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ADENINE METABOLISM AND NUCLEIC ACID SYNTHESIS: APPLICATIONS TO MICROBIOLOGICAL OCEANOGRAPHY

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INTRODUCTION

Marine microbial ecology is currently one of the least developed areas of microbiological research. This situation has been, in part, due to the limited availability of methods for evaluating the in situ rates of metabolism and growth of naturally occurring microbial populations. In fact, it may be fair to state that our present understanding of the integrated functioning of marine ecosystems is methods limited. A period of rapid advance in our understanding of microbiological oceanographic processes following the successful development and application of each new experimental approach, is evidence of this limitation.

Classical microbiological methods (e.g., plate count, light-dark bottle) have proven unsatisfactory for enumerating the sparse populations and for assessing the relatively low rates of production which occur in most marine environments. During the past 20 years a significant effort has been invested in the development and evaluation of new experimental approaches, and a variety of methods (some as yet untested) are now available to evaluate the distribution, abundance and metabolic activities of microorganisms in nature. Many of these techniques make use of our expanding fundamental knowledge of biochemistry, physiology and cell biology. One area where significant progress has been made is in the study of cellular nucleotides and nucleic acid metabolism. The well-established and obligatory role of nucleotides in cellular energetics, biosynthesis and metabolic regulation has provided the motivation for initiating laboratory and field studies. During the past few years, a coordinated study of cellular nucleotide concentrations, their intermediary

metabolism, turnover rates and rates of polynucleotide biosynthesis has been undertaken in marine and freshwater microbial assemblages. This approach, referred to as "environmental nucleotide fingerprinting" (Karl 1980), provides non-selective, corroborative, and redundant information on total microbial biomass, physiological potential, growth, and cell division rates. It should be emphasized from the start that the methods described herein are aimed at evaluating the combined activities of all microorganisms in the sea. Previous attempts to separate bacteria from unicellular algae, protozoa, and other microorganisms, whether by physical or chemical means, have generally been unsuccessful due to overlapping size spectra and to the non-specificity of antibiotics and inhibitors. Although certain critics have focused their attention on the inability of our methods to measure strictly bacterial processes, we feel that the non-selectivity of nucleotide fingerprinting is one of the most unique and important aspects of this approach. Obviously, investigators must look to different methods for information on how the total biomass carbon and metabolic activity are partitioned among the individual microbial taxa in nature. In this regard, each methodological approach should be viewed as a separate "experiment" that yields novel information on the ecosystem as a whole. The most obvious conclusion which can be drawn from the extensive literature on marine microbiological methods is that there is no single approach which is universally acceptable. A second conclusion is that despite the numerous methods related publications on microbial growth in the sea, it is nearly impossible to calibrate any single measurement *in situ* with any degree of accuracy due to uncertainties regarding the validity of the assumptions which accompany each approach. Intercalibrations are equally difficult since each method measures a slightly different aspect of metabolism, growth or cell division. Hence there is no *a priori* reason for different methods to yield comparable ecological information. The need for conducting multiple "experiments" to define the growth state of natural microbial assemblages cannot be overemphasized.

One of the most useful indices in our environmental nucleotide fingerprinting approach is the measurement of nucleic acid, particularly DNA, synthesis. In general, two distinct approaches have been used to measure microbial DNA synthesis in aquatic environments. One approach utilizes [methyl-^{3}H] thymidine incorporation as a unique measure of bacterial DNA synthesis (Brock 1967; Tobin and Anthony 1978; Fuhrman and Azam 1980, 1982; Moriarty and Pollard 1981, 1982; see also Moriarty this volume). The assumptions implicit in this approach have been discussed in the above references, and by Karl (1982). The second approach uses [2-^{3}H] adenine as a precursor to nucleic acid synthesis (Karl 1979, 1981; Karl et al. 1981a) and differs significantly from the ^{3}H-thymidine method both in theory and practice. The most noteworthy differences are: (1) ^{3}H-adenine is used to simultaneously measure rates of both RNA and DNA synthesis, ^{3}H-thymidine measures only DNA synthesis, (2) ^{3}H-adenine

is assimilated by both bacteria and eucaryotic microalgae so that experiments with natural microbial assemblages yield nucleic acid synthesis rates for the entire microbial community, and (3) a sensitive assay for the immediate precursor to adenine incorporation into nucleic acid exists so that its specific activity can be measured directly.

The present paper is intended to summarize the recent work from our laboratory on adenine metabolism and rates of nucleic acid synthesis and demonstrate how these methods might be used to address specific hypotheses in microbiological oceanography.

DISCUSSION

Metabolic Considerations and Assumptions

Background. RNA and DNA synthesis in microorganisms is required for growth and cell division and the rates of synthesis are closely coupled to these processes. The cell content of RNA in bacteria and microalgae (expressed as mg RNA per mg dry weight) is positively correlated with growth rate and is regulated with great precision (see reviews by Maaloe and Kjeldgaard 1966; Kjeldgaard 1967; Nierlich 1978). By comparison, DNA concentrations (expressed as mg DNA per mg dry weight) are relatively insensitive to changes in growth rate, chemical composition of the growth medium or to the nature of the growth limiting nutrient. Correlations between rates of RNA and DNA synthesis and cell growth appear to be universally applicable to microorganisms and lend themselves well to the study of the growth of mixed populations encountered in marine ecosystems.

