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Laboratory Calibrations of the [^{3}H]Adenine Technique for Measuring Rates of RNA and DNA Synthesis in Marine Microorganisms

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The nucleic acid synthesis rates of several marine phytoplankton and bacteria grown in chemostat and batch cultures were measured by using [^{3}H]adenine. The [^{3}H]adenine synthesis rates showed excellent agreement with the known rates of synthesis estimated from chemical RNA and DNA data. Under certain conditions, RNA turnover and ATP pool compartmentalization produce inaccuracies in synthesis measurements made with [^{3}H]adenine. However, accurate measurements of the rates of microbial RNA and DNA synthesis can be made in any environmental situation provided a few simple precautions are observed. First, time course experiments are recommended. Second, experiments should be conducted for periods long enough to avoid problems arising from disequilibria of internal ATP pools. Finally, exogenous [^{3}H]adenine should remain in the medium over the length of the time course.

In microorganisms, nucleic acids are vital cellular constituents with well-known metabolic functions (10). Consequently, measurements of total microbial RNA and DNA production should provide useful information regarding microbial growth and metabolism in nature. In general, attempts to measure nucleic acid synthesis in aquatic environments have employed labeled nucleic acid precursors. To estimate absolute rates, radiotracer experiments require two independent measurements: (i) the rate of accumulation of radioactivity in either RNA or DNA, or both (i.e., nanocuries milliliter^{-1} hour^{-1}) and (ii) the specific activity (i.e., nanocuvies picomole^{-1}) of the direct precursor. Only with this information, can the actual rate of nucleic acid synthesis (i.e., nanocuries milliliter^{-1} hour^{-1}/nanocuries picomole^{-1} = picomoles milliliter^{-1} hour^{-1}) be derived.

In recent years, a number of studies of nucleic acid synthesis in aquatic environments have been conducted. For the most part, two separate isotopes have been used in these investigations. The incorporation of [*methyl*-^{3}H]thymidine has been used as a measure of bacterial growth (1, 8, 9, 22, 23, 27). In some investigations (8), it was assumed that only bacteria incorporate [^{3}H]thymidine, that the radiolabel is incorporated exclusively into DNA, and that the specific activity of the thymidine incorporated into DNA is equal to that of the added precursor. More recent studies, however, have demonstrated the nonspecificity of macromolecular labeling with [^{3}H]thymidine (14, 25). In other investigations (22, 23), isotope dilution has been used to evaluate the dilution of the specific activity of the added [^{3}H]thymidine by exogenous and endogenous pools of thymidine-like molecules and de novo synthesis. This approach has shown that dilution of the added [^{3}H]thymidine occurs in certain environments. These observations obviously cast doubt upon the validity of the assumption that the specific activity of the direct precursor to thymidine incorporation into DNA is equal to the specific activity of the added [^{3}H]thymidine. Unfortunately, isotope dilution theory (5, 7) is not valid under natural conditions (19, 29). However, the estimates of of the dilution of added [^{3}H]thymidine by unlabeled thymidine derivatives, using isotope dilution theory, can be interpreted as a maximum (i.e., yielding a minimum specific activity estimate) and are the best estimates currently available. Fuhrman and Azam (9) have recently examined the assumption that the specific activity of thymidine incorporated into DNA by bacterioplankton is equal to the specific activity of the added thymidine. They conclude that the specific activity of the TTP incorporated into DNA is less than that of the added precursor and suggest that the rate of DNA synthesis measured with thymidine in some previous experiments be increased by a factor of ca. 3 to 7. The above results have clearly complicated the interpretation of experiments utilizing [^{3}H]thymidine in aquatic environments. However, Kirchman et al. (18) have employed a novel approach to the use of [^{3}H]thymidine incorporation as a measure of bacterial growth and production which avoids the problem of the specific activity of the direct precursor and appears to have promise.

The second radiotracer frequently used to quantify nucleic acid synthesis rates in aquatic environments is [2-^{3}H]adenine (11, 13, 15, 16; D. M. Karl and C. D. Winn, *in* J. E. Hobbie and P. J. LeB. Williams, ed., *Heterotrophic Activity in the Sea*, in press; C. D. Winn and D. M. Karl, Biol. Oceanogr., in press). This approach differs significantly from those utilizing [*methyl*-^{3}H]thymidine. First of all, adenine is a precursor to both RNA and DNA, so that rates of synthesis of both macromolecules can be measured simultaneously. Second, adenine is assimilated by both bacteria and eucaryotic microalgae, so that experiments with natural microbial populations yield nucleic acid synthesis rates for the entire microbial community (15, 23; Karl and Winn, in press). The fact that [^{3}H]adenine can be assimilated by the entire microbial community can be used to advantage in certain environmental applications. Third, and most importantly, methods exist for the isolation and measurement of the immediate intracellular precursor to adenine incorporation into nucleic acids, so that its specific activity can be measured directly (11).

A number of assumptions are inherent in experiments in which [^{3}H]adenine is used to measure rates of nucleic acid synthesis. These include: (i) all microorganisms are capable of assimilating adenine, (ii) the ATP and dATP pools are in isotopic equilibrium, and (iii) the precursor pools are not

