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Evidence against incorporation of exogenous thymidine by sulfate-reducing bacteria

Abstract—The uptake of exogenous [^{3}H]thymidine by SO_4^{2-}-reducing bacteria (SRB) was tested in both pure culture and in marine sediments. Ten cultures isolated from mesohaline sediments of Chesapeake Bay, plus ATCC strain *Desulfovibrio desulfuricans aestuarii,* were incubated in SO_4^{2-}-reducing media with [^{3}H]TdR concentrations ranging from 2 to 100 nM. In most cases [^{3}H]TdR uptake levels were no more than a few times higher than Formalin-killed controls; SRB cells took up a maximum of 1.7×10^{-22} moles TdR cell^{-1} h^{-1}. Across all incubations, an average of 3.3×10^{23} SRB cells were produced (mol [^{3}H]TdR)$^{-1}$ taken up, compared to an average value of $\sim 2 \times 10^{18}$ cells (mol [^{3}H]TdR)$^{-1}$ in natural, oxic waters. In anoxic sediments the addition of molybdate, an inhibitor of SO_4^{2-} reduction, significantly reduced amino acid metabolism but did not decrease [^{3}H]TdR incorporation into TCA-insoluble material. These data suggest that the thymidine uptake method may drastically underestimate bacterial production in samples where SO_4^{2-} reduction is quantitatively important, such as nonsurficial marine and estuarine sediments and anoxic saline waters.

Measurement of the incorporation of exogenous tritiated thymidine into DNA by aquatic bacterial communities is becoming a standard technique for estimating bacterial productivity. Implicit to use of the TdR incorporation method is the assumption that all of the metabolically active bacteria in the ecosystem of interest are assimilating exogenous TdR into DNA. This assumption has proven valid for several aquatic systems, particularly oxic seawater (Fuhrman and Azam 1982; Moriarty 1986). Measurements of TdR incorporation by cells in the anoxic hypolimnion of a freshwater lake (McDonough et al. 1986) and in muddy sediments (Fallon et al. 1983) suggest, however, that anaerobic bacteria take up less exogenous TdR per mole of DNA produced than do aerobes, and that a smaller percentage of TdR taken up is incorporated into DNA.

During a study of the spatial and temporal distribution of bacterial activity in surficial Boston Harbor sediments, in which [^{3}H]TdR uptake was used as a measure of bacterial activity, we encountered several sediment sites that were anoxic and sulfitic to within millimeters of the sediment surface. A suggestion by Moriarty (1984) that SO_4^{2-}-reducing bacteria (SRB)—dominant bacteria in this type sediment—do not seem to take up exogenous thymidine prompted us to test that hypothesis. SO_4^{2-} reduction can account for the majority of C flux from shallow-water marine sediments (Martens and Klump 1984) so that measurement of the productivity of these microorganisms must be included in any estimate of microbial production in such environments. Moriarty (1984) suggested that anaerobes with strict nutrient requirements may lack transport systems for TdR, citing in particular a personal communication from G. W. Skyring

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Table 1. Characteristics of the SRB species used to test [^{3}H]TdR uptake. All species were isolated from midsalinity Chesapeake Bay sediments.

		Growth substrates*										
		$+SO_4^{2-}$								$-SO_4^{2-}$		
Isolate	Tentative identification	lac	fum	frm	pyr	mal	EtOH	ace	other	lac	pyr	Morphology
B203	*Desulfovibrio* sp.	+	+	+	+	+		−		−	+	Tiny vibrio
C106	*Desulfovibrio* sp.	+	+	+	+	+	+	−		−	+	Vibrio
C107	*Desulfovibrio* sp.†	+	+					−			+	Tiny rod
C202	*Desulfotomaculum* sp.	+		+				−			+	Vibrio, spores
C204	*Desulfovibrio* sp.	+	+	+	+		+	−		+	+	Vibrio
ND132	*Desulfovibrio* sp.‡	+	+	+		+	+	−		−	+	Vibrio
T2	*Desulfovibrio* sp.§	+			+			−	palmitate	−	+	Bulbous vibrio
W3A	*Desulfobacterium* sp.	+						+		−	−	Rods, chains
X2	*Desulfobacterium* sp.	+		+	+	+	+	+		+	−	Fat rods
X8	*Desulfovibrio* sp.	+		+	+	+	+		benzoate	−	−	Fat rods

* +, growth; −, no growth; blank, not tested.
† 91% sequence homology (16S RNA) with *D. salexigens*.
‡ 90% sequence homology with *D. africanus*.
§ 92% sequence homology with *D. desulfuricans aestuarii*.

that *Desulfovibrio* does not appear to utilize exogenous TdR.