Most microorganisms possess the ability to utilize exogenous supplies of adenine, and certain other nucleic acid bases, as a supplement to de novo synthesis. The mechanisms and implications of these so-called "salvage pathways" have been reviewed elsewhere (Hochstadt 1974). When adenine is transported into a cell, it is rapidly incorporated into a number of derivatives including ATP, dATP (deoxy-adenosine triphosphate), ADP and AMP, and eventually into the metabolically stable nucleic acids. It has been demonstrated that adenine uptake is directly coupled to nucleic acid synthesis such that the rate of entry of adenine into the cell (k_1, Fig. 1) does not exceed the biosynthetic requirements for ATP and dATP (sum of k_2 plus k_3). However, the actual quantity of adenine taken up by microbial cells is much less than the value k_2 plus k_3 due to variable but substantial contributions of adenine from de novo synthesis and internal recycling of nucleic acid bases. This is true not only for adenine but for all nucleic acid precursors.

When radioactive adenine is added to a culture or seawater sample, it is diluted to an unknown extent by existing pools of

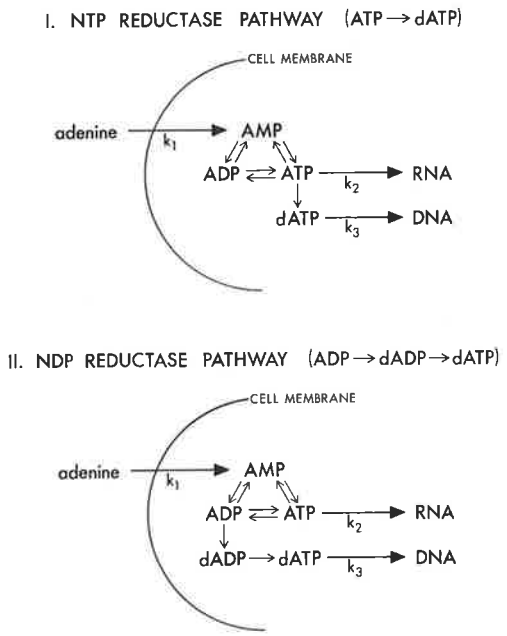


Figure 1. Known pathways for the flow of exogenous adenine into RNA and DNA of microorganisms (From Karl 1981).

adenine and adenine-containing compounds (e.g., ATP, ADP, AMP, adenosine). This process decreases the specific radioactivity of the introduced radiotracer prior to transport into the cells. Since the salvage pathways generally cannot supply the total amount of adenine required by the cells, this process must be supplemented by de novo synthesis of adenine. This further decreases the specific activity of the introduced radiotracer after transport into the cells but before incorporation into nucleic acids. Consequently, before an absolute rate of RNA or DNA synthesis can be obtained, it is necessary to determine the specific radioactivity of the immediate nucleotide triphosphate precursor, as well as the total amount of radioactivity incorporated into the individual nucleic acid pools. Without knowledge of both precursor specific activity (ATP in the case of ^{3}H-adenine assimilation) and the extent of end product labeling, rates of nucleic acid synthesis in seawater samples cannot be measured. This concern, of course, is not unique to ^{3}H-adenine experiments but is true for the use of all stable or radioisotopic tracers.

The two alternative schemes presented in Figure 1 indicate the known pathways by which adenine nucleotides are reduced to the corresponding deoxyadenosine derivatives prior to incorporation into DNA (Thelander and Reichard 1979). It has been demonstrated

experimentally that ATP and ADP achieve isotopic equilibrium (i.e., internal specific activity of ATP pool = internal specific activity of ADP pool) over time periods of less than 1 min (Karl 1981). Consequently, we assume that the specific radioactivity of the cellular dATP is at all times equal to that which we measure for ATP, regardless of whether the reduction occurs at the diphosphate or triphosphate level (see Fig. 1). This assumption is consistent with all existing theoretical models and experimental data.

The detailed stepwise procedures and rationale for measuring rates of microbial RNA and DNA synthesis in environmental samples have been presented elsewhere (Karl 1979, 1981, 1982; Karl et al. 1981a, b). Briefly, a seawater sample is incubated with ^{3}H-adenine for a predetermined time period. For reasons which will be discussed later, a time course is preferred to single end-point measurements. Following the incubation, a subsample is extracted for isolation of nucleic acids. By thin layer chromatography, the ATP is purified and radioassayed. The mean ATP pool specific radioactivity is then calculated from the chemical and radiochemical ATP data. RNA and DNA are also separated and purified, and their total radioactivities measured. From the incorporation and precursor specific radioactivity data, the rates of RNA and DNA synthesis can be calculated and expressed as mass of adenine (or deoxyadenine) incorporated into RNA (or DNA) per unit volume per unit time. Biomass-specific rates of RNA and DNA synthesis can also be derived by dividing the total measured rates by biomass (ATP). Alternatively, relative RNA-to-DNA synthesis rate ratios can be determined directly from the incorporation data, thereby eliminating the necessity for ATP pool specific radioactivity measurements. If time course data are available, an independent calculation of the rates of RNA and DNA synthesis can be performed using the total radioactivity accumulated in RNA and DNA and the integral of the ATP specific activity over the length of the time course experiment (Yamazaki and Leung 1981; Winn and Karl 1983).

