APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1988, p. 2213–2219
0099-2240/88/092213-07$02.00/0

Vol. 54, No. 9

Calculation of Cell Production from [^{3}H]Thymidine Incorporation with Freshwater Bacteria†

JAN D. SMITS[1] AND BO RIEMANN[2]*

Department of Nature Conservation, Agricultural University, Ritzema Bosweg 32 a, 6703 AZ Wageningen, The Netherlands,[1] and Freshwater Biological Laboratory, University of Copenhagen, Helsingørsgade 51, DK-3400 Hillerød, Denmark[2]

Received 8 February 1988/Accepted 6 June 1988

The conversion factor for the calculation of bacterial production from rates of [^{3}H]thymidine incorporation was examined with diluted batch cultures of freshwater bacteria. Natural bacterial assemblages were grown in aged, normal, and enriched media at 10 to 20°C. The generation time during 101 growth cycles covered a range from 4 to >200 h. The average conversion factor was 2.15×10^{18} cells mol^{-1} of thymidine incorporated into the trichloroacetic acid (TCA) precipitate (standard error = 0.29×10^{18}; $n = 54$), when the generation time exceeded 20 h. At generation times of <20 h, the average conversion factor was 11.8×10^{18} cells mol^{-1} of thymidine incorporated into TCA precipitate (standard error = 1.72×10^{18}; $n = 47$). The amount of radioactivity in purified DNA increased with decreasing generation time and increasing conversion factor (calculated from the TCA precipitate), corresponding to a decrease in the percentage in protein. The conversion factors calculated from purified DNA or from the TCA precipitate gave the same variability. Conversion factors did not change significantly with the medium, but were significantly higher at 20°C than at 15 and 10°C. A detailed examination of the [^{3}H]thymidine concentrations that were needed to achieve maximum labeling in DNA was carried out 6 times during a complete growth cycle. During periods with low generation times and high conversion factors, 15 nM [^{3}H]thymidine was enough for the maximum labeling of the TCA precipitate. This suggests that incorporation of [^{3}H]thymidine into DNA is probably limited by uptake during periods with generation times of <20 h and that freshwater bacterioplankton cell production sometimes is underestimated when a conversion factor of 2.15×10^{18} cells mol^{-1} of thymidine incorporated is used.

During the past few years, [^{3}H]thymidine incorporation into bacterial DNA has often been used to determine bacterial cell production in natural environments (2, 7, 18).

Calculations of bacterial cell production from rates of [^{3}H]thymidine incorporation are based on several assumptions which have been discussed previously (for a review, see reference 9). An important assumption is that the factor for the derivation of bacterial cell production from rates of [^{3}H]thymidine incorporation is constant (3; D. J. W. Moriarty, Ergeb. Limnol., in press). Many workers have derived conversion factors which differ by a factor of 10 (6, 10, 12, 17). However, most of these results are based on a few experiments. Recently, Riemann et al. (14) found a conversion factor of 1.1×10^{18} cells mol^{-1} of thymidine incorporated from coastal marine environments (standard error [SE] = 0.05×10^{18}; $n = 63$). This conversion factor was surprisingly uniform. It did not change significantly with medium (aged, normal, and enriched media of natural origin were used), temperature (6 to 30°C), or generation time (1 to >200 h). From the data matrix ($n = 63$), three high values indicated that bacterial cell production is occasionally underestimated when the mean conversion factor of 1.1×10^{18} cells mol^{-1} of thymidine incorporated is used. A similar comprehensive evaluation has not been carried out in fresh water.

In this study, we examined the size and variability of the factor for the derivation of bacterial cell production from [^{3}H]thymidine incorporated into cold trichloroacetic acid (TCA)-precipitable material and into purified DNA from diluted batch cultures of freshwater bacteria. Natural bacterial assemblages were grown at temperatures covering a range from 10 to 20°C in prepared media representing aged (depleted by bacteria 7 to 10 days before use), normal, and enriched (with glucose) substrates.

MATERIALS AND METHODS

Water samples were taken from eutrophic Lake Frederiksborg Slotssø, which is situated in the center of the city of Hillerød, Denmark, on 31 July (19°C), 10 August (15°C), 5 October (11°C), and 12 October (10°C) 1987.

Immediately after sampling, 50-ml portions were filtered through 1.0-μm-pore-size filters (Nuclepore Corp., Pleasanton, Calif.) and incubated for 4 to 6 h with cycloheximide (100 mg liter^{-1}; Sigma Chemical Co., St. Louis, Mo.), to prevent the growth of eucaryotic bacteriovores which might have passed through the 1.0-μm-pore-size filter (4).