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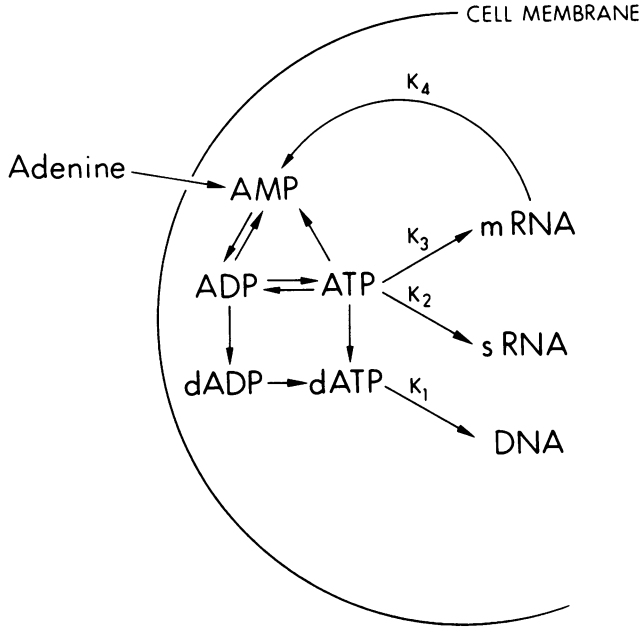


FIG. 1. Schematic diagram of adenine incorporation into nucleic acids.

compartmentalized. Previous investigations (13, 15; Karl and Winn, in press) have shown that, whereas the first two assumptions are not violated, the ATP precursor pool is compartmentalized during the initial period of labeling.

Several researchers have attempted to correlate bacterial growth rates measured by [^{3}H]thymidine or [^{3}H]adenine incorporation into natural microbial assemblages with estimates derived from other techniques (4, 18). However, attempts have not been made to calibrate the rates of microbial nucleic acid synthesis measured by using either of these radiotracer techniques with marine microorganisms maintained at known growth rates. This communication presents the results of a series of laboratory experiments designed to evaluate the accuracy of nucleic acid rate measurements made with [^{3}H]adenine. Special attention has been paid to the potential analytical problems of intracellular compartmentalization so that they can be avoided during environmental applications of this technique.

Theoretical considerations. The complex series of biochemical reactions involved in nucleic acid metabolism are similar in all microorganisms thus far investigated and are represented in a relatively simplistic schematic diagram (Fig. 1). Adenine is assimilated in microorganisms via a group transport process and is simultaneously converted into AMP. Intracellular AMP is then rapidly interconverted into several adenine derivatives, including ADP, ATP, dADP, and dATP. The intracellular turnover of these compounds is extremely rapid (3, 13), so that it can be assumed that their specific activities remain equal (further evidence for this will be presented below). The immediate precursors to adenine incorporation into RNA and DNA are ATP and dATP, respectively. ATP is reduced to dATP at either the triphosphate or the diphosphate level (Fig. 1).

DNA is a stable molecule and is not recycled within the cell. RNA is made up of three components: mRNA, tRNA, and rRNA. Whereas tRNA and rRNA, henceforth referred to collectively as stable RNA (sRNA), are stable molecules and generally do not turn over at a significant rate, mRNA is unstable and is rapidly recycled within the cell. DNA and all three forms of RNA are precipitated in cold acid so that the radiolabel found in RNA includes [^{3}H]adenine contained in both the stable and unstable forms. The relative concentrations and synthesis rates of mRNA and sRNA can vary widely depending upon the organism and its growth conditions. The concentration of mRNA is generally small relative to sRNA, but its synthesis rate can often be a significant proportion of total RNA synthesis. In bacteria, for example, mRNA typically accounts for 2 to 10% of the total RNA and 50% of the instantaneous synthesis rate (21).

The [^{3}H]adenine assimilation model (Fig. 1) assumes that adenine flows through a single, well-mixed precursor pool and then into nucleic acids. If this representation is accurate, the relationship between nucleic acid labeling and the specific activity of its direct precursor can be defined precisely as:

$$d^*NC/dt = K_s\,(^*A/A) - K_b\,(^*NC/NC) \quad (1)$$

where *NC and NC are the radiolabel and chemical concentration of nucleic acid, respectively, K_s is the rate of synthesis of nucleic acid, K_b is the rate of breakdown of nucleic acid due to cell death, and $^*A$ and A are the radiochemical and chemical concentrations of the direct precursor. During short-term labeling experiments (i.e., less than the doubling time of the population), the specific activity of the end products (i.e., *NC/NC) will be much less than the specific activity of the precursor (i.e., $^*A/A$) so that the second term in the equation can be ignored. Equation 1 can then be simplified to:

$$d^*NC/dt = K_s\,(^*A/A) \text{ or } ^*NC = K_s \int_0^t (^*A/A) \quad (2)$$

and can be rearranged to solve for K_s:

$$K_s = {}^*NC/\textstyle\int_0^t (^*A/A) \quad (3)$$

Ignoring for the moment the turnover of mRNA, equation 3 can be used to calculate the rates of either RNA or DNA synthesis.

Compartmentalization of ATP precursor pools has been identified in both eucaryotic and procaryotic microorganisms (17, 24, 26, 28). The use of the term compartmentalization is unfortunate in that it is somewhat of a misnomer. As used here, the term implies only that two or more intracellular pools of precursor (ATP or dATP), with unequal specific activities, are used to synthesize nucleic acids. The term is not intended to imply that intracellular compartments, separated by membrane systems, must exist. One might imagine that ATP pool compartmentalization cannot exist in procaryotes since these organisms do not possess internal compartments. However, ATP compartmentalization has been clearly demonstrated in procaryotes (24, 26). Although we cannot rule out the existence of separate internal ATP pools in the eucaryotic organisms used in this study, we view compartmentalization as being due to diffusive or kinetic processes in both eucaryotes and procaryotes.