Assumption. Several testable assumptions are implicit in the application of this method to the analysis of natural microbial assemblages, the most critical of which are:

1. All (or in practice, most) microorganisms in the ocean assimilate exogenous adenine by a common pathway and exhibit a similar "salvage" response under *in situ* conditions.
2. Addition of radioactive adenine does not affect the ATP cell quota, ATP turnover rate, or the rate of microbial RNA and DNA synthesis.
3. There is no intracellular compartmentalization of microbial ATP pools, or if there is, such compartmentalization does not affect the accuracy of the measured rates.

Experimental Verification of Assumption

The ability of *E. coli* to utilize exogenous adenine supplies in

preference to de novo synthesis has been extensively investigated (Hochstadt 1974). In 49 out of 50 strains of marine and freshwater bacteria examined, nanomolar concentrations of exogenous adenine were utilized by a similar mechanism (Karl et al. 1981a). This is not altogether surprising since the biosynthesis of adenine is highly endergonic. The existence of these salvage pathways enables microorganisms to conserve energy that would otherwise be required for purine biosynthesis (Karl 1979).

More recently, we have investigated the occurrence and extent of adenine salvage in marine alage. Twenty-two unialgal, but not axenic, cultures of marine phytoplankton representing most major taxa (see Table 1) were grown to early logarithmic phase under constant light on f/2 medium (Eppley et al. 1967), at which time they were incubated with 1 μCi ^{3}H-adenine·ml^{-1} (specific radioactivity = 15 Ci·mmol^{-1}) for a 6 h period. ATP, ^{3}H-ATP, ^{3}H-RNA and ^{3}H-DNA were measured as described previously (Karl et al. 1981a). Since the cultures were not axenic and since bacteria are known to assimilate ^{3}H-adenine, we endeavored to set an upper limit on the contribution of bacteria to the total uptake and assimilate of ^{3}H-adenine. A subsample of each culture was fixed in glutaraldehyde, stained with acridine orange, and the bacteria enumerated with UV fluorescence microscopy (Hobbie et al. 1977). The majority of the cells were large rods (1.0 - 1.5 μm x 0.5 μm). Biovolume was calculated by assuming that all cells conformed to the largest dimensions (1.5 x 0.5 μm) which biased the estimate in favor of bacterial biovolume. This value was converted to biomass (Ferguson and Rublee 1976) and finally to ATP (Karl 1980). This upper bound on the contribution of bacterial ATP was compared to the total ATP concentration measured for each culture. Bacterial ATP ranged from 0.14 - 27% of the total ATP and only those cultures where bacterial ATP was 1% of the total are reported in this study (14 out of 22 cultures). As an independent confirmation of algal uptake, subsamples of each culture were also prepared for microautoradiography (Meyer-Reil 1978). Labeled phytoplankton were observed in all 22 cultures. Table 1 summarizes the results of the uptake experiments. The ATP pool's specific radioactivity data, by themselves, provide convincing indirect evidence for the uptake of ^{3}H-adenine. If the metabolism of ^{3}H-adenine was restricted to contaminating bacteria (< 1% of total ATP in the culture) it would have been impossible to attain the specific activities presented, since bacterial specific activities rarely exceed values of 1.0 nCi·pmol^{-1} (Karl et al. 1981a; see also Fig. 5 for waters > 150 m), even under saturating conditions of ^{3}H-adenine with a specific activity of 15 nCi·pmol^{-1} (Karl et al. 1981a). Finally, the uptake and incorporation of ^{3}H-adenine into ATP and nucleic acids in environmental samples proceeds without a detectable lag period indicating that microbial communities in nature are probably utilizing these salvage pathways under in situ conditions.

Table 1. Evidence for ^{3}H-adenine uptake and assimilation by marine phytoplankton.

Culture[1]	^{3}H-RNA Produced (nCi/ml)	^{3}H-DNA Produced (nCi/ml)	DNA:RNA	ATP Pool Specific Radioactivity (nCi/pmol)
CHRYSOPHYTA				
Bacillariophyceae				
Chaetoceros pelagicus	79.2	13.7	0.17	0.70
Cyclotella nana	149.5	27.0	0.18	0.68
Lauderia borealis	163.4	31.3	0.19	0.73
Nitzchia sp.	75.7	17.9	0.24	1.79
Rhizosolenia robusta	43.6	7.1	0.16	2.14
Chrysophyceae				
Cricosphaera carterae	245.7	24.4	0.10	0.61
Emiliania huxleyi	218.1	42.2	0.19	1.50
Monochrysis lutheri	247.9	26.9	0.11	0.61
Phaeocystis sp.	35.9	6.0	0.17	1.13
PYRROPHYTA				
Desmophyceae				
Exuviella sp.	60.9	7.4	0.12	1.28
Dinophyceae				
Gymnodinium simplex	35.0	6.1	0.17	0.81
Gymnodinium sp.	39.3	6.5	0.17	1.94
Peridinium foliaceus	23.1	2.5	0.11	0.25
UNIDENTIFIED				
Microcell MC-1	17.3	9.7	0.56	0.66

[1] Phytoplankton cultures courtesy of J. Jordan, Food Chain Research Group, Scripps Institution of Oceanography.