Experiments were carried out at the in situ temperature, except for the samples from 5 October, for which the temperature was raised from 11 to 20°C.

At the start of each experiment, 450-ml portions of particle-free (<0.2 μm) medium were inoculated with 50 ml of the 1-μm-pore-size filtrate.

Bacteria were grown in three different media, which were prepared as followed. Aged medium was prepared by storing 1.0-μm-pore-size filtered lake water for 7 to 10 days at room temperature. Then, it was filtered through a 0.2-μm-pore-size filter capsule (12122; Gelman Sciences, Inc., Ann Arbor, Mich.). Normal medium was lake water filtered through a 0.2-μm-pore-size filter capsule. Enriched medium was prepared the same way as the normal medium, but D-glucose was added at each sampling time to a final concentration of

* Corresponding author.

† Contribution no. 480 from the Freshwater Biological Laboratory, University of Copenhagen, Hillerød, Denmark.

50 μg liter^{-1}. The media were stored frozen (−18°C) prior to use.

During each growth cycle, the bacteria were allowed to grow to the carrying capacity of the medium, after which they were diluted 10 times with the original medium. Three growth cycles were studied with each of the three media, and triplicates of each medium were used.

During each growth cycle, [^{3}H]thymidine incorporation was measured 5 to 6 times, as described by Fuhrman and Azam (2) with the following exceptions. Samples of 10 ml (20 ml when macromolecular fractionations were carried out) were incubated for 20 min with 15 nM [^{3}H]thymidine (20 μCi nmol^{-1}; Dupont, NEN Research Products, Boston, Mass.). In incorporation experiments with [^{3}H]thymidine and with [^{3}H]thymidine concentrations ranging from 1 to 50 nM, 15 nM gave maximal labeling of the bacteria. Incubation was stopped by adding Formalin to a final concentration of 1%. Blanks were prepared from samples, with Formalin added immediately before the addition of [^{3}H]thymidine.

During the saturation experiment, replicate samples were incubated with 5, 7, 10, 15, 20, or 50 nM [^{3}H]thymidine.

After incubation, samples were filtered through 0.45-μm-pore-size filters (Sartorius) and washed 9 times with 4.5 to 5.5 ml of ice-cold 5% TCA.

The macromolecular fractions were carried out as described by Fuhrman and Azam (3), with minor modifications. Briefly, samples were divided into three 5-ml subsamples. The first subsample was heated for 30 min to 95 to 100°C with an equal volume of 40% TCA. After samples were chilled, they were filtered and rinsed as described above. This hot TCA treatment is assumed to hydrolyze DNA and RNA and leaves the radioactivity in proteins. The second subsample was heated for 1 h at 60°C with 0.55 ml of 5 N NaOH. After chilling, 1.4 ml of ice-cold 100% TCA was added and the samples were filtered and rinsed with TCA. This base treatment hydrolyzes RNA and leaves the radioactivity in proteins and DNA. The third subsample was filtered directly and rinsed with TCA; it was assumed to contain DNA, RNA, and protein. All fractionated samples were filtered through 0.45-μm-pore-size filters (HA; Millipore Corp., Bedford, Mass.) (when Sartorius cellulose-nitrate filters were used, background levels were often unacceptably high in the base and hot TCA-treated samples). The amount of radioactivity in DNA, RNA, and protein fractions was calculated. Filters were dissolved in 1 ml of ethyl acetate, and after 10 min 10 ml of scintillation cocktail (Aqualyte; Lumac) was added. Samples were counted by using a scintillation counter (Rack-beta; LKB Instruments, Inc., Rockville, Md.).

The number of bacteria was determined at the beginning and the end of each growth cycle by using the standard acridine orange direct count method (5). The number of nanoflagellates was determined from the same filters at the end of each growth cycle. In seven samples, a small number of flagellates were observed, and the results obtained with these bottles were removed from the data.

Conversion factors (cells moles^{-1} of thymidine) were calculated by dividing the difference in cell number at the beginning and end of each growth cycle (cells milliliter^{-1}) by the integrated [^{3}H]thymidine incorporation (moles milliliter^{-1}).

The generation time (T) was calculated from the following equation: $T = \{\ln(2)/[\ln(n_2) - \ln(n_1)]\} \times t$, where n_1 and n_2 are the number of bacteria (milliliters^{-1}) at the beginning and end of the growth cycle, respectively, and t is the duration of the experiment (in hours).

Linear regressions were performed on radioactivity in DNA, RNA, and protein fractions versus generation time or conversion factor. Natural logarithm transformation was used to correct for nonhomoscedasticity (samples along the regression that did not have a common variance). Differences in the conversion factor obtained when different temperatures and media were used were tested with F tests after natural logarithm transformation to achieve homogeneity of variances.