MATERIALS AND METHODS

Culture conditions. A marine bacterium, *Serratia marinorubra*, and representatives from three separate classes of marine algae were used in this study. *S. marinorubra* was grown in shake culture at 24 ± 1°C in medium containing 1.0 g of glucose and 1.5 g of Bacto-Peptone (Difco Laboratories) liter^{-1} in 80% seawater supplemented with 500 μM NH_4Cl and 5 μM NaH_2PO_4. *Pavlova lutheri*, *Chlorella cordata*, and *Cylindrotheca* sp. were used as the eucaryotic test organisms. Algal chemostat cultures were maintained at

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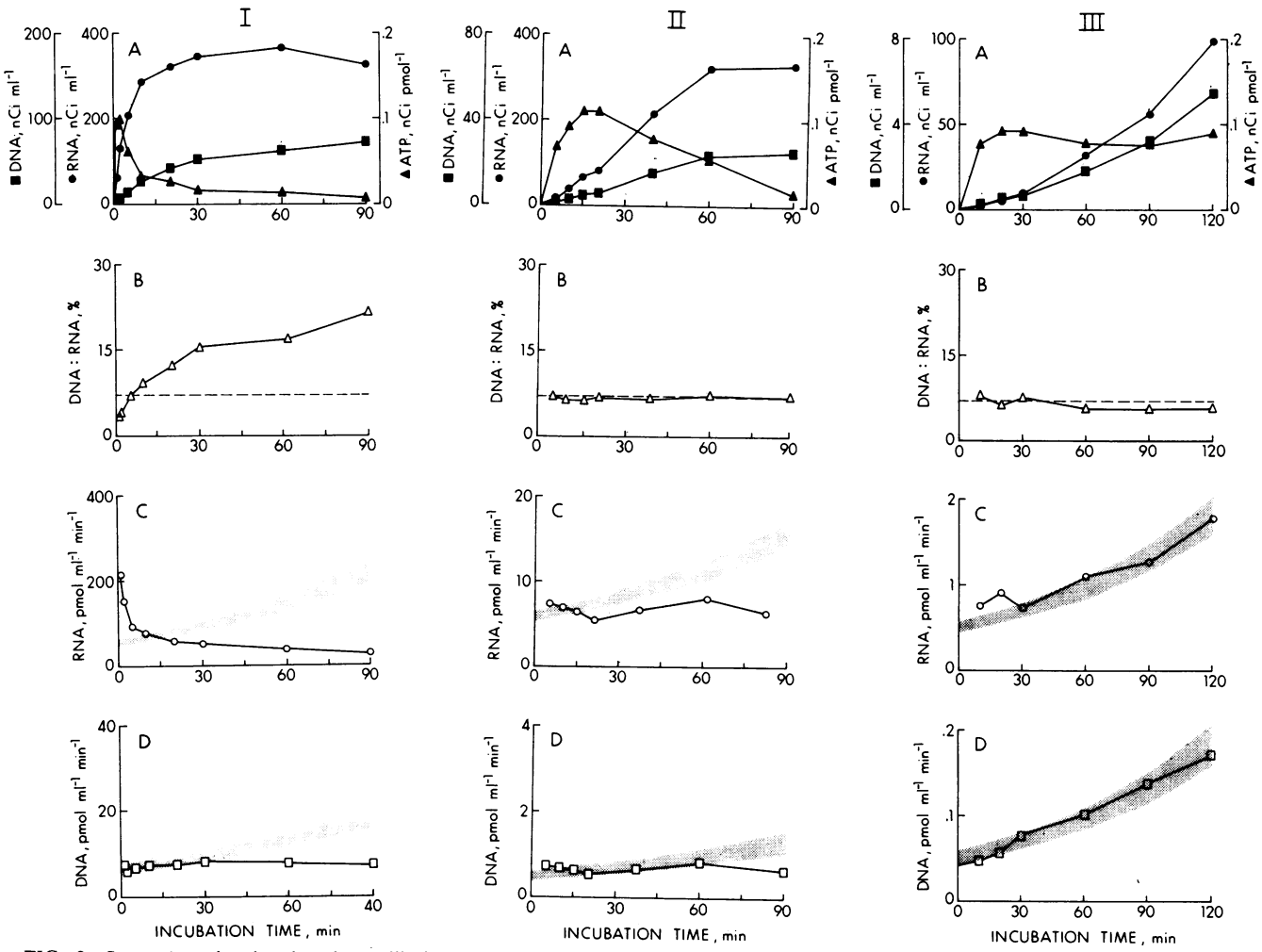


FIG. 2. *S. marinorubra* batch culture dilution experiment. Panels I, II, and III show the results of the uptake and incorporation of [^{3}H]adenine into cultures containing initial concentrations of 120, 6, and 0.6 ng of ATP ml^{-1}, respectively. The dashed lines in frames B indicate the mean DNA:RNA rate ratio as determined by independent chemical measurements, and the stippled areas in frames C and D indicate the known rates of RNA and DNA synthesis (±1 standard deviation unit). Culture doubling time was 1 h.

22°C under continuous light on modified FCRG medium (6). The phosphorous-limited medium contained 40 μM NH_4 and 2 μM PO_4. The nitrogen-limited medium contained 50 μM NO_3 and 5 μM PO_4. Algal batch cultures were grown on standard FCRG medium (500 μM NO_3, 50 μM PO_4) under continuous light at 24 ± 1°C. All batch culture experiments were conducted at mid-log phase so that growth rates were well defined.

Nucleic acid rate measurements. Rates of nucleic acid synthesis were measured with [2-^{3}H]adenine (15 to 20 Ci mmol^{-1}) by the techniques outlined previously (11, 14, 15). Nucleic acid synthesis rates were calculated from [^{3}H]adenine incorporation data by dividing the label in nucleic acids by the integral ATP precursor specific activity (i.e., equation 3). The known rates of nucleic acid synthesis were determined from the doubling time and the concentration of particulate RNA and DNA in the culture. RNA and DNA concentrations were measured in *S. marinorubra* by incorporation of $^{32}PO_4$ into RNA and DNA after labeling to isotopic equilibrium (at least six generations) in medium containing $^{32}PO_4$ of a known and constant specific activity. The RNA and DNA fractions were isolated for radiochemical counting as described previously (13). RNA and DNA concentrations in algal cultures were also determined by the methods described above, with orcinol (20) for RNA and diphenylamine (2) for DNA.