In order to assess the applicability of the [^{3}H]TdR uptake method to bacterial activity measurements in sulfitic sediments, we examined the ability of SRB to take up exogenous TdR, both in pure cultures and in sediments. Specific inhibitors were used to assess uptake by SRB and methanogens. In no case did we find evidence of significant thymidine incorporation into macromolecules by SRB.

The SRB examined in pure culture (Table 1) were originally isolated on various organic substrates from sediments in the mesohaline region of Chesapeake Bay. Cultures were maintained with strict anaerobic techniques on a modified Starkey's medium with estuarine salts under O_2-free N_2 (Gilmour et al. 1987) and given lactate, formate, fumarate, or ethanol as the C source. Resazurin was used as an indicator of anaerobiosis in cultures and during manipulations.

[^{3}H]TdR uptake experiments were performed under various culture conditions. "Low" organic C experiments were performed in media containing 1 mM organic C substrate without yeast extract (YE) but with added vitamins (Laanbroek and Pfennig 1981). "High" organic C experiments were performed at 20 or 31 mM C, plus 0.1 g liter^{-1} of YE.

Thymidine incorporation assays for pure cultures were performed in sealed serum bottles containing 25 or 50 ml of culture under O_2-free N_2. A sterile aqueous solution of [*methyl*-^{3}H] thymidine (2.96 GBq mol^{-1}) was added to sealed cultures by syringe after the onset of logarithmic growth, and incubations were carried out for 4–24 h at 27° or 30°C. After incubation, the entire culture volume was mixed with two volumes of ice-cold 10% trichloroacetic acid (TCA). After 45 min on ice, samples were filtered through a 0.2-μm Nuclepore polycarbonate filter, washed twice with ice-cold 5% TCA, and air dried. Filters were then wet-combusted with 200 μl of perchloric acid and 400 μl of hydrogen peroxide at 75°C for 1–2 h.

Cultures tested under conditions of low organic C received [*methyl*-^{3}H]TdR to a final concentration of 3 nM; high organic C cultures received 10, 50, or 100 nM [^{3}H]TdR. Cell growth was monitored by sulfide production, optical density of cultures, or acridine orange direct counts. [^{3}H]TdR added to cultures containing 2% buffered Formalin and to sterile medium represented killed controls and blanks, respectively. *Escherichia coli* K-12 cultured in nutrient broth (Difco) served as a positive control for [^{3}H]TdR uptake. Tritium levels in filtered and TCA-washed SRB cultures were generally only a few times greater than in killed controls, even after overnight incubations with dense cultures of log-phase cells.

The differential effects of inhibitors of specific bacterial activities on the uptake of [^{3}H]TdR and [^{14}C]amino acids and on

[$^{35}SO_4^{2-}$] reduction in sediments were examined in various depth horizons. Sediment samples were taken from a muddy intertidal site in Savin Hill Cove, adjacent to the University of Massachusetts in Boston Harbor. This site is influenced by pulses of untreated sewage outfall and has fine, organic-rich sediments (Shiaris et al. 1987). Sediment cores were collected by hand with 2-cm-i.d. polycarbonate tubing in May 1987. The water temperature at collection was 8°C, but incubations were carried out at 15°C. SO_4^{2-} concentration in the overlying water was 17 mM and decreased to 12 mM in pore water at a sediment depth of 10 cm, suggesting sediment SO_4^{2-} reduction at relatively slow winter rates. Sediments were reduced to within millimeters of the surface, however. Redox measurements with a Pt wire electrode gave Eh readings below zero at 1-cm depth. Cores were kept at in situ temperature until use.