RESULTS

The conversion factors calculated from the amount of [^{3}H]thymidine incorporated into TCA precipitate and cell counts from 101 growth cycles were plotted versus the generation time (Fig. 1). The conversion factor varied considerably, particularly at low generation times. Thus, at generation times of <20 h, the conversion factor ranged from 1.2×10^{18} to 59.0×10^{18} cells mol^{-1} ($\bar{x} = 11.8 \times 10^{18}$ cells mol^{-1}; SE = 1.72×10^{18}; $n = 47$), but at generation times of >20 h, the average conversion factor was 2.15×10^{18} cells mol^{-1} of thymidine incorporated (SE = 0.29×10^{18}; $n = 54$). A cumulative distribution of (cold TCA) conversion factors was made. In 82% of the batch cultures, conversion factors were lower than 10×10^{18}, in 62% they were lower than 5.0×10^{18}, and in 38% they were lower than 2.0×10^{18} cells mol^{-1}.

Conversion factors did not differ significantly ($P > 0.05$) with the medium used. However, conversion factors found at 19 and 20°C were significantly higher ($P < 0.001$) than those found at 15 or 10°C. Conversion factors were not different at 15 and 10°C ($P > 0.05$).

Macromoelcular fractionations of the samples were carried out at 10 and 20°C (Fig. 2). The radioactivity in the DNA fraction (percentage of TCA precipitate) decreased with increasing generation time ($r = 0.52$; $P < 0.01$; $n = 52$) (Fig. 2A) and increased with increasing conversion factor ($r = 0.59$; $P < 0.01$; $n = 52$) (Fig. 2B). The correlation between radioactivity in RNA and generation time or conversion factor was not significantly different from zero ($r = 0.17$; $P > 0.05$; $n = 52$) (Fig. 2C and D). The radioactivity in the protein fraction was not significantly correlated with generation time, but it decreased significantly with increasing conversion factor ($r = 0.42$; $P < 0.01$; $n = 52$) (Fig. 2E and F). Because a lower amount of radioactivity was found in the DNA fraction than in the TCA precipitate, conversion factors calculated from the amount of [^{3}H]thymidine incorporated into DNA were higher (Table 1). At 20°C we found the same coefficient of variation in conversion factors, whether they were derived from radioactivity incorporated into DNA or into the TCA precipitate. At 10°C coefficients of variation were somewhat lower when conversion factors were calculated from incorporation into DNA than when they were based on incorporation into the TCA precipitate.

We examined whether the high variability of the conversion factor was caused by concentrations of [^{3}H]thymidine that were too low during periods with a low generation time. During a complete growth cycle, [^{3}H]thymidine incorporation into the TCA precipitate was carried out 6 times by using concentrations from 5 to 50 nM (Fig. 3). High conversion factors were observed during the exponential growth phase; and [^{3}H]thymidine incorporation with a concentration of 15 nM, as was used during the other experiments, was the same as the rates obtained from 20 or 50 nM. At the end of the growth cycle, [^{3}H]thymidine incorporation rates were lower when 15 nM than 50 nM was used, and conversion

