we rejected the homogeneous model for most unperturbed cultures. The traditional model, which has not explicitly been studied before, failed to fit the kinetics of perturbed cultures satisfactorily. Only the 4-compartment model gave consistently acceptable fit over the full range of growth conditions.

As judged by the residual SS the difference among the models was usually small, and a variance test would likely not justify the extra complexity of the 4-compartment model in many cases. This is because much of the observed kinetics would be accounted for by any model which predicts a movement of <sup>14</sup>C from DIC to POC; this was much the largest flux even over 24 h in most cultures. However, the 4-compartment model was consistently superior under all growth conditions and its greater realism led to different predictions concerning the nature of standard estimates of production and excretion (discussed below). The choice of model is therefore important.

Li and Goldman (1981) and Goldman et al. (1981) examined the relationship between short-term <sup>14</sup>C uptake and net production as a function of nitrogen-limited growth rate, and failed to find strong systema-



tic changes with growth rate in most species. Similarly, we found that the exchanging pool remained small and rapidly-cycling at all steady-state growth rates, usually producing kinetics more resembling the traditional than the homogeneous model. Under perturbation, the pool enlarged and cycled more slowly, producing kinetics more like those of the homogeneous model. Lower steady-state growth rates might have produced a result similar to that observed in the perturbed cultures, but our lowest growth rate was only about one-tenth of the observed maximum. Such relative growth rates appear most meaningful to algal physiology (Goldman, 1977) so our steady-state observations probably covered the ecologically relevant range for this species at least.

By contrast, Peterson (1978) reported systematic changes in the relationship between short-term  $^{14}\text{C}$  uptake and net production as a function of phosphorus-limited growth rate in mixed, freshwater cultures.  $^{14}\text{C}$  assimilation progressively overestimated net production as growth rate decreased, a result consistent with the homogeneous model of carbon exchange (Li and Goldman, 1981; Smith, 1982). This result could reflect a stress response similar to that observed in our perturbed cultures, because the mixed cultures were undergoing a species succession which entailed the decline of many initially abundant species under excessive phosphorus limitation (Peterson et al., 1974).

Harris and Piccinin (1977) and Savidge (1978) have also reported that  $^{14}\text{C}$  uptake commonly approximates gross production at sub-saturating light intensities in natural populations. Stress on their populations was not evident from the time course of assimilation in Savidge's case at least. We are currently testing the alternative explanation that light-limited populations differ from nutrient-limited ones in possessing a larger and more slowly cycled pool of respiratory and excretory substrates.

Dring and Jewson (1982) developed a model which postulated no respiration of recent, labelled photosynthate, a result very different from our 4-compartment model. Dring and Jewson did not fit most of their models to the  $^{14}\text{C}$  uptake observed in batch culture, but performed simulations to permit subjective comparisons with the observations. The model of choice was arrived at primarily because it could reproduce the observed quasi-linear uptake for 6 to 8 h. However, our 4-compartment model also produces quasi-linear uptake for low to moderate growth rates, and for a perturbed culture (Fig. 4). Harris and Piccinin (1983) demonstrated that new, labelled photosynthate contributes significantly to respiration in phosphorus-limited *Chlamydomonas reinhardtii* after only 10 to 40 min of contact with the tracer. While they suggested that mingling of recent photosynthate with older

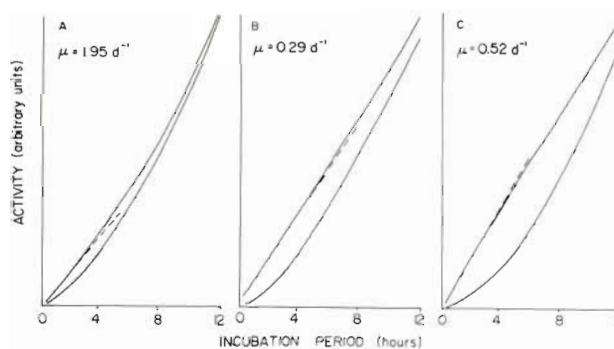


Fig. 4. Time courses of particulate and DOC activities expected in a hypothetical batch incubation. Curves generated by assuming that batch populations grow exponentially and conform to 4-compartment model. Parameters were those actually fitted to chemostat populations growing at the indicated rates. In each panel, the upper curve is particulate activity, the lower DOC; dashed line extrapolates the initially pseudo-linear particulate time course to indicate point of departure from linearity. (A) and (B): steady-state cultures; (C): second of the perturbed cultures

respiratory substrates would generally be slower in nutrient-sufficient populations, their results are consistent with our 4-compartment model and inconsistent with Dring and Jewson's. It is therefore not clear whether Dring and Jewson observed a pattern of carbon exchange different from ours, or whether a more formal fitting procedure would have led them to results similar to ours.