Dilution experiments. Dilution experiments were conducted with *S. marinorubra* and *P. lutheri* to examine the relationship between microbial biomass and the time course kinetics of [^{3}H]adenine incorporation. In these experiments, the organisms were grown to mid-log phase and then serially diluted with fresh medium. Care was taken to insure that the cells were in exponential growth when the dilutions were made so that the growth rate and therefore the rates of RNA and DNA synthesis were not altered by the addition of fresh medium.

Field experiment. A surface sample was collected at 17° 46′ N; 108° 57′ W, ca. 350 km off the west coast of Mexico. The sample was incubated on deck under in situ light and temperature conditions.

RESULTS AND DISCUSSION

Bacterial dilution experiment. The results of the time course of [^{3}H]adenine incorporation into a dilution series of exponentially growing *S. marinorubra* cells are shown in Fig. 2. The ATP concentration in the most dilute culture was

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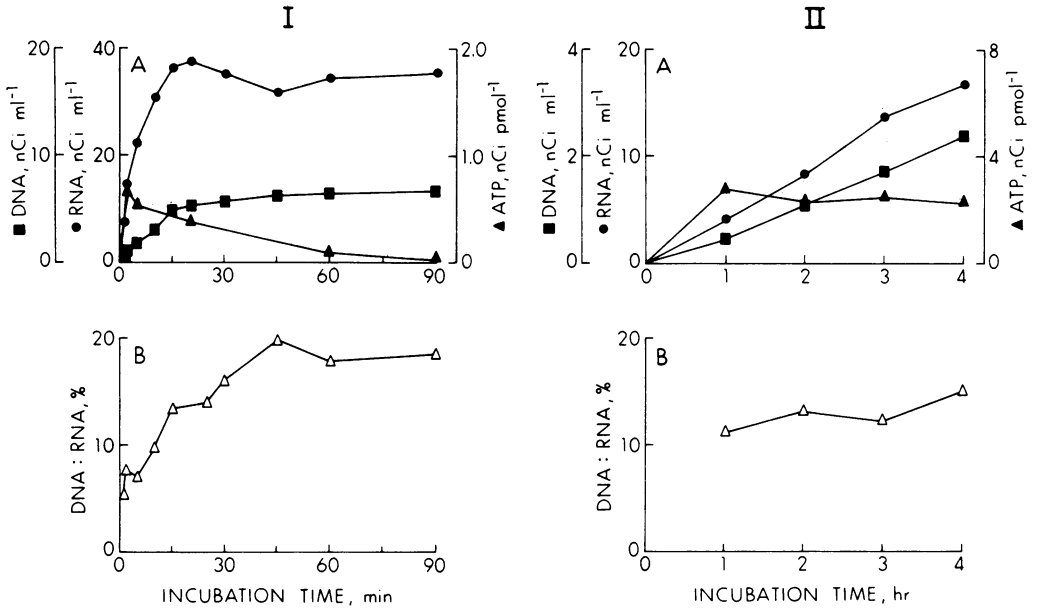


FIG. 3. *P. lutheri* batch culture dilution experiment. Panels I and II show the results of [^{3}H]adenine uptake into cultures containing initial concentrations of 8 and 0.3 ng of ATP ml^{-1}, respectively. Culture doubling time was 18 h.

approximately equal to that typically found in mesotrophic aquatic environments (12). Since the known rates of synthesis were calculated from the concentrations of RNA and DNA and the doubling time of the culture, it is assumed that the rate of accumulation of nucleic acid is equal to the rate of synthesis. This is a reasonable assumption, since the cells are in exponential growth and the rate of DNA and stable RNA turnover due to cell death and lysis is therefore small relative to the rate of synthesis of nascent nucleic acid.

Estimates of DNA synthesis from [^{3}H]adenine incorporation data are accurate at all cell densities. The rate of RNA synthesis, however, is accurate only in the two dilute cultures. The radiochemical DNA:RNA ratio also accurately reflects RNA and DNA cell quotas only in the diluted cultures. The error in the RNA synthesis rate and the DNA:RNA rate ratio at extremely high cell densities are interpreted as being due to the turnover of mRNA in combination with the rapid exhaustion of [^{3}H]adenine from the medium. When exogenous [^{3}H]adenine is depleted from the medium, the specific activity of the total ATP precursor pool increases and then decreases rapidly and the specific activity of mRNA does not remain equal to the specific activity of the ATP precursor pool. The underestimate in the rate of RNA synthesis in the concentrated culture can also be viewed as a result of disequilibrium between the specific activities of mRNA and ATP. The reason for this can be made clear by expressing the precursor-product relationship involved in RNA synthesis in precise terms. The rate of radiolabel accumulation in the two RNA pools can be expressed as:

$$\mathrm{d^*sRNA/d}t = K_2\,(\mathrm{^*ATP/ATP}) \text{ and} \quad (4)$$

$$\mathrm{d^*mRNA/d}t = K_3\,(\mathrm{^*ATP/ATP}) - K_4\,(\mathrm{^*mRNA/mRNA}) \quad (5)$$

where K_2, K_3, and K_4 are as shown in Fig. 1, and where * denotes a radiochemical concentration. The rate of radiolabel accumulation in the total RNA pool can then be expressed as:

$$\mathrm{d^*RNA/d}t = \mathrm{d^*mRNA/d}t + \mathrm{d^*sRNA/d}t \quad (6)$$

where *RNA represents the radiolabel in the total RNA pool. By substitution:

$$\mathrm{^*RNA/d}t = K_2\,(\mathrm{^*ATP/ATP}) + K_3\,(\mathrm{^*ATP/ATP}) - K_4\,(\mathrm{^*mRNA/mRNA}) \quad (7)$$