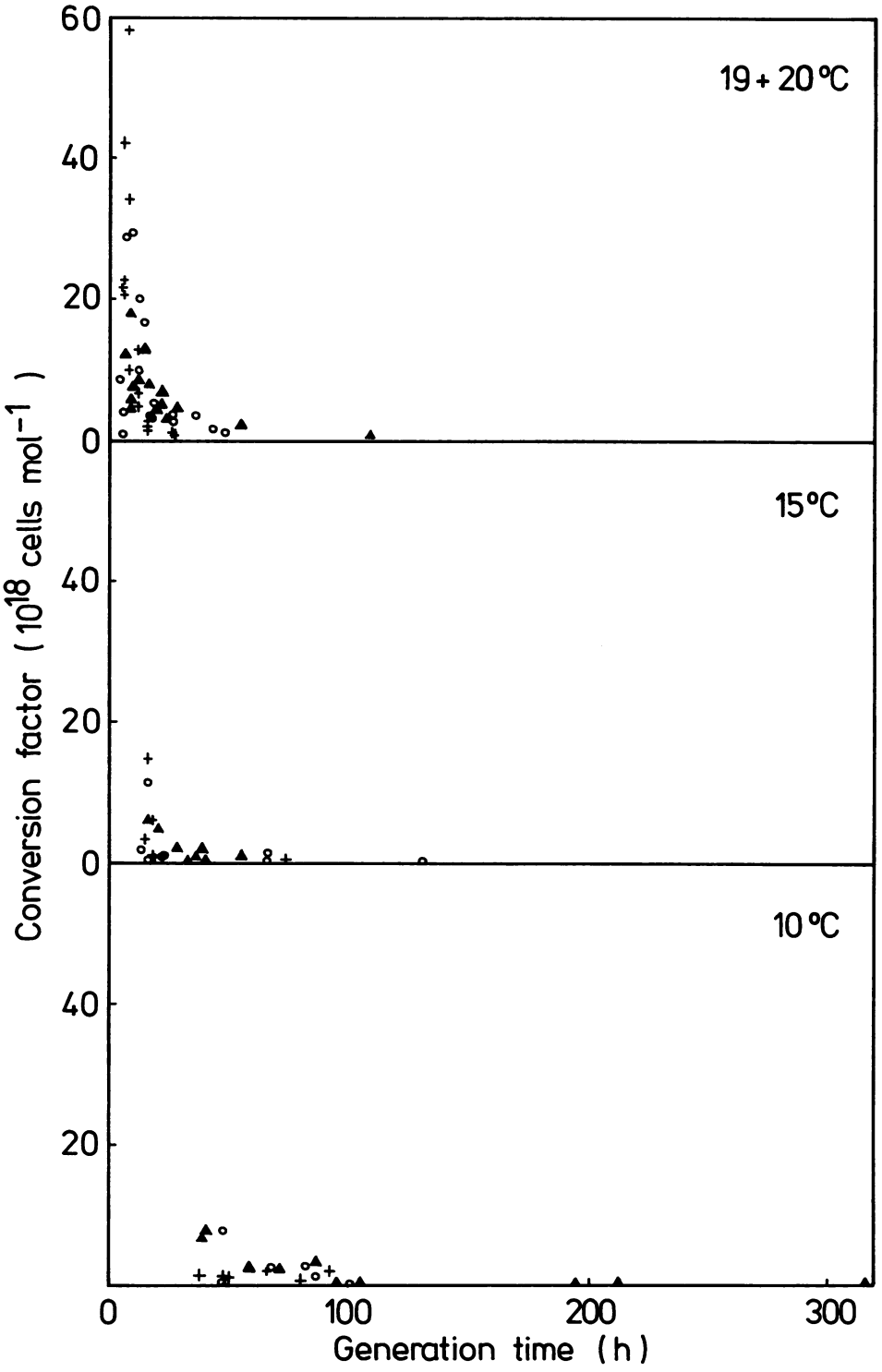

FIG. 1. Conversion factors (10^{18} cells mol^{-1} of thymidine incorporated into TCA precipitate) obtained from aged (▲), normal (○), and enriched media (+) versus bacterial generation time (in hours) at 19 and 20°C (A), 15°C (B), and 10°C (C). In two growth cycles, generation times exceeded 300 h and conversion factors were 0.033×10^{18} and 0.12×10^{18} cells mol^{-1} (data not shown).