Cores were sliced with depth into three sections. Each section was then homogenized and combined with similar sections from other cores. Sediments were mixed in order to compare treatments. The top sections contained the visibly oxidized (brown) surface layer (usually <1 cm), while the two bottom sections included sediments from 3 to 10 and 7 to 15 cm below the oxidized layer. All manipulations of the two bottom sections were performed under O_2-free N_2 inside an anaerobic chamber (Coy Laboratory Products) at room temperature; the top sections were manipulated under air. The average pore-water SO_4^{2-} concentration in the top two sections was 16 mM; the bottom section averaged 13 mM SO_4^{2-}. After vigorous stirring, aliquots from each section were separated for addition of inhibitors before various activity measurements. Sediments were amended with either $NaMoO_4$ or bromoethanesulfonic acid (BES) (or both) to a final pore-water concentration of 20 mM, with 2% Formalin, or with a comparable small volume of distilled water (five treatments). Additions were made assuming 50% porosity.

After a 30-min incubation with the inhibitors, subsamples were taken and amended with one of three isotopically labeled substrates. [^{3}H]TdR (2.96 GBq mol^{-1}) was added to obtain a final pore-water concentration of ~10 nM. We estimate this concentration to be 2–3 times the in situ TdR level, based on isotope dilution measurements (Leavitt 1988). [^{14}C] amino acids [NEC-445E amino acid mixture containing the L-forms of 15 amino acids, sp act 2.07 GBq (atom C^{-1}), New England Nuclear Corp.] were added to achieve about 9 μM total; 37 KBq of $^{35}SO_4^{2-}$ (14.8 GBq mol^{-1}) was added to each 5 ml of sediment. Amino acid additions should have swamped ambient amino acid levels, while SO_4^{2-} was a trace addition. Final sample incubation volumes were 2 cm^3 of sediment, with each inhibitor treatment-activity measurement combination made in triplicate.

Sediment samples for SO_4^{2-} reduction and thymidine uptake measurements were packed into 3-cm^3 cutoff syringes for incubation. Anaerobic samples were sealed with serum stoppers, allowing no headspace. Amino acid mineralization samples were incubated in test tubes with serum stoppers and a hanging center well for later CO_2 collection. Top-section sediments were incubated under air, and bottom sediments were incubated under O_2-free N_2. Based on previous experience with [^{3}H]TdR uptake by surface sediments from this site, we stopped "aerobic," top-section incubations after 1 h. Anaerobic incubations lasted 3 h. Uptake of [^{3}H]TdR (Leavitt 1988) and reduction of $^{35}SO_4^{2-}$ were linear through the endpoints for both incubation times.

SO_4^{2-}-reduction experiments were stopped by freezing intact samples. SO_4^{2-}-reducing activity was estimated by production of [^{35}S] acid-volatile sulfides (AVS). Although measurement of AVS underestimates true SO_4^{2-}-reduction rates (Howarth 1979; King et al. 1985), we felt that this measurement was sufficient for comparisons in the context of this study. Reduction of labeled SO_4^{2-} to acid-volatile sulfides was measured by sparging stirred, acidified samples (30 ml of deoxygenated 6 N HCl per sample) with O_2-free N_2 for 2 h. Sulfide was trapped in 25 ml of 10% zinc acetate plus 1 drop Antifoam B and measured by liquid scintillation analysis. All scintillation measurements were corrected for quench with external standards. Pore water was separat-

ed by centrifugation from aliquots of each of the three combined sediment sections, and SO_4^{2-} analyzed by the method of Howarth (1978). SO_4^{2-}-reduction rates were calculated according to Jørgensen (1978).

Amino acid uptake experiments were terminated by injecting 2 ml of 10% TCA into the sealed tubes. $^{14}CO_2$ resulting from mineralization of [^{14}C]amino acids was trapped over 18 h on filter-paper wicks saturated with ethanolamine. The wicks were placed in hanging center wells (Kontes) attached to the serum cap.