or the equivalent expression:

$$\mathrm{RNA} = K_2 \int_0^t (\mathrm{^*ATP/ATP}) + K_3 \int_0^t (\mathrm{^*ATP/ATP}) - K_4 \int_0^t (\mathrm{^*mRNA/mRNA}) \quad (8)$$

is derived. It is apparent from equation 8 that, without a simplifying assumption, the rate of stable RNA synthesis (K_2) cannot be quantified precisely without knowledge of the integral specific activities of both ATP and mRNA. However, given that K_t equals the total rate of RNA synthesis:

$$K_t = K_2 + K_3 - K_4 \quad (9)$$

and if it is assumed that the specific activities of ATP and mRNA are equal over the length of the experiment, then equation 8 can be rearranged to yield:

$$K_t = \mathrm{^*RNA}/\int_0^t (\mathrm{^*ATP/ATP}) \quad (10)$$

which is equivalent to equation 3.

Under most environmental conditions, the assumption of equal specific activities of mRNA and ATP is valid (see below). Inaccuracies in the measured rate of RNA synthesis may result, however, if the specific activities of mRNA and ATP diverge significantly. This will occur only when [^{3}H]adenine is exhausted from the medium and the absolute value of the ATP precursor specific activity initially increases and then decreases rapidly. The conditions appropriate for calculation of RNA synthesis rates are when the DNA:RNA rate ratio remains constant with time and, most importantly, when the ATP specific activity initially increases and then remains constant (Fig. 2C). These prerequisites are met when microbial biomass is low (e.g., <10 μg of ATP liter^{-1}), as is most often the case in natural microbial assemblages (12). In situations where microbial biomass is high, it should be possible to use sufficient amounts of [^{3}H]adenine, or to

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add sufficient amounts of nonradioactive adenine, to insure that exogenous [^{3}H]adenine remains free in the medium over the length of the incubation period.

Equation 3 can be employed without simplifying assumptions when calculating the rate of DNA synthesis since DNA does not have a rapidly recycling fraction. However, a potential complicating factor in the calculation of DNA synthesis is that dATP, not ATP, is the direct precursor to dAMP incorporation into DNA (Fig. 1). Karl (13) has pointed out that the specific activities of dATP and ATP are expected to be equal at all time intervals due to the extremely rapid intracellular turnover of common precursors. The results of the present experiment clearly show that this is the case, since accurate rates of DNA synthesis are derived from ATP specific activities at all cell densities tested. It should be emphasized that even the most dilute cultures are more concentrated than most marine environments. Consequently, the conditions under which these measurements are made in the oceanic environment are consistent with those yielding the most accurate estimates of RNA and DNA synthesis.

Algal dilution experiment. A dilution experiment similar to that conducted with *S. marinorubra* was conducted with *P. lutheri*. The results (Fig. 3) are consistent with those of the bacterial dilution experiment. When the label is rapidly exhausted from the medium, the DNA:RNA rate ratio rises with incubation time, again presumably due to radiochemical disequilibrium between mRNA and ATP. The rate ratio remains constant in the dilute culture where the ATP specific activity indicates that [^{3}H]adenine was not exhausted from the medium.

The algal cultures used in this study were not axenic (i.e., they contained small quantities of bacteria). However, direct microscopic observations showed that bacterial biomass was always low (<2 to 3% of total biomass). One might argue that our measurements of [^{3}H]adenine uptake in these cultures reflect only the activity of the bacterial cells. However, two lines of evidence show conclusively that this is not the case. First, accurate rates of nucleic acid synthesis are derived in the algal cultures (see below). If only the contaminating bacteria assimilated adenine, accurate rates of synthesis would not be obtained since the algae are, for all practical purposes, the sole contributors to chemical RNA and DNA in the culture. Second, the ATP precursor specific activities measured in the algal cultures are as high, and usually higher, than in the bacterial cultures alone (compare Fig. 3 through 7 with Fig. 2). If only the bacteria assimilated adenine, the ATP specific activities in the algal cultures would be much lower than in the bacterial cultures since the bacterial ATP represents a small proportion of the total ATP present in the culture. In fact, a simple calculation will show that it is theoretically impossible for bacteria alone to have assimilated adenine since this would require that the specific activity of the bacterial ATP be greater than the specific activity of the added [^{3}H]adenine.

Algal chemostat calibration. *P. lutheri* chemostat experiments were conducted under both NO_3 and PO_4 limitation. The results from the NO_3-limited experiment are shown in Fig. 4. It is apparent from these data that the ATP precursor pool is compartmentalized, particularly during the initial period of labeling, when the ATP pool specific activity rises rapidly while the rate of incorporation of [^{3}H]adenine into both RNA and DNA remains constant (Fig. 4A). The compartmentalization of the ATP precursor pool results in an overestimate of both RNA and DNA synthesis during the early time period. However, accurate rates of synthesis are