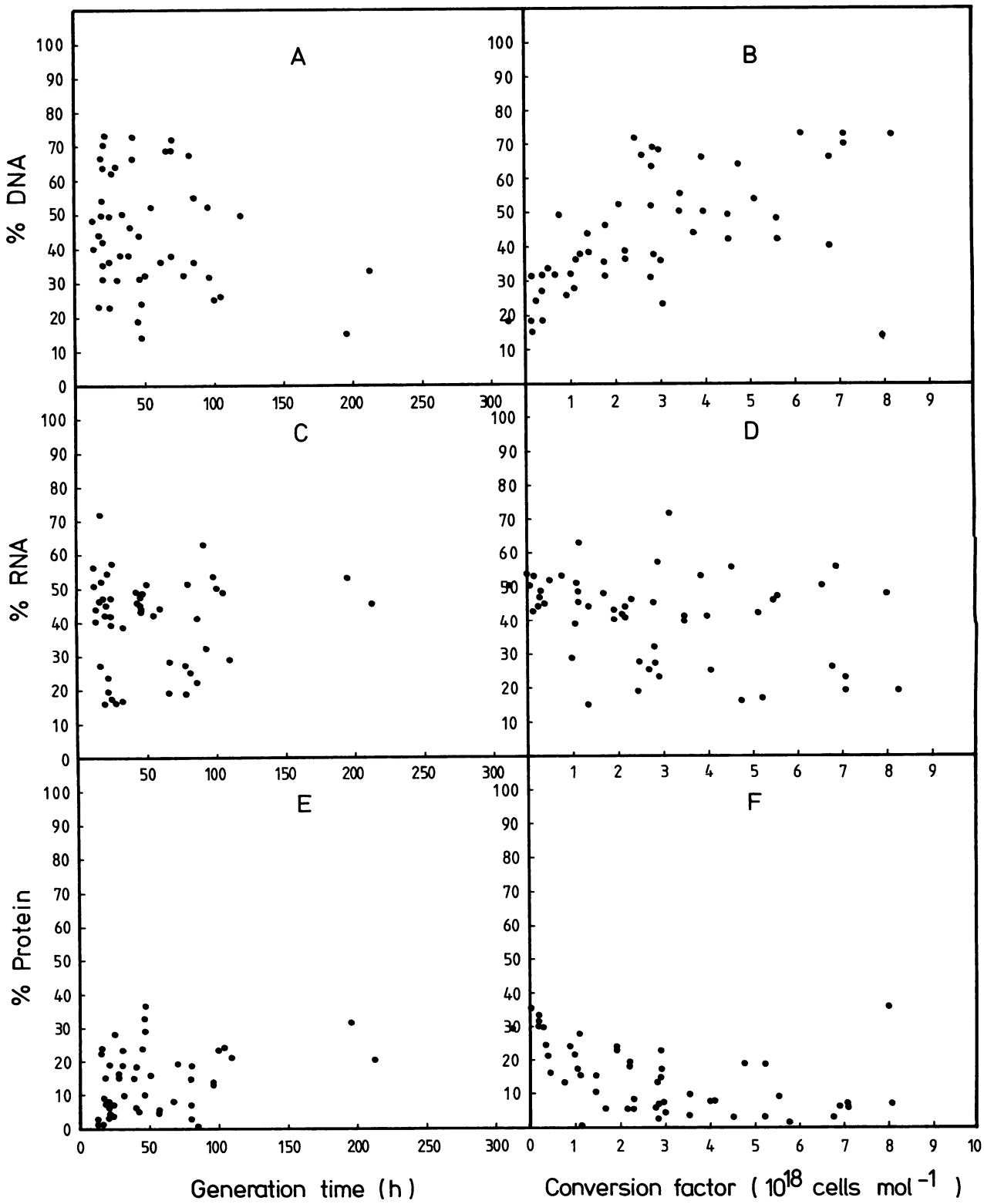

FIG. 2. Incorporation of [^{3}H]thymidine into DNA (A and B), RNA (C and D), and protein (E and F) versus generation time (A, C, and E) or conversion factor (B, D, and F) based on incorporation into TCA precipitate.

TABLE 1. Conversion factors calculated from [^{3}H]thymidine incorporated into TCA precipitate or purified DNA from the experiments carried out at 10 and 20°C

Temp (°C)	Conversion factors (cells/mol; 10^{18}) for:					
	TCA precipitate			DNA		
	$\bar{x}$	CV[a]	n	$\bar{x}$	CV	n
20	3.66	51	27	7.06	49	27
10	2.09	113	25	3.35	81	25

[a] CV, Coefficient of variation.

factors were also lower. As a result of the large changes in the conversion factor, absolute values of disintegrations per minute were highest after exponential growth ceased.

DISCUSSION

The standard technique for the determination of [^{3}H]thymidine incorporation (2) includes the addition of a single concentration of [^{3}H]thymidine (normally, 5 to 10 nM) and assumes a constant conversion factor to achieve an estimate of the cell production. Our data matrix, based on 101 growth cycles, demonstrated that conversion factors varied by more than a factor of 100 when average generation times of the freshwater batch cultures were <20 h. In contrast, conversion factors were lower and gave a better reproducibility when generation times exceeded 20 h ($\bar{x}$ = 2.15 × 10^{18}; SE = 0.29 × 10^{18}; n = 54). Variability in the conversion factors was almost the same when they were calculated from purified DNA as when they were calculated from the amount of radioactivity in the total TCA precipitate.

Previous estimates of the conversion factors from freshwater environments range from 0.9 × 10^{18} to 8.7 × 10^{18} cells mol^{-1} of thymidine incorporated (1, 7, 10, 12, 13), although even larger ranges (5 × 10^{18} to 68 × 10^{18} cells mol^{-1}) have been published based on the procedure described by Kirchman et al. (6) and Scavia and colleagues (17, 18).