The second assumption is that the addition of ^{3}H-adenine to environmental samples neither alters the steady-state concentration and turnover rate of cellular ATP, nor affects the rate of nucleic acid synthesis. We have evaluated this assumption by: (a) measuring the total ATP pool levels in natural microbial assemblages with time following the addition of ^{3}H-adenine (Karl et al. 1981a), (b) measuring the rate at which ^{3}H-ATP is "chased" out of the cellular ATP pools upon the addition of saturating concentrations of non-radioactive adenine to a microbial community previously labeled with tracer levels of ^{3}H-adenine, (Karl et al. 1981a), and (c) measuring the effects of ^{3}H-adenine isotope dilution on measured rates of RNA

and DNA synthesis (Karl 1982). From these experimental data we conclude that there is no luxury uptake of adenine, even at saturating concentrations, and no apparent expansion of ATP cell quotas or stimulation of RNA or DNA synthesis upon the addition of ^{3}H-adenine to the sample. These results support our previous model indicating that the uptake of adenine by the cells (k_1, Fig. 1) does not exceed the net removal of ATP and dATP required for macromolecular synthesis.

The final assumption concerns intracellular compartmentalization of the adenine nucleotide pool which might be expected to arise through either barrier (membrane) or kinetic processes (see discussion in Karl 1979). We have now performed several hundred time course experiments on the rates of RNA and DNA syntheses in cultures and environmental samples and have observed what we interpret to be kinetic compartmentalization of the ATP precursor pool during the initial time periods of labeling ($<$30 s for cultures and $<$ 1 h for most environmental samples; see Karl et al. 1981a, b; Karl 1981). For this reason we have stressed the utility of performing time course experiments. The effects of compartmentalization decrease with increasing incubation time suggesting that it might arise, in part, from diffusion-controlled processes. This is supported by the fact that we have observed effects of near equal magnitude in cultures of bacteria and eucaryotic algae. If the effect were due to the existence of organelles (barrier compartmentalization processes), one might argue that it would not be observed in bacteria. Intracellular compartmentalization and time-dependent changes in precursor specific radioactivity are problems inherent to all tracer experiments and certainly are not unique to the use of ^{3}H-adenine. This technique simply provides a convenient means of assessing the problem.

For ecological applications, the assumption regarding intracellular compartmentalization is inevitably further complicated by the heterogeneity of natural populations, all containing ATP pools. As currently employed, our method assumes that all of the individual cellular ATP pools are in isotopic equilibrium. This assumption is also implicit, although not often stated, in all radiotracer experiments with mixed populations. It is very difficult to evaluate this assumption directly but it is encouraging to note that laboratory cultures of algae yield ATP pool specific activities which are comparable to those measured in bacteria grown under identical laboratory conditions. This implies that the salvage pathways in both bacteria and unicellular algae supply similar percentages of the required cell quota of adenine. In fact, this appears to be true under field conditions as well, since there is no substantial difference in the magnitude of the ATP pool specific activities between different size fractions of seawater samples analyzed to date (Karl et al. 1981b) which might be expected if only a portion (e.g., bacteria) of the community was actively assimilating

^{3}H-adenine. We have performed "nutrient addition" perturbation experiments to selectively stimulate the rates of nucleic acid synthesis of photoautotrophs (by the addition of inorganic nutrients) or chemoheterotrophs (by the addition of organic nutrients), and have not been able to detect any substantial changes in the mean specific activity of the total microbial ATP following 12 h incubations (relative to control samples), despite biomass increases of up to 60% (Table 2). Finally, we have recently performed a series of $^{32}PO_4$ and ^{3}H-adenine double label experiments to investigate further the assumption regarding uniform isotope equilibrium among the various microbial taxa. Figure 2 summarizes data resulting from these double label experiments, which demonstrate: (1) a significant ($p > 0.05$) and positive correlation between the ^{32}P rate ratios and those derived from ^{3}H-adenine, (2) a numerical correspondence between the two data sets (i.e., slope of the regression line is not significantly different from 1.0). If we tacitly assume that all micro-

Table 2. Effects of nutrient additions on the measured ATP concentrations and ATP pool specific radioactivities following incubation with ^{3}H-adenine.