TdR incubations were stopped by slicing 2-mm sections from the syringe samples into 2 ml of cold 10% TCA. Samples were then processed in the same way as cultures. Activity was measured by liquid scintillation counting and corrected with [^{3}H]toluene as the internal standard. Incubation of [^{3}H]TdR with Formalin-killed control sediments resulted in an average of 158 dpm per sample.

After incubation with [^{3}H]TdR, ^{3}H levels in filtered and TCA-washed SRB cultures were generally only a few times greater than in killed controls, even after overnight incubations with dense cultures of log-phase cells (Table 2). [^{3}H]TdR additions were always made to batch cultures in early log phase, as determined by cell counts or optical density compared with previous growth curves in the same medium. Initial experiments were performed on cultures growing on 31 mM lactate, formate, fumarate or ethanol, plus 0.1 g liter^{-1} of yeast extract, with 2 nM added [^{3}H]TdR. In this medium, cellular growth was limited by SO_4^{2-} rather than C.

Because these cultures did not seem to take up [^{3}H]TdR, cells were then grown with lower concentrations of organic substrate (as little as 1 mM) in an attempt to reduce intracellular dilution of the label and to stimulate uptake of exogenous nucleosides. Cells were also grown in defined medium with vitamins to preclude possible addition of nucleosides in yeast extract. Additional cultures were incubated with higher levels of added [^{3}H]TdR (up to 100 nM) to minimize dilution of the label and possibly block de novo synthesis of TdR. None of these measures substantially increased the amount of label taken up by the cells, and there was no correlation of changes in apparent uptake rate with TdR or organic substrate concentration.

Although the amount of uptake was always very low, we used measured cell numbers to calculate the amount of [^{3}H]TdR taken up per cell (Table 2). Cell numbers at the beginning of incubation with [^{3}H]TdR were used to estimate moles [^{3}H]TdR taken up per cell. This approach gives an upper bound, as cell numbers increase during the long incubation times. In some experiments, cells were counted at the beginning and end of incubations, allowing calculation of number of cells produced per mole [^{3}H]TdR taken up.

When used as a positive control, *E. coli* accumulated [^{3}H]TdR about 10^4 times faster than SRB (Table 2). SRB assimilated an average of 9.2×10^{-24} mol [^{3}H]TdR cell^{-1} h^{-1}, while *E. coli* assimilated 6.5×10^{-20} mol TdR cell^{-1} h^{-1}. Data for [^{3}H]TdR uptake by *E. coli* fall within the large range of literature values for [^{3}H]TdR uptake by bacteria in natural waters, but uptake rates for SRB do not (Table 3). Although [^{3}H]TdR uptake per cell is a function of growth rate, the ~50-fold difference in average growth rate between *E. coli* and the SRB tested does not account for the 7,000-fold difference in TdR uptake rate.

The number of cells produced per mole TdR incorporated is a better value for comparison of [^{3}H]TdR uptake between cultures, as it theoretically should not change with growth rate of cells. At least 1,000 times more SRB cells were produced in culture per mole TdR taken up than would theoretically be produced if cellular DNA was produced solely from exogenous TdR. On the basis of an average procaryotic genome size of 2.6×10^{-15} g DNA cell^{-1} in seawater (Fuhrman and Azam 1982) and assuming 25% thymidine nucleotides, 3.8×10^{17} cells would be produced for every mole of TdR incorporated into DNA. For the SRB cultures tested, an average of 3.3×10^{23} cells were produced per mole TdR precipitated in cold TCA.

In practice, <100% of the exogenous TdR taken up by cells results in DNA production

Table 2. [^{3}H]TdR uptake and productivity conversion factors for SRB species growing in pure culture under a range of culture conditions.