High conversion factors are obtained when (i) the added [^{3}H]thymidine concentration is too low for maximal labeling of DNA, (ii) a rapid degradation of [^{3}H]thymidine by means of thymidine phosphorylase occurs, and (iii) incorporation of thymidine is uptake limited. Concerning the first point, bacteria regulate nucleotide biosynthesis to prevent the unnecessary buildup of nucleotide triphosphates (9). When the bacteria are supplied with sufficient thymidine in the medium, de novo synthesis of thymidine is supposed to be inhibited (2, 9). All thymidine triphosphate used for the synthesis of DNA comes through the salvage pathway from the external pool. Concentrations of labeled thymidine that are too low mean an incomplete inhibition of de novo synthesis (9) and a dilution of the labeled thymidine. Unless a correction for this dilution is made, the conversion factors will be in error. Our saturation experiment demonstrated that during the exponential phase of the growth cycle, the addition of 15 nM [^{3}H]thymidine presumably was sufficient for maximum labeling of the DNA. It is not clear why the addition of 50 nM [^{3}H]thymidine at the end of the growth cycle gave radioactivity in macromolecules that was nearly 2 times higher than 15 nM additions did. As a result of the marked changes in the conversion factor during periods of exponential growth (factor, 24 to 36) and after exponential growth had ceased (factor, 0.7), absolute values of disintegrations per minute were highest after the exponential growth phase ceased (Fig. 3). The calculated conversion factor with 15 nM thymidine was 0.7 × 10^{18} cells mol^{-1} at the end of the growth cycle. By using the incorporation rates obtained from the addition of 50 nM thymidine, the conversion factor was reduced to 0.38 × 10^{18} cells mol^{-1}, probably because of the accelerated degradation of thymidine by thymidine phosphorylase (see below). In fact, increased radioactivity was found in the protein fraction in batch cultures with low conversion factors (Fig. 2F). It can be concluded that the added concentration of thymidine was not too low during the periods with high conversion factors. Bell (R. T. Bell, Ph.D. dissertation, Uppsala University, Uppsala, Sweden, 1986) has demonstrated that with samples from humic lakes, concentrations up to 50 nM are needed to saturate the incorporation. However, additions of 10 to 15 nM [^{3}H]thymidine seem to be enough in most fresh waters to inhibit de novo synthesis completely (15).

Concerning the second point, the breakdown of labeled thymidine occurs in the cell by means of thymidine phosphorylase, which cleaves thymidine into thymine phosphorolytically. The labeled methyl group is thereby lost into the general pool of metabolic reactions (9), and a subsequent labeling of RNA and protein occurs. According to Moriarty (9), this nonspecific labeling is not important in short-term (10 to 20 min) experiments.

In the fractionation experiment, radioactivity in DNA (percentage of TCA precipitate) decreased with increasing generation time (Fig. 2). Fast-growing bacteria presumably use thymidine more efficiently for the synthesis of DNA than slow-growing bacteria do. Over all generation times, a large and variable percentage (42%; standard deviation [n − 1] = 12) of label was found to be incorporated into RNA. Thus, nonspecific labeling is a common feature in slow-growing bacteria. Robarts et al. (16) have reported a comparable variation in the distribution of radioactivity over the different fractions. However, they found a negative correlation between the radioactivity in the DNA and activity in the bacteria.

In our experiments, conversion factors were often high in fast-growing bacteria. This raises the question of whether uptake can limit the incorporation of [^{3}H]thymidine. The enzymes used in the transport and catabolism of thymidine are controlled by the same regulatory genes (8, 9). The activities of both transport and catabolic enzyme complexes are influenced by the cellular cyclic AMP level. In *Escherichia coli*, high cyclic AMP levels increase transport and catabolic activities, and cyclic AMP levels reveal an inverse relationship with generation time (J. Neuhard, personal communication).

Assuming that results from *E. coli* can be applied to natural freshwater bacteria, it might be hypothesized that in slow-growing bacteria, thymidine is transported across the cell membranes and a substantial fraction is cleaved phosphorolytically. However, the thymidine concentration that is present in the cell may still be high enough to inhibit de novo synthesis. In accordance, conversion factors (~2 × 10^{18} cell mol^{-1}) are close to the theoretical factors (2). In fast-growing bacteria, however, uptake may limit incorporation. Although phosphorolysis is also reduced, up to 30% of the radioactivity was found in compartments other than DNA (Fig. 2C through F). Consequently, after a short time, incorporation of [^{3}H]thymidine into DNA ceases because it has been broken down to thymine (11). Moriarty (9) has found that thymidine breakdown exceeds thymidine uptake after 8 min in warm sediments, and with lower temperatures it does so after 20 min. If incomplete inhibition of de novo