Treatment[1]	ATP Concentration[2] (ng/l)	ATP Pool Specific Radioactivity[3] (nCi/pmol)
Control	44.5 50.5	2.14 ± 0.19
+NH_4	40.3 42.3	1.85 ± 0.51
+PO_4	30.3 40.3	1.42 ± 0.71
+Glucose	73.2 63.8	1.84 ± 0.11

1 Nutrients were added to yield final concentrations of 5 μM.
2 ATP concentration at end of 12 h incubation period. ATP concentration at start of the experiment was 43.6 ± 0.16 ng/l.
3 Measured at end of 12 h incubation period. Values represent mean ± 1 standard deviation (n = 4).

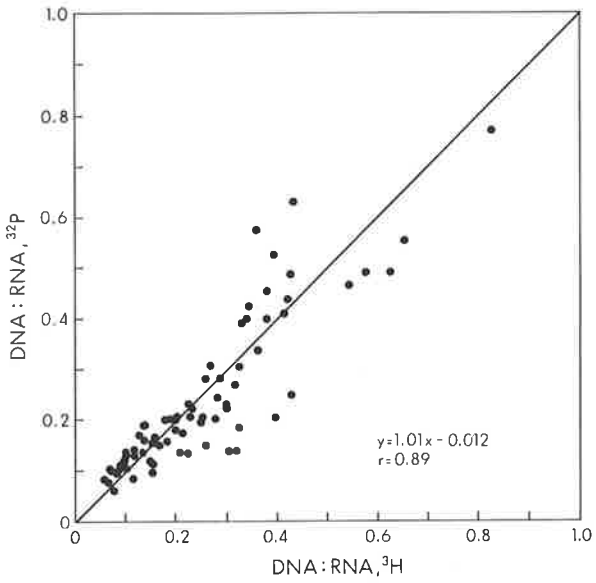


Figure 2. DNA:RNA incorporation rate ratios determined using $^{32}PO_4$ and ^{3}H-adenine in double-label tracer experiments. All data are from 4 h incubations with surface seawaters (0-90 m) collected at the VERTEX I station (36°48'N, 121°46'W) (From Orrett and Karl, in prep.).

organisms are capable of assimilating $^{32}PO_4$, then we must conclude that all, or at least an identical subset of microorganisms is also capable of assimilating ^{3}H-adenine. Otherwise it would be extremely difficult to explain the correspondence between the two data sets. This excellent agreement between the two independent methods also supports our assumption that adenine and deoxyadenine comprise 25 mol percent of the nucleotide bases in RNA and DNA, respectively, if we acknowledge that the α-P specific activity of all nucleotide bases is equivalent. All of the above observations and data support the assumption that marine microorganisms assimilate adenine by a common and uniform pathway under in situ conditions.

Laboratory Calibration of the Method

We have recently completed a series of laboratory experiments to evaluate the accuracy of the ^{3}H-adenine method for estimating rates of RNA and DNA synthesis in algal and bacterial cultures. The experiments were designed to compare the nucleic acid synthesis rates using ^{3}H-adenine with the actual rates determined from direct measurements of RNA and DNA concentrations and culture doubling times. A complete discussion of the experiments, results, and interpretations will be presented elsewhere (Winn and Karl,

submitted for publication); however, a selected summary of the results is presented in Figures 3 and 4. The most important result is the fact that our radiochemical technique provides an accurate estimate of the actual rate of RNA and DNA synthesis in both bacterial and algal cultures. The overestimate during the initial period of labeling observed with the algal cells (Fig. 4) is consistent with our previous data on intracellular compartmentalization (Karl et al. 1981a) and with our current model of the assimilation and metabolism of adenine by marine microorganisms. However, it should be emphasized that accurate rates of both RNA and DNA synthesis are achieved after incubation periods as short as 10-20% of the doubling time.

Selected Ecological Applications

Several of our publications present and discuss the rates of seawater RNA and DNA synthesis (Karl 1979, 1981, 1982; Karl et al. 1981a, b; Fellows et al. 1981). Table 3 presents a summary of ATP biomass, rates of RNA and DNA syntheses and biomass-specific rates of nucleic acid production for several additional marine environ-