Isolate	Added TdR* (nM)	Initial cell density (10^6 cells ml^{-1})	Generation time† (h)	TdR uptake rate (dpm h^{-1})‡	TdR uptake rate (10^{-24} mol cell^{-1} h^{-1})	Cell production [10^{22} cells (mol TdR)$^{-1}$]
B203	2	16		73	0.53	
	2	16		29	0.21	
	100	47		9	0.04	
	100	53		5	0.02	
Avg					0.2	
C106	2	6.8		262	4.33	
	2	6.8		23	0.37	
	50	3.0	13.3	90	6.76	0.64
	50	3.0	3.6	90	88.1	0.51
	100	20	7.9	8	0.10	162
	100	17	8.4	99	1.31	10.8
	100	0.2		1	0.01	
	100	51		0	0.00	
Avg					12.6	43.5
C107	10	170		0	0.00	
C202	2	7.1		50	0.80	
	10	170		24	0.03	
Avg					0.42	
C204	2	11		41	0.41	
	2	51		91	0.20	
	2	51		24	0.05	
	2	11		65	0.64	
	10	170		27	0.04	
	50	0.4	8.2	298	168.0	0.05
Avg					28.2	
ND132	100	170		5	0.01	
	10	170		6	0.01	
	100	13	4.3	11	0.01	255
	100	11		0	0.00	
Avg					0.07	
T2	2	12		30	0.27	
	2	12		19	0.17	
	50	0.4	13.0	151	85.0	0.053
	100	1.7	5.8	10	1.38	19.6
	100	2.6	8.7	45	3.86	3.48
Avg					18.1	7.71
W3A	10	170		88	0.11	
	100	2.9	5.1	45	3.49	9.95
	100	14	121	1	0.02	32.1
Avg					1.21	21.1
X2	50	2.0	6.4	142	16.0	0.83
X8	50	1.0	4.9	176	39.6	0.57
	100	5.6	29.8	18	0.74	3.6
	100	4.3	17.6	95	4.97	1.01
Avg			17.4	96	15.1	1.73
Overall SRB avg			17.2	48§	9.2§	33.3‖
Escherichia coli	2	100	<1	115,060	64,800	

* [^{3}H]TdR sp act 7.88 Ci mmol^{-1}, except 50 and 100 nM additions at 0.788 Ci mmol^{-1}.
† Generation times not measured for all incubations.
‡ Total [^{3}H]TdR uptake by 25 or 50 ml of culture, from 0.9 to 9 × 10^6 dpm added TdR.
§ Average derived from individual isolate averages (n = 10).
‖ Average of all measurements (n = 15).

Table 3. Conversion factors for calculating bacterial production from [^{3}H]TdR uptake.

Type of sample	10^{18} cells (mol TdR)$^{-1}$	10^{-20} mol TdR cell^{-1} h^{-1}	Reference
SRB cultures			
Range	522–2,550,000	0–0.017	This study
Avg	333,000	0.00092	This study
Escherichia coli		6.48	This study
Natural waters and sediments			
Range	1–68	0.05–450	Staley and Konopka 1985
Avg	2	1	Moriarty 1986

(Findlay et al. 1984; Carman et al. 1988), and intra- and extracellular isotope dilution occurs; both of these factors serve to increase the empirical conversion factor above the theoretical. Empirical conversion factors for bacterial communities in natural waters, however, are generally in the range of 1–9 × 10^{18} cells (mol TdR)$^{-1}$, although higher conversion factors have been measured for cells inoculated into filtered seawater (Kirchman et al. 1982) (Table 3). In contrast, SRB produced an average of 3.3 × 10^{23} cells (mol TdR)$^{-1}$—four orders of magnitude more cells per mole [^{3}H]TdR taken up than even eutrophic or enriched water samples. Empirical conversion factors for sediment bacteria are not readily available for comparison because direct determination of the increase in bacterial numbers in sediments over time is difficult. Findlay et al. (1984) noted, however, that bacteria from surficial sediments generally incorporate $>10^4$ dpm (0.1 mg dry wt cells)$^{-1}$ h^{-1} into DNA; our SRB cultures incorporated <25 dpm (0.1 mg dry wt cells)$^{-1}$ h^{-1}.

Intracellular isotope dilution may be greater in culture than in natural waters, but the lack of increased uptake of [^{3}H]TdR by SRB at higher added concentrations argues against isotope dilution as an explanation for the very high cell (mol TdR)$^{-1}$ conversion factors. Another possible explanation for low uptake is TdR catabolism during the long incubation times. Some shorter incubations were performed (4 h, *see Table 2*), however, without increased uptake.