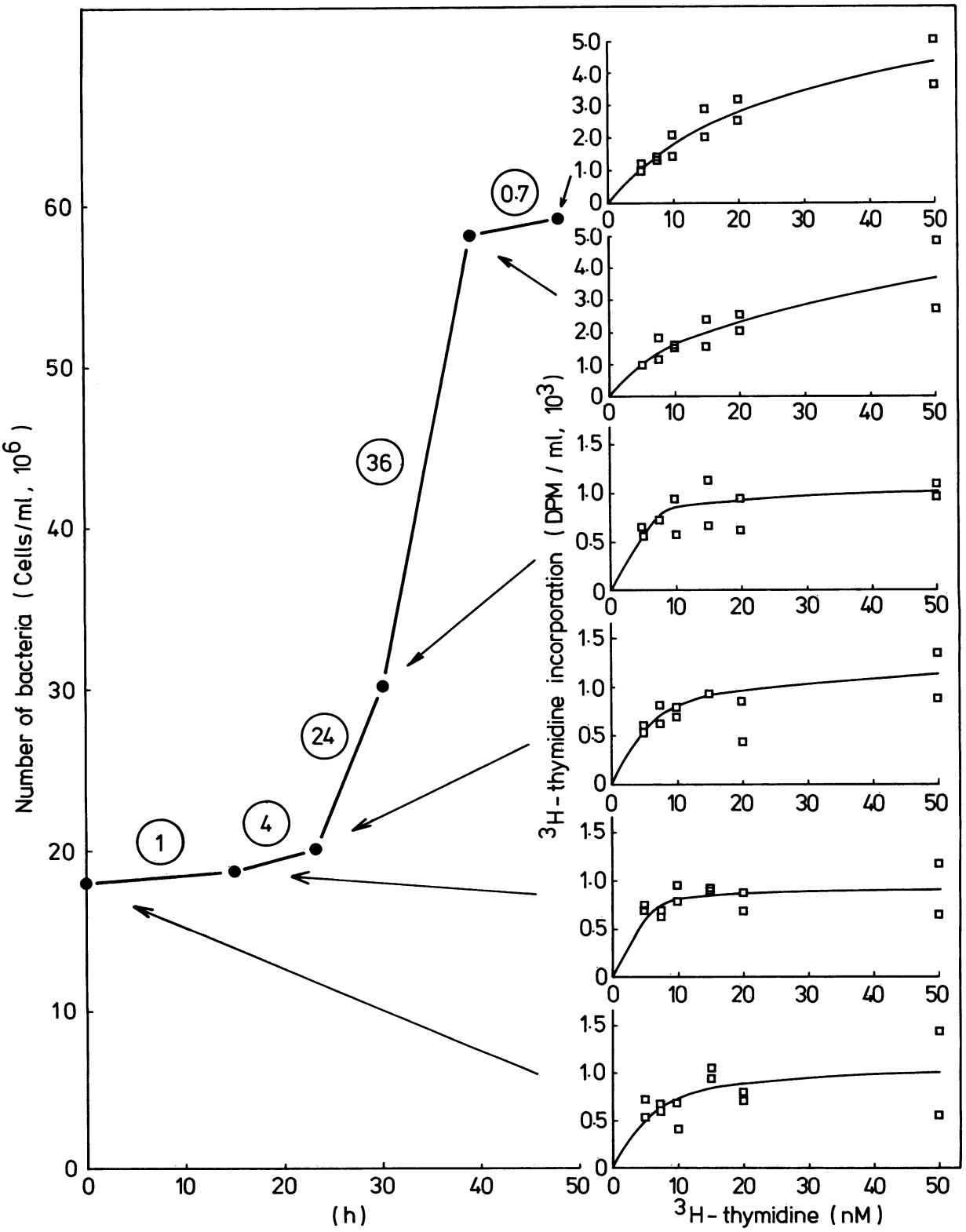

FIG. 3. Incorporation of [^{3}H]thymidine into TCA precipitate by using 5 to 50 nM during a complete growth cycle of freshwater bacteria. The actual conversion factors (10^{18} cells mol^{-1}) are given in the circles.

synthesis occurs, [^{3}H]thymidine is diluted and the conversion factor is high.

Robarts and Wicks (R. D. Robarts and R. J. Wicks, Limnol. Oceanogr., in press) have concluded that thymidine uptake does not limit the incorporation of [^{3}H]thymidine into DNA, because the total uptake of [^{3}H]thymidine always exceeds incorporation into macromolecules. This method, however, does not prove whether the difference between uptake and incorporation is sufficient to reduce the de novo synthesis of thymidine.