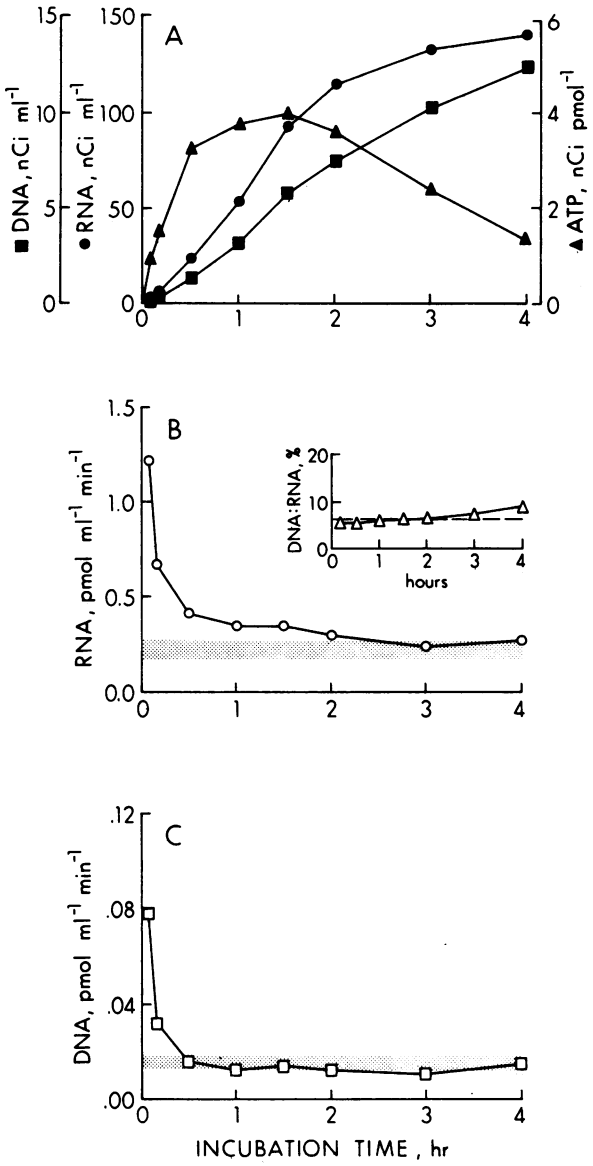


FIG. 4. *P. lutheri* NO_3-limited chemostat experiment. The stippled area in panels B and C indicate the rates (±1 standard deviation unit) of RNA and DNA synthesis as determined by chemical measurements. The dashed line in the inset of panel B indicates the mean DNA:RNA rate ratio as determined by chemical measurements. Initial ATP concentration was 6.3 ng of ATP ml^{-1}. The culture dilution rate was 0.54 day^{-1}.

derived after ca. 30 min for DNA synthesis and 2 h for RNA synthesis. This is equivalent to ca. 2 and 10% of the doubling time, respectively. The measured rate of RNA synthesis is overestimated by a larger factor and declines more slowly than DNA synthesis, presumably due to the turnover of mRNA. The results for the PO_4-limited chemostat were essentially identical to those shown in Fig. 4, with true rates of DNA and RNA synthesis being established after ca. 30 min and 2 h, respectively.

It has been reported that internal compartments containing different ATP precursor specific activities are responsible for ATP compartmentalization in some eucaryotes (17). Since accurate rates of synthesis are obtained in these experiments, it is apparent that, despite the presence of

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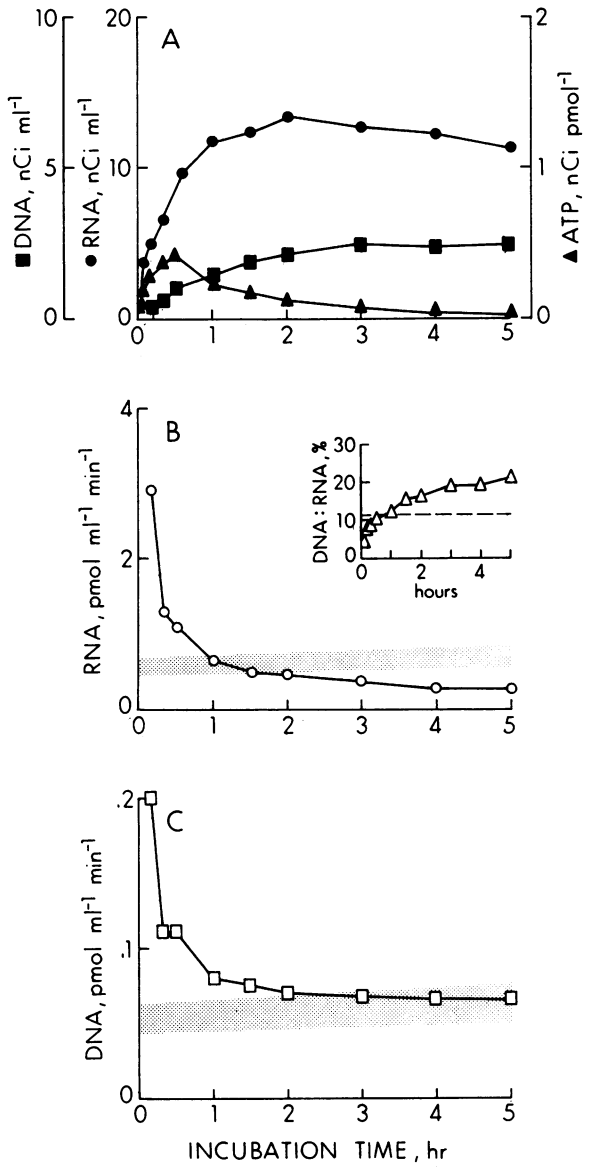


FIG. 5. *P. lutheri* batch culture experiment. The data presentation is as shown in Fig. 2. Initial ATP concentration was 8.3 ng ml^{-1}. Culture doubling time was 18 h.

membrane-bound internal compartments, the ATP precursor specific activity reaches isotopic equilibrium in these organisms over short time intervals. Compartmentalization can therefore be viewed as a result of restricted diffusion within the cell, rather than arising from intracellular barriers. In this view, the presence of internal compartments, along with the larger size of the cell, may be important in producing more pronounced compartmentalization in eucaryotes than in procaryotes. However, our data indicate that this does not preclude the measurement of accurate rates of nucleic acid synthesis in algal cultures or in environmental samples containing both eucaryotes and procaryotes. It may be difficult to imagine that diffusion alone can account for compartmentalization; however, it should be pointed out that the viscosity of the intracellular environment in microorganisms can be ca. 600 times the viscosity of water (24).