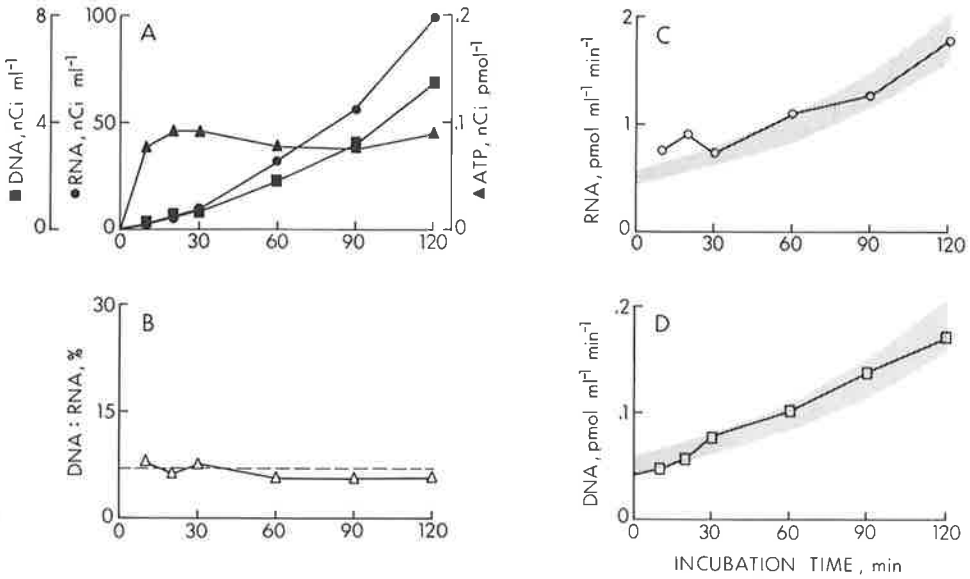


Figure 3. ^{3}H-adenine calibration experiment performed with the marine bacterium Serratia marinorubra. The dashed line in frame B indicates the mean DNA: RNA rate ratio as determined by independent chemical determinations and the stippled areas in frames C and D indicate the known rates of RNA and DNA synthesis (mean $\pm$ 1 standard deviation). Initial ATP concentration of culture was 600 ng ATP·l^{-1} and the doubling time was 60 min (From Winn and Karl, submitted).

Table 3. Summary of representative euphotic zone measurements of microbial biomass and rates of RNA and DNA synthesis.

Sample Location	ATP-Biomass (ng/l)	TOTAL RATE RNA Synthesis (pmol A/1/h)	TOTAL RATE DNA Synthesis (pmol dA/1/H)	DNA:RNA (x 100%)	TOTAL RATE RNA Synthesis (pmol A/ng ATP/h)	TOTAL RATE DNA Synthesis (pmol dA/ng ATP/h)
Kaneohe Bay, Hawaii (21°26'N, 157°47'W)						
1 m	418	1,440	144	10.0	3.44	0.34
Ke-ahole Point, Hawaii (19°55'N, 156°10'W)						
1 m	53.1	150	*	--	2.90	--
North Pacific Ocean near Point Sur, California (36°07'N, 122°47'W)						
1 m	205	822	--	--	4.00	--
Equatorial Pacific Ocean near Galapagos Islands (0°48'N, 86°09'W)						
1 m	63	240	--	--	3.81	--
Central Pacific Ocean near Hawaii (18°43'N, 156°50'W)						
1 m	27.2	35	12.8	36.6	1.29	0.47
50 m	32.3	28	11.3	40.4	0.87	0.35
130 m	19	61	17.5	28.7	3.21	0.92
North Pacific Ocean, VERTEX I Station (36°48'N, 121°46'W)						
1 m	175	190	42	22.1	1.09	0.24
20 m	210	305	69	22.6	1.45	0.33
50 m	228	270	60	22.2	1.18	0.26

*DNA synthesis data not available.

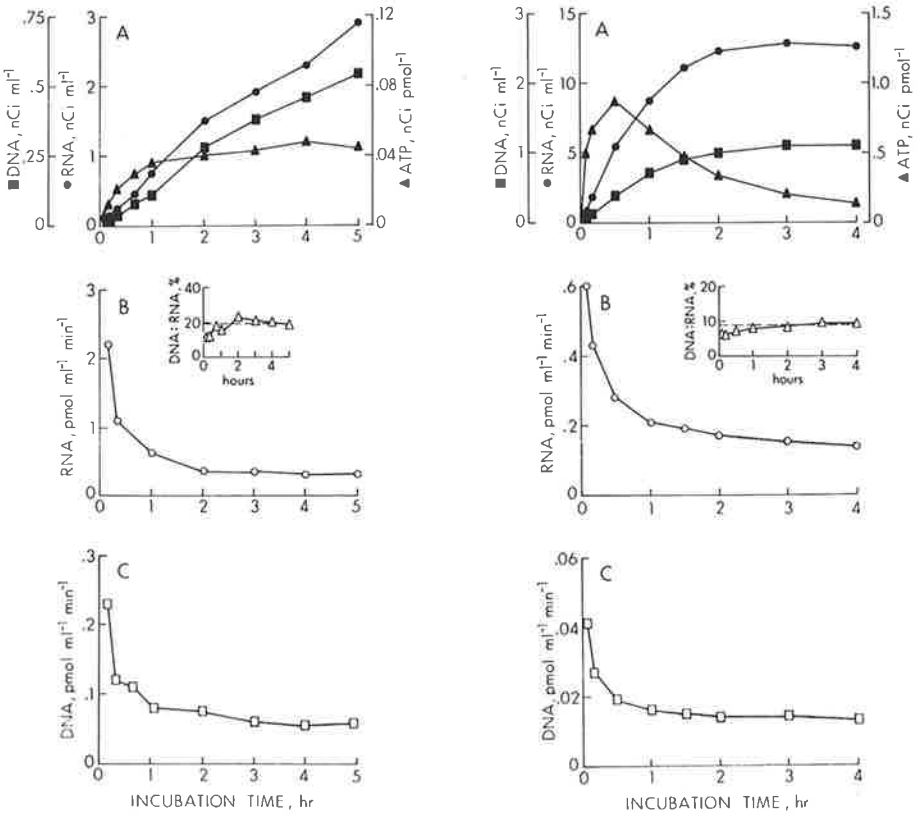


Figure 4. ^{3}H-adenine calibration experiments performed with Chorella cordata (left) and Cylindrotheca sp. (right). Details as per Figure 3. ATP concentrations and culture doubling times were: 10 ng·ml^{-1}, 20.5 h; and 4 ng·ml^{-1}, 17 h, for C. cordata and Cylindrotheca respectively (From Winn and Karl, submitted).