Although reported conversion factors for production of bacteria based on [^{3}H]TdR incorporation vary somewhat among natural environments (Table 3), conversion factors do not seem to vary by a factor of >10 with medium, growth rate, or temperature for a given community (Riemann et al. 1987). For enriched and unenriched batch cultures of natural assemblages of coastal marine bacteria with generation times varying from 1 to >200 h, [^{3}H]TdR uptake averaged 1.1±0.05 × 10^{18} cells per mole [^{3}H]TdR incorporated into TCA precipitate. In a similar study of a freshwater bacterial community, conversion factors for populations with relatively fast growth rates (<20 h) averaged 10 times higher than conversion factors for slower growing cells (Smits and Riemann 1988). In contrast, conversion factors derived for SRB cultures with generation times of 4–120 h varied over more than three orders of magnitude, from 5.2 × 10^{20} to 2.5 × 10^{24}. We feel that this range is mostly noise in measurement of nonspecific background [^{3}H]TdR binding to cells and filters, as the variability represents a difference of only ~100 dpm h^{-1} of incubation.

The organic substrates used by SRB in this study may not be the major substrates used by SRB in marine sediments, although sediment studies described below support the conclusion that natural marine SRB populations do not assimilate significant amounts of exogenous TdR. SO_4^{2-}-reducing activity, amino acid mineralization, and thymidine uptake were measured in three depth horizons from an intertidal marine sediment, and the differential effects of inhibitors of SO_4^{2-} reduction (molybdate) and methanogenesis (BES) on each activity examined (Oremland and Capone 1988). As expected, overall bacterial activity as measured by either amino acid mineralization or thymidine uptake was highest in oxidized surficial sediment, while SO_4^{2-} reduction

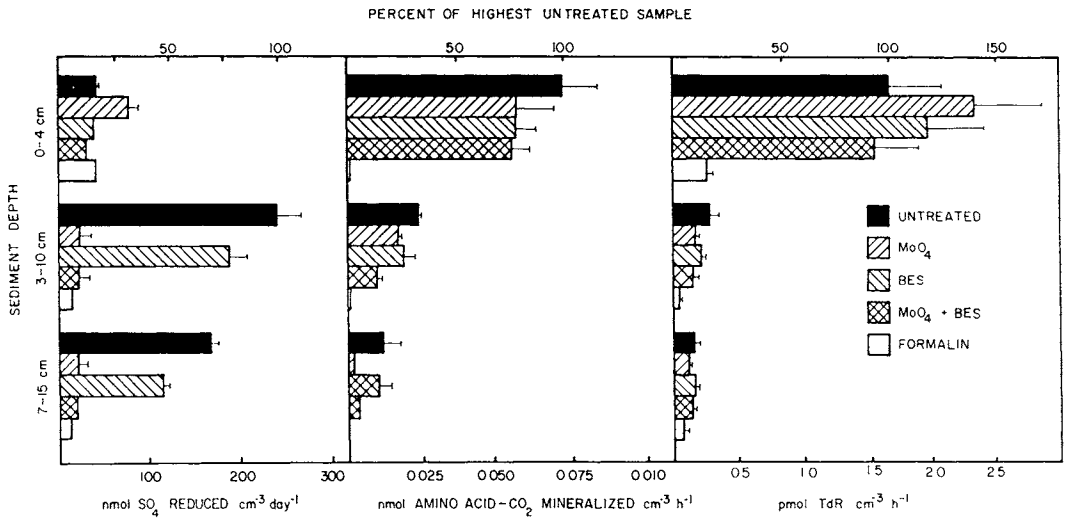

Fig. 1. Effect of specific inhibitors of SO_4^{2-} reduction and methanogenesis on rates of SO_4^{2-} reduction, amino acid mineralization, and thymidine uptake in aerobic and anaerobic sediments collected from Savin Hill Cove.

was higher in reduced, buried sediments (Fig. 1).