Algal batch culture calibrations. Calibrations of the rates of nucleic acid synthesis with [^{3}H]adenine were also conducted with representatives of the *Chrysophyceae*, *Chlorophyceae*, and the *Bacillariophyceae*. The results of these experiments are shown in Fig. 5, 6, and 7. It is apparent from these data that the uptake and metabolism of [^{3}H]adenine is similar in all three groups and that accurate rates of both RNA and DNA synthesis can be measured with [^{3}H]adenine. In the *P. lutheri* experiment, [^{3}H]adenine is assimilated for a brief period (<1 h) before being exhausted from the medium. This resulted in a significant disequilibrium between the specific radioactivities of mRNA and ATP, as evidenced by the changing DNA:RNA rate ratio, and an underestimate of the rate of RNA synthesis. The rate of DNA synthesis is overestimated during the early portion of the time course because of the compartmentalization of the ATP precursor pool, but an accurate estimate is obtained if the precursor specific activity is integrated over the entire time course.

The results for the green alga, *C. cordata*, are shown in Fig. 6. Accurate rates of both RNA and DNA synthesis are

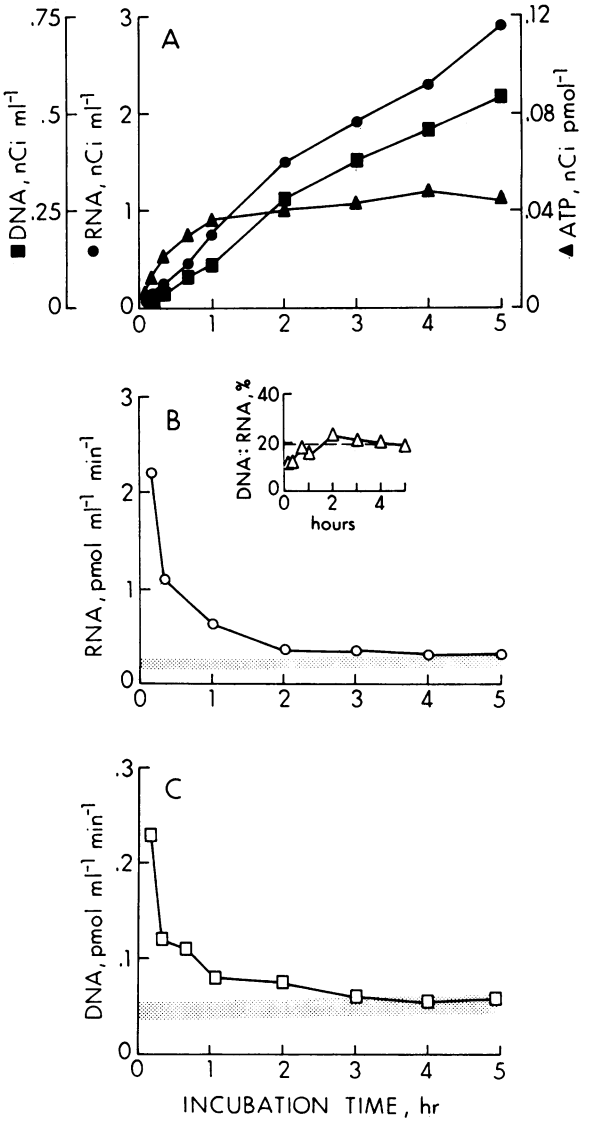


FIG. 6. *C. cordata* batch culture experiment. The data presentation is as shown in Fig. 2. Initial ATP concentration was 9.8 ng ml^{-1}. Culture doubling time of the culture was 20.5 h.

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also derived with this species. It appears that [^{3}H]adenine is assimilated more slowly by this organism than by either of the other two algal species. The result is a specific activity that is low relative to the other laboratory cultures. The slower rate of assimilation also results in [^{3}H]adenine being incorporated over the entire length of the time course and an ATP specific activity which reaches isotopic equilibrium (i.e., the precursor specific activity initially increases and then remains constant; Fig. 6A). True rates of RNA and DNA synthesis are achieved after ca. 10 and 15% of the doubling time, respectively.

The results for the diatom, *Cylindrotheca* sp. (Fig. 7), again show that accurate rates of synthesis are achieved after ca. 5 and 10% of the doubling time for DNA and RNA, respectively. In this experiment the DNA:RNA rate ratio rises only slightly during the time course, indicating that the specific activities of mRNA and ATP are nearly equal.

Kinetics of [^{3}H]adenine uptake in the pelagic environment. Our research interests center on microbial processes in the open ocean, and it is in this environment that the [^{3}H]adenine technique has generally been applied. Due to logistic constraints at sea, time series analyses have not always been feasible. Under these conditions the rate of nucleic acid synthesis has been determined by dividing the label incorporated at the endpoint by the product of the specific activity of the precursor at the endpoint and the length of the experiment: i.e., (nanocuries liter^{-1})/[(nanocuries picomole^{-1}) × time]. This expression is precisely equal to the true rate of synthesis only when the specific activity of the precursor is constant and the incorporation is linear over the length of the experiment. In reality, this situation never exists, since the precursor specific activity always starts at zero. However, a time course where the precursor specific activity is constant with time is often closely approximated in the ocean where microbial biomass is low (Fig. 8). As is typical in these experiments, the DNA:RNA rate ratio remains constant over the length of the experiment, and the ATP specific activity rises rapidly and then remains relatively constant with time. It is clear that the synthesis rate measured as an endpoint after 6 or 8 h would closely approximate the rate measured as an integral (i.e., equation 3). In environmental applications we routinely confirm that [^{3}H]adenine is not exhausted during the incubation period (15). This is not meant to imply that time series analyses are not important in environmental applications. On the contrary, exactly the opposite is true. Without some knowledge of the time series of events in these experiments, little confidence can be placed in the accuracy of the measured rates of nucleic acid synthesis.