Other models of phytoplankton growth, which explicitly deal with intracellular compartmentation of carbon and other elements, have been developed to account for compositional response of phytoplankton to environmental change. Williams (1971) described a model which comprised one intracellular pool associated with carbon assimilation and another associated with cell synthesis, and showed that the model successfully simulated features of chlorophyll and cell concentrations observed in chemostat experiments with *Chlorella* sp. Shuter (1979) developed a much more detailed model of allocations among storage, functional and structural pools. The model did not explicitly consider respiration and excretion, postulating only a net flow of carbon into the cell via a storage pool. This particular assumption was not critical to the model's predictions of compositional response (Shuter, 1979). However, our results show that the kinetics of carbon flow through the cell correspond to passage first through a rapidly exchanging and metabolically-active pool, rather than a storage compartment.

Our results give additional support to some recently developed methods for measuring primary production and nutrient assimilation. Redalje and Laws (1981) and DiTullio and Laws (1983) have proposed techniques

that require chlorophyll and protein, respectively to be synthesized from precursor pools which exchange and equilibrate rapidly with exogenous  $^{14}\text{C}$ - $\text{CO}_2$ . These assumptions appear to be well justified for our steady-state populations. More information on populations in transient states (e.g. the nutrient shift-down experiment, present study) is most desirable to determine how robust the assumptions might be for natural populations.

The 4-compartment model can be used to calculate errors expected in standard  $^{14}\text{C}$  production and excretion estimates. As previously described for the homogeneous model (Smith, 1982), these calculations reveal the consequences of ignoring the finite time required to bring respiratory and excretory substrates to tracer equilibrium. With the current model, we are concerned with equilibration in the exchanging pool only. Accordingly, we have:

$$\hat{\mu} = \mu + L \cdot e^{-P' \cdot t} \quad (9)$$

$$\hat{E} = E \cdot (1 - e^{-P' \cdot t}) \quad (10)$$

where  $t$  = incubation period (d);  $\hat{\mu}$  = estimated net production rate ( $\text{d}^{-1}$ );  $\mu$  = true net production rate ( $\text{d}^{-1}$ );  $\hat{E}$  = estimated excretion rate ( $\text{d}^{-1}$ );  $E$  = true excretion rate ( $\text{d}^{-1}$ );  $L$  = loss (respiration + excretion) rate ( $\text{d}^{-1}$ );  $P'$  = gross assimilation rate ( $\text{d}^{-1}$ ); and all rates are normalized to the exchanging pool. These calculations assume that at least the true rate of instantaneous tracer uptake or release is known from the experimental incubation. If tracer kinetics are non-linear (e.g. DOC labelling in most cases, Fig. 4), then a fixed length incubation will generate further error.

Using Equation 9, a 6 h incubation would overestimate net production by, at most, 1.1 % among our steady-state cultures. For the perturbed cultures, the 6 h overestimate would be 63 and 13 % for the first and second culture respectively; these errors would decline to 25 and 5.8 % for a 12 h incubation. Although the error in the first perturbed culture is substantial, it is certainly much smaller than other apparent uncertainties in  $^{14}\text{C}$  production estimates for some waters (Shulenberger and Reid, 1981; Jenkins, 1982).

Comparable predictions from the homogeneous model would be 6 h overestimates of 3.0 % or less for steady-state cultures, and 92 and 23 % for the 2 perturbed cultures. The traditional model predicts no error at all. The models accordingly can lead to quite different interpretations of standard production estimates.

The excellent agreement between  $^{14}\text{C}$  uptake and net production in our steady-state cultures resulted from very low loss rates, as well as rapid equilibration in the exchanging pool. Our apparent respiration rates in the chemostats are among the lowest ever reported; values of 10 to 20 % of maximum gross photosynthesis seem typical for dark respiration (Humphrey, 1975;

Burris, 1980; Harris, 1980). There are at least 3 possible explanations for this.

Intracellular re-fixation of respired carbon (Stemann-Nielsen, 1955; Andersen and Sand-Jensen, 1980) in the light could have made respiration invisible to our kinetic methods. We do not rule out such an effect, although it would have no bearing on the validity of short-term  $^{14}\text{C}$  uptake as a measure of net production in the light. The much larger respiration rates observed in the dark bottle incubations (Table 5) could be construed as evidence of considerable re-assimilation in the light.

Other evidence indicates that the dark bottles greatly overestimated the respiration rates of the illuminated chemostat cultures. Although very low, the range of our respiration estimates was quite consistent with earlier estimates for this species (Sharp et al., 1980), made by electron transport activity assays (i.e. maximum potential respiration rate) which were free from re-assimilation effects. Further, the 24 h nutrient deprivation stimulated apparent respiration, even in the light, in the second perturbed culture (Table 3). The bottle incubations entailed an equivalent nutrient deprivation, and the enhancement of respiration in the bottle was comparable in magnitude to that observed in the perturbed chemostat. The slowing of cell division also indicated that bottle incubations were stressful, so it seems likeliest that this marine diatom does possess very low respiration rates relative to many species, but responds to stresses such as sudden nutrient deprivation with greatly elevated respiration rates.