Therefore, as the high conversion factors were restricted to bacteria with fast growth rates and the external addition of [^{3}H]thymidine seemed sufficient to label DNA maximally, we suggest that [^{3}H]thymidine uptake limits the incorporation in fast-growing cells (generation times of <20 h). In a number of our growth cycles, however, the conversion factor remained low during generation times of <20 h (Fig. 1); the reasons for this variation are not clear. We realize, however, that the exponential phase of the whole growth cycle was not constant in the three subsequent growth cycles that were carried out. If a lag or stationary growth phases constituted a larger part of the second and third growth rates, lower conversion factors could occur. Riemann et al. (14) have cautioned about the high conversion factors in a study with coastal marine bacteria. Of 63 growth cycles, however, only 3 deviated significantly from the normal distribution of the data set, and the high values of these 3 growth cycles were all found in populations with generation times below 20 h. Thus, it is fair to conclude that cell production by aquatic bacteria with generations of <20 h can be severely underestimated when a conservative conversion factor is used.

ACKNOWLEDGMENTS

We thank Jan Neuhard for chemical supplies, the loan of laboratory space, and valuable comments on an earlier version of the manuscript. We also thank Jed Fuhrman for valuable suggestions. Technical assistance was performed by Winnie Martinsen. Hanne Møller typed the manuscript.

LITERATURE CITED

1. **Bell, R. T., G. M. Ahlgren, and I. Ahlgren.** 1983. Estimating bacterioplankton production by measuring [^{3}H]thymidine incorporation in a eutrophic, Swedish lake. Appl. Environ. Microbiol. **45**:1709–1721.
2. **Fuhrman, J. A., and F. Azam.** 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. Appl. Environ. Microbiol. **39**:1085–1095.
3. **Fuhrman, J. A., and F. Azam.** 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar. Biol. **66**:109–120.
4. **Fuhrman, J. A., and G. B. McManus.** 1984. Do bacteria sized marine eukaryotes consume significant bacterial production? Science **224**:1257–1260.
5. **Hobbie, J. E., J. Daley, and S. Jasper.** 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. **33**:1225–1228.
6. **Kirchman, D., H. Ducklow, and R. Mitchell.** 1982. Estimates of bacterial growth from changes in uptake rate and biomass. Appl. Environ. Microbiol. **44**:1296–1307.
7. **Lovell, C. R., and A. Konopka.** 1985. Seasonal bacterial production in a dimictic lake as measured by increase in cell number and thymidine incorporation. Appl. Environ. Microbiol. **49**:492–500.
8. **Møllgaard, H., and J. Neuhard.** 1983. Biosynthesis of deoxythymidine triphosphate, p. 145–201. *In* A. Munch-Petersen (ed.), Metabolism of nucleotides, nucleosides and nucleobases in microorganisms. Academic Press, Inc., London.
9. **Moriarty, D. J. W.** 1986. Measurement of bacterial growth rates in aquatic systems using rates of nucleic acid synthesis. Adv. Microb. Ecol. **9**:245–292.
10. **Murray, R., and R. E. Hodson.** 1985. Annual cycle of bacterial secondary production in five aquatic habitats of the Okefenokee Swamp ecosystem. Appl. Environ. Microbiol. **49**:650–655.
11. **Neuhard, J.** 1983. Pyrimidine salvage pathways, p. 95–148. *In* A. Munch-Petersen (ed.), Metabolism of nucleotides, nucleosides and nucleobases in microorganisms. Academic Press, Inc., London.
12. **Riemann, B.** 1984. Determining growth rates of natural assemblages of freshwater bacteria by means of [^{3}H]-thymidine incorporation into DNA: comments on methodology. Ergeb. Limnol. **19**:67–80.
13. **Riemann, B.** 1985. Potential importance of fish predation and zooplankton grazing on natural populations of freshwater bacteria. Appl. Environ. Microbiol. **50**:187–193.
14. **Riemann, B., P. Koefoed Bjørnsen, S. Newell, and R. Fallon.** 1987. Calculation of cell production of coastal marine bacteria based on measured incorporation of [^{3}H]thymidine. Limnol. Oceanogr. **32**:471–476.
15. **Riemann, B., and M. Søndergaard.** 1986. Bacteria, p. 127–197. *In* B. Riemann and M. Søndergaard (ed.), Carbon dynamics in eutrophic temperate lakes. Elsevier/North-Holland Publishing Co., Amsterdam.
16. **Robarts, R. D., R. J. Wicks, and L. M. Sephton.** 1986. Spatial and temporal variations in bacterial macromolecule labeling with [*methyl*-^{3}H]thymidine in a hypertrophic lake. Appl. Environ. Microbiol. **52**:1368–1373.
17. **Scavia, D., and G. A. Laird.** 1987. Bacterioplankton in Lake Michigan: dynamics, controls and significance to carbon flux. Limnol. Oceanogr. **32**:1017–1033.
18. **Scavia, D., G. A. Laird, and G. L. Fahnenstiel.** 1986. Production of planktonic bacteria in Lake Michigan. Limnol. Oceanogr. **31**: 612–626.