It is evident from these data that the rates of nucleic acid synthesis in marine microorganisms can be accurately estimated by using [^{3}H]adenine. This is true for representative

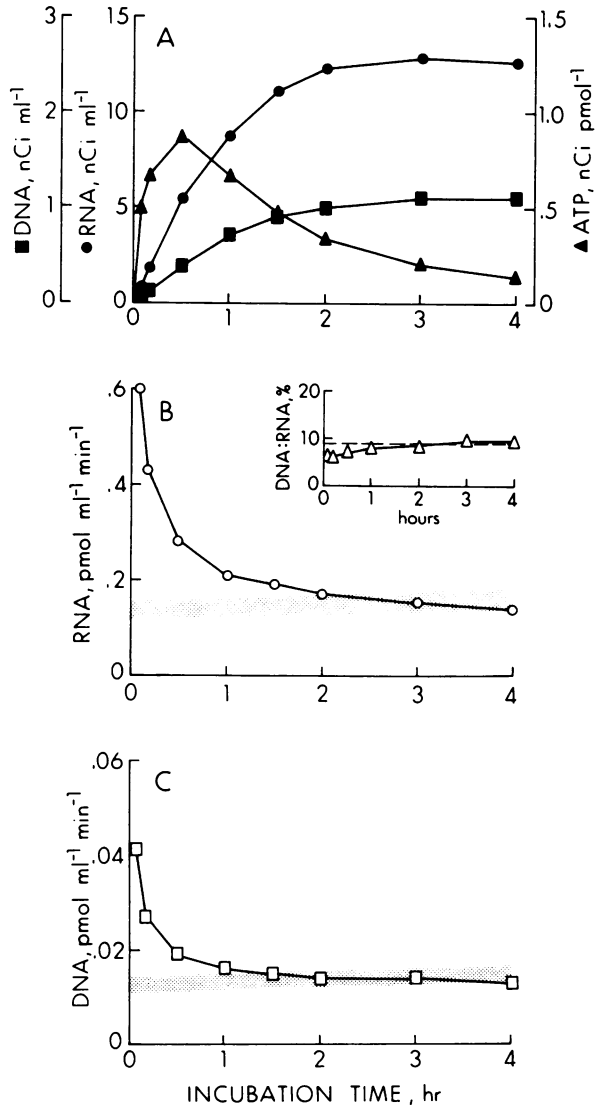


FIG. 7. *Cylindrotheca* sp. batch culture experiment. The data presentation is as shown in Fig. 2. Initial ATP concentration was 4.3 ng ml^{-1}. Culture doubling time was 17 h.

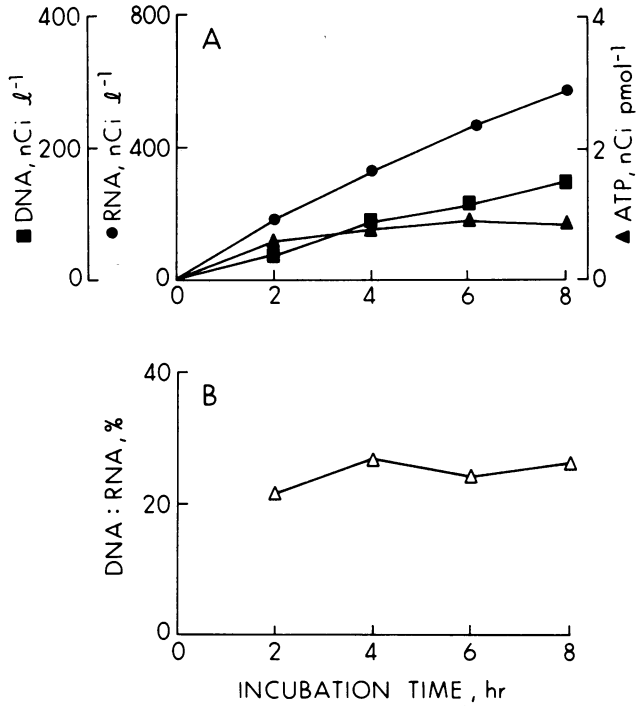


FIG. 8. Time course of RNA, DNA, and ATP labeling in seawater sample. Panel A shows [^{3}H]adenine incorporation into RNA, DNA, and ATP. Panel B shows the DNA:RNA as a function of time during the incubation. [^{3}H]adenine was added at an initial radiochemical concentration of 0.1 μCi ml^{-1}.

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eucaryotic and procaryotic marine microorganisms grown in either steady-state chemostat or in exponential batch culture, and is true for marine algae grown under either NO_3 or PO_4 limitation. Furthermore, it is clear that the assumption of equal ATP and dATP specific activities is valid. This must be true since accurate rates of both RNA and DNA synthesis can be derived by using the specific activity of ATP. It is also evident that some precautions must be observed to ensure that accurate rates of nucleic acid synthesis are measured. This is necessary since correct rates of nucleic acid synthesis are not derived under all conditions or at all points during a time course. We would like to stress that without some knowledge of the time course of [^{3}H]adenine metabolism, or at least a postincubation radiochemical inventory (15), little confidence can be placed in the accuracy of the results.

In nature, the time course kinetics of nucleic acid and precursor pool labeling will depend upon many factors, including temperature, microbial biomass, turnover of the exogenous adenine pool, and microbial growth rate. To ensure that accurate rates of synthesis are derived in natural samples, the ATP precursor specific activity should rise and remain constant, or at least not drop precipitously. For this to occur, exogenous [^{3}H]adenine must remain in the medium for the duration of the incubation period. The time course should also be long enough to avoid the effects of ATP pool compartmentalization. The true rates of synthesis of both RNA and DNA are then derived when the rate becomes constant with time. Our results show that the time required for this to occur is a maximum of ca. 10% of the generation time.

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