The third possibility is that respiration was actually inhibited by light. There seems to be no clear consensus on when and to what extent such inhibition occurs (Falkowski and Owens, 1978; Burris, 1980; Graham, 1980; Harris, 1980), and many would argue that photorespiration increases the total respiration rate under many conditions (Harris and Piccinin, 1977; Stone and Ganf, 1981). For *Thalassiosira pseudonana* in particular, 2 earlier studies have indicated that respiration continues, albeit at a very low rate, in the light (Falkowski and Owens, 1978; Sharp et al., 1980), and that mitochondrial respiration in the light is about equal to rates in the dark (Falkowski and Owens, 1978). Illumination, or lack of it, does not itself seem very important to dark respiration rate in this species. This may not be true for other species, however (Burris, 1980), and photoperiod and light regime may also influence respiration rates (Casper, 1982). Extrapolation of short-term measurements to 24 h production rates must accordingly entail considerable uncertainty, and demands ingenuity in design of controls (e.g. Li and Harrison, 1982).

Excretion rates were only 1.2 to 2.7 % of gross

assimilation in our cultures (Table 4). This is in the range previously reported for this species (Sharp et al., 1980). By contrast, Sharp (1977) measured very large excretion (42 to 95 %) in a nitrogen-limited cyclostat, although parallel short-term  $^{14}\text{C}$  release again yielded low rates (1 to 10 %). This difference was attributed to perturbation of flow to the cyclostat, but we could not reproduce the effect by interrupting nutrient supply to our chemostats while maintaining flow, so the mechanism underlying Sharp's observation is unclear. Our extreme perturbation of 24 h batch incubation also failed to accelerate release.

Our low excretion rates are consistent with the majority of estimates for actively growing, and even some senescent, algal cultures (Nalewajko and Marin, 1969; Laws and Caperon, 1976; Laws and Wong, 1978; Laws and Bannister, 1980; Cospér, 1982). Most studies, like ours, have also failed to find substantially larger release in response to increased limitation of growth rate and it has been postulated that a special pathway or metabolic controls constrain excretion rates to near constancy (Smith and Wiebe, 1976). The low release rates, and lack of strong relationships with growth rate, contradict the situation often stated to exist in nature (Watanabe, 1980; Fogg, 1983). One explanation that has been offered for this discrepancy is that the excretion of low molecular weight compounds follows diffusion gradients which are much stronger in natural populations than in relatively concentrated culture populations (Fogg, 1983). Our cultures were about one order of magnitude more dilute than most, however, yet still yielded very low excretion rates.

Despite this, our results indicate that kinetic considerations must cause most field estimates of excretion to be, if anything, underestimates. Using Equation 10, excretion estimated from a 6 h incubation would range from 69 to 100 % of true among steady-state cultures (with error largest at low growth rates), and 59 to 54 % for the perturbed cultures. For a 2 h incubation, as sometimes suggested (Nalewajko et al., 1976; Lancelot, 1979), the corresponding estimates would be 32 to 100 % for steady-state, and 23 to 26 % for perturbed, cultures. These errors are much more substantial than those in production estimates, and pose a difficult problem for field measurements.

Very short (2 h) excretion estimates are intended to avoid underestimation of excretion rate by minimizing heterotrophic uptake and metabolism of labelled exudate. Such utilization can be rapid enough to influence apparent excretion rates after several hours of incubation (Nalewajko et al., 1976; Lancelot, 1979; Billen et al., 1980). It has generally been assumed that excreted carbon equilibrates so quickly with added tracer that underestimates due to tracer disequilibrium would not be an important problem in short incuba-

tions, and there is experimental support for this view (Wiebe and Smith, 1977; Wolter, 1982). However, our results show that short incubations can generate serious underestimates, depending on algal growth conditions, due to disequilibrium. There is therefore no single, ideal incubation period which can be relied upon to give a simple and accurate estimate of excretion rate. Rather, the kinetics of excretion (Wiebe and Smith, 1976) and the extent of heterotrophic activity (Jensen, 1983) should be explicitly considered if underestimates of algal excretion rates are to be avoided.

We have not attempted here to identify the 2 intracellular functional pools of the 4-compartment model with specific classes of biochemical compounds. It is tempting to associate the small, rapidly-cycling pool of exchanging carbon largely with the intermediates of photosynthetic carbon reduction and carbon oxidation cycles. Unfortunately, the routing of newly-assimilated carbon through much of these pathways is still poorly understood and controversial (Burriss, 1980; Graham, 1980), and it is a formidable problem to interrelate kinetics at the gross level with pathways at the biochemical level even when the latter are well known (Oaks and Bidwell, 1970). At a less demanding level, the labelling of coarsely-defined biochemical fractions (e.g. Morris et al., 1981) such as proteins and carbohydrates may be susceptible to simple kinetic treatments similar to those we have applied at the cell level. Better knowledge of the functional significance of these broadly defined classes of compound, and their labelling patterns, would further increase the usefulness of their study in the field.

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