Addition of molybdate or BES to surface sediments resulted in no significant changes in any of the activity measurements, probably due to the lack of significant methanogenesis or dissimilatory SO_4^{2-} reduction in surface sediments incubated aerobically. Addition of molybdate blocked SO_4^{2-} reduction occurring in deeper sediments incubated anaerobically and also significantly reduced amino acid metabolism in both the 3–10- and 7–15-cm sediment horizons examined. BES did not significantly decrease amino acid mineralization at any sediment depth; either methanogens were not mineralizing amino acids in these sediments or, more likely, methanogens were not active in sediments where SO_4^{2-} reduction was proceeding.

Although SO_4^{2-}-reducing bacteria appeared to be important contributors to bacterial metabolism in the two deeper sediment horizons examined, molybdate did not significantly decrease the amount of thymidine uptake in deeper sediments. Also, the decrease in thymidine uptake rates with depth was much greater than the decrease in amino acid mineralization rates with depth. Amino acid mineralization in 3–10-cm sediments decreased to ~30% of its level in surficial sediment, while thymidine uptake was decreased to ~15% of its surficial sediment maximum. The decrease in TdR uptake was also greater between the surface and the 7–15-cm section than was the decrease in rate of amino acid mineralization. These data suggest that SO_4^{2-}-reducing bacteria take up exogenous TdR at rates lower than those of aerobic bacteria and possibly at lower uptake rates than other anaerobes. The greater decrease in thymidine uptake relative to amino acid mineralization in reducing sediments also suggests that anaerobes in general incorporate exogenous TdR inefficiently relative to aerobes.

Other studies suggest that TdR uptake per unit of production is decreased in anaerobic sediments and waters relative to aerobic systems. McDonough et al. (1986) reported that the TdR method seriously underestimated bacterial activity in the anoxic hypolimnion of Lake Oglethorpe, Georgia, although at least some proportion of the anaerobic community seemed to take up TdR. Fallon et al. (1983) concluded that estimates of bacterial productivity in nearshore marine and marsh sediments based on thymidine incorporation into DNA were reasonable with respect to oxygen uptake data for these sediments, but cell-specific uptake rates for thymidine were lower in anaerobic muds relative to aerobic sands (0.81 vs. 4.8×10^{-21} mol [^{3}H]TdR cell^{-1}

h^{-1}, respectively). Also, the ratio of the frequency of dividing cells to [^{3}H]TdR uptake was much higher in the mud than in the sand.

The finding that individual species or groups of procaryotes do not incorporate exogenous thymidine is not unique. Other procaryotes which do not take up TdR include cyanobacteria (Bern 1985), a few species of *Pseudomonas* (Ramsey 1974; Moriarty 1984) and five chemolithotrophs including NO_2^--, NH_4^+-, and CH_4-oxidizing bacteria (Johnstone and Jones 1989). In addition, fungi (Grivell and Jackson 1968) and other eucaryotic microorganisms, including algae (Moriarty 1984), seem to lack thymidine kinase. Cells lacking thymidine kinase do not incorporate TdR into DNA by salvage pathways, acquiring the nucleotide through de novo synthesis alone. It is possible that SRB are capable of TdR uptake but lack thymidine kinase, preventing incorporation of the nucleoside into DNA. The cold-precipitation step in our assay would remove any intracellular pools of labeled thymidine. The limited substrate range of most SRB suggests, however, that thymidine transport systems are not present.

In conclusion, these data suggest that total heterotrophic bacterial activity should not be measured with the [^{3}H]TdR uptake method in environments where SRB are important contributors to total microbial growth, including most estuarine and shallow-water marine sediments (other than surficial sediment flocs: Fallon et al. 1983; Bell and Ahlgren 1987), anoxic saline waters, and sediments of freshwaters in which SO_4^{2-} levels are raised. Use of thymidine uptake in these systems may grossly underestimate bacterial production, depending on the relative amount of SO_4^{2-}-reducing activity in the sample. Most of the cultures listed in Table 1 assimilate exogenous leucine (Henry and Gilmour unpubl. data), however, suggesting that [^{3}H]leucine incorporation (Kirchman et al. 1985) might be used as a measure of bacterial protein synthesis in sulfitic sediments. Lack of TdR incorporation by heterotrophs with limited substrate utilization capacity suggests that the capability for TdR incorporation by methanogens and acetogens should be checked if the technique is to be applied to anoxic freshwater systems.

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