ments. It is interesting to note that despite substantial variations in microbial biomass (ATP) and total rates of nucleic acid synthesis (variations greater than 10 to 50-fold), the biomass-specific rates of RNA and DNA synthesis display only minor variation among the different ecosystems.

Figure 5 presents a detailed depth profile of ATP and rates of RNA and DNA synthesis for water samples collected during the Vertical Transport and Exchange ("VERTEX" I) experiment off Point Sur, California. As might be expected, the total rates of RNA and DNA synthesis decrease with increasing water depth and closely follow the depth distribution of ATP. However, the biomass-specific

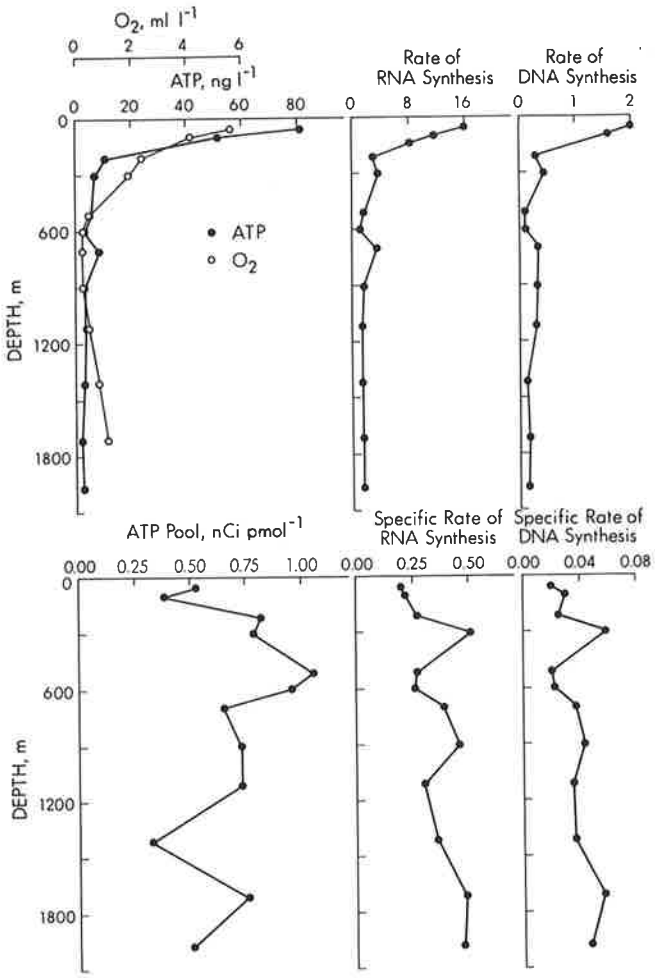


Figure 5. ATP, O_2, rates of RNA and DNA synthesis, specific activity of the ATP pool, and specific rates of RNA and DNA synthesis for the water column at the VERTEX I station (36°48'N, 121°46'W). All water samples were incubated in situ for 10 h. Rates of RNA and DNA synthesis are expressed as pmol A (or dA) incorporated into RNA (or DNA)·l^{-1}·h^{-1} and specific rates are expressed as pmol A (or dA) incorporated·ng ATP^{-1}·h^{-1} (From Karl and Knauer, in press).

rates of RNA and DNA synthesis are nearly uniform throughout the entire water column, suggesting that the microbial community growth rate is uniform with depth from 0-2000 m. This was contrary to our expectations of the vertical distribution of microbial growth in the ocean; however, we have recently confirmed these observations with additional data from the tropical North Pacific Ocean (Winn and Karl

1983) and eastern tropical Pacific Ocean (Karl and Knauer, in preparation). We believe this relationship could well be a common feature of the mesopelagic zone of all oceanic environments.

Extrapolation to Growth Rate and Carbon Production Estimates

The majority of investigations concerned with the production, flux, and turnover of organic matter in natural ecosystems have relied upon carbon as the basic unit of cell mass and growth. If the assumptions inherent in the application of nucleic acid precursors to ecological studies were determined to be valid under in situ conditions the ^{3}H-adenine method as described would yield reliable estimates of the total rates of microbial RNA and DNA synthesis under natural environmental conditions. While we would argue that nucleic acid synthesis rate data, by themselves, provide useful information regarding the growth of microorganisms in nature, it is often necessary to extrapolate the DNA synthesis data to estimates of total microbial production (μg C produced per unit volume per unit time) for comparison with other experimental data. Of course, the precision and accuracy of the estimates are adversely affected by the common practice of extrapolation, since the "conversion factors" are, for the most part, highly variable. Our calculations to date have assumed a mean DNA:C ratio of 50 which is based upon laboratory experiments (Holm-Hansen 1969; Mandelstam and McQuillen 1976) and direct field measurements of total DNA and organic carbon. The latter approach is acceptable only where the amount of non-living particulate DNA and carbon are expected to be negligible, which may severely limit the use of an empirically determined extrapolation factor. In a recent intercomparison of methods conducted in Hawaiian coastal waters (PRPOOS experiment; Eppley et al., in prep.) the DNA extrapolated microbial carbon production estimates were, on the average, 160% of the values obtained by the standard ^{14}C primary production technique. This is consistent with the presumed importance of microheterotrophic production (i.e., POC produced from detrital DOC and/or POC) since the DNA synthesis technique measures both autotrophic and heterotrophic processes. Finally, measurements of RNA and DNA synthesis, when used in conjunction with steady-state RNA and DNA concentration data, may be used to estimate the mean community turnover time and specific growth rate (μ). This must be considered a minimum value for the growth rate since the presence of detrital (i.e., non-living) RNA or DNA would tend to overestimate the value for microbial standing stock and underestimate the growth rate.

Table 4 presents a summary of ATP concentrations, rates of DNA synthesis and carbon production (extrapolated from rates of DNA synthesis) and specific growth rates (measured by two independent procedures). It is evident that the specific growth rates calculated from the DNA-biomass data are substantially lower than those based on the ATP-biomass data (assuming a DNA:ATP ratio of 5).

Table 4. Carbon production and community specific growth rate estimates extrapolated from measurements of DNA synthesis at VERTEX II station (154°30'N, 107°30'W).

Depth (m)	ATP Concentration (ng/l)	ATP Pool Specific Radioactivity (nCi/pmol)	Rate of DNA Synthesis (pmol/l/hr)	Total Microbial Production[1] (µg C/l/hr)	Specific Growth Rate[2] (day^{-1})	DNA Concentration (µg/l)	Specific Growth Rate[3] (day^{-1})
5	57	0.76	12.7	0.84	0.98	3.0	0.09
10	59	1.32	15.0	0.99	1.16	2.5	0.13
15	68	1.66	29.7	1.97	1.92	4.0	0.16
20	60	1.28	24.1	1.60	1.77	2.6	0.21
25	69	2.34	53.0	3.51	3.38	2.1	0.55
35	84	2.51	48.6	3.22	2.54	2.1	0.51
45	71	1.40	14.2	0.94	0.88	4.6	0.07
55	71	1.27	24.5	1.62	1.51	2.7	0.20
65	63	0.70	14.3	0.95	1.00	1.9	0.16

[1] Carbon production extrapolated from DNA synthesis measurements by converting pmol DNA to pg DNA and by assuming that DNA is 2% of the cell carbon.

[2] Calculated from DNA synthesis and ATP-biomass data as, μ = (doublings day^{-1}) x 0.693, assuming a DNA:ATP ratio of 5.

[3] Calculated from DNA synthesis and DNA-biomass data as, μ = (doublings day^{-1}) x 0.693.

We interpret this to be due to the presence of "detrital" (i.e., non-living) DNA (Holm-Hansen et al. 1968; Sutcliffe et al. 1970) in the marine environment.

The development of new methods to distinguish between "living" and "non-living" nucleic acids in environmental samples would undoubtedly be useful in this regard and might also provide more accurate direct estimates of the mean C:DNA ratios of living microorganisms. Finally, it should be emphasized that extrapolation of nucleic acid synthesis data to estimates of carbon production assumes that marine microorganisms in nature exhibit "balanced growth", with all cellular components increasing at the same rate. As Eppley (1981) points out, the concept of balanced growth is fundamental (but generally not explicitly stated) to the use of nutrient assimilation rates as measures of rate constants for growth. Karl (1981) has already demonstrated that cell growth (as measured by RNA synthesis) and cell division (as measured by DNA synthesis) may be uncoupled in mixed assemblages of asynchronously growing marine microorganisms over incubation periods as short as 1 h.

CONCLUSION

This paper has attempted to summarize the theoretical principles involved in the application of ^{3}H-adenine as a measure of microbial RNA and DNA synthesis in the marine environment. We have not discussed methods employing other nucleic acid precursors, such as ^{3}H-thymidine or ^{3}H-uridine. In our view nucleic acid synthesis measurements employing ^{3}H-adenine and ^{3}H-thymidine are not mutually exclusive methods in microbiological oceanography, since each approach yields different information regarding microbial growth in the oceans. Unless evidence is presented to suggest otherwise, they should be considered complimentary techniques in experimental microbiological oceanography. Finally, the potential significance of ecological information to be derived from estimates of *in situ* rates of RNA and DNA synthesis more than justifies the level of research effort that is required to ensure a proper interpretation and accurate extrapolation to estimates of microbial growth and production.

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