Nitrogen Cycling in Coastal Marine Environments
Edited by T. H. Blackburn and J. Sørensen

CHAPTER 7

Distribution and Metabolism of Quaternary Amines in Marine Sediments

GARY M. KING

7.1 INTRODUCTION

The metabolism of organic nitrogen in most ecosystems is typically discussed in terms of primary or secondary amines, with amino acids and proteins receiving the greatest attention. This focus is largely due to the fact that amino acids and proteins constitute a major fraction of organic nitrogen in most organisms. Other pools, such as nucleic acids, lipids or polysaccharides, are usually only a small fraction of the total. Notable exceptions include organisms with an extensive chitin exoskeleton; however, even in these organisms the nitrogen is contained in a form similar to that of proteins.

While primary amines are of obvious importance in the organic nitrogen cycle, it has recently become apparent that another class of organic nitrogen may be of significance as well. Surveys of a large diversity of marine organisms have established that alkyl amines, and quaternary amines (QA) in particular, are nearly ubiquitous within the marine biota, and in some cases are present at concentrations rivaling those of the most abundant amino acids (Wyn Jones and Storey, 1981; Yancey *et al.*, 1982). The major function of these compounds is supposedly in osmoregulation (Hochachka and Somero, 1984). Like several amino acids, QAs are accumulated by cells in response to salinity or water stresses. They are accumulated presumably because they are compatible solutes which do not disrupt protein structure or inhibit enzyme activity at high concentrations. QAs, with the exception of choline, do not contribute to structural polymers as do amino acids, and exist almost entirely in intracellular solution. It has also become apparent that the patterns of metabolism of QAs differ from those of amino acids. Of particular interest are the relationships between QAs and fluxes of gases to the atmosphere. QA metabolism may be responsible for the fluxes from marine systems of several volatile amines (e.g. methyl-, dimethyl-, and trimethylamine) and more importantly, methane (King,

1984). The metabolism of analogs of QAs is also responsible for fluxes of dimethylsulfide, and for at least one component of arsenic biogeochemistry. Thus, QAs and their analogs represent a somewhat unique and relatively unstudied component of the nitrogen cycle. The general significance of these compounds in marine ecosystems is as yet unclear, but it is likely that they are important in coastal systems which experience extremes in salinity and which tend to have a relatively high biomass of QA-containing organisms.

The subject of this paper then, will be the distribution and significance of QAs (and related compounds) in coastal ecosystems. There will be no attempt to review thoroughly the literature associated with the topic; instead an effort will be made to discuss some of the more pertinent papers related to various biochemical, physiological and ecological problems. Attention will be given to the role of QAs in osmoregulation and the utilization of QAs as osmotica by a wide diversity of the marine biota since this forms the basis for any ecological significance. The discussion of ecological aspects of QAs will be drawn from studies in intertidal systems, such as mudflats and saltmarshes, with which the author is most familiar. An emphasis will be placed on pathways of microbial metabolism, especially anaerobic metabolism, and the involvement of methanogenesis. Within the constraints of the available data, two questions will be examined from an ecosystem perspective: (1) where and under what conditions are QAs important? (2) what pathways are involved in the utilization of QAs? The answers to these questions will be necessarily speculative, in part due to the lack of definitive research.

7.2 QUATERNARY AMINE CHEMISTRY

Before discussing the biology and ecology of quaternary amines it is useful to consider their chemistry at least briefly, since it differs markedly from the more familiar primary amines. QAs by definition consist of nitrogenous compounds in which the nitrogen atom is substituted by four alkyl or aryl groups (Miller and Springall, 1966; Allinger *et al.*, 1971). The resulting compounds have no unshared electron pairs as do other amines, are ionic with a permanent positive charge residing on the nitrogen atom, are very soluble in aqueous solutions and insoluble in ether, and participate in a much more restricted range of reactions than other simpler amines (Miller and Springall, 1966; Allinger *et al.*, 1971).

A characteristic reaction of many QAs is the formation of trimethylamine (TMA) and an olefin via the Hoffman elimination (eqn. (1); Miller and Springall, 1966; Allinger *et al.*, 1971). The presence of a α-hydrogen on one of the substituent groups promotes a base-catalyzed elimination resulting in the least, rather than the most, substituted olefin as is usually observed in the reactions of alkyl halides. Formation of the least substituted product is due in part to the fact that the trimethylamino function is a poor leaving group, and that the direction of the substitution depends on the acidity of the α-hydrogen. An example of this reaction

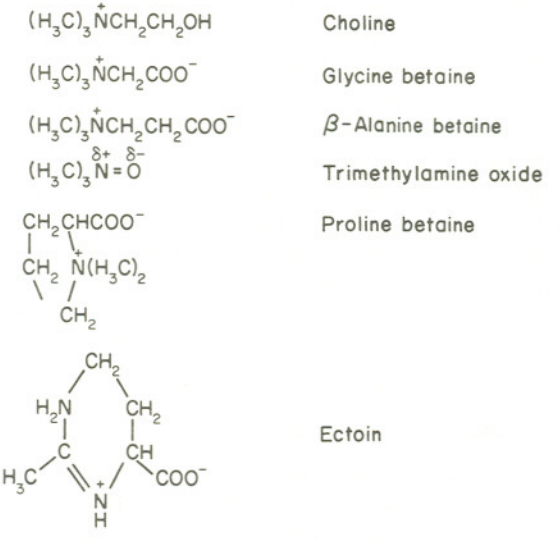
is the formation of TMA and acrylic acid by base hydrolysis of β-alanine betaine (eqn. 2). Note that glycine betaine (Figure 7.1) forms TMA and ethenoic acid in only relatively low yields on base hydrolysis due to the absence of a α-hydrogen. The Hoffman elimination also occurs with the related sulfonium compound dimethylsulfoniopropionate (DMSP, Figure 7.1) resulting in dimethylsulfide and acrylic acid (White, 1982 and references therein). DMSP, which is an osmoticum, has been proposed as the major precursor of dimethylsulfide in marine systems (e.g. Andreae and Barnard, 1984).

$$B + R{-}C{-}C{-}NR_3 \rightarrow BH + R{-}C{=}C + NR_3 \quad (1)$$

$$OH{-} + {-}O_2C(CH_2)_2N(CH_3)_3 \rightarrow H_2O + {}^{-}O_2CCH{=}CH_2 + N(CH_3)_3 \quad (2)$$

The structures of some QAs of special interest in biological systems are indicated in Figure 7.1. In general, the QAs possess a trimethylamino function and a fourth aliphatic substituent. However some significant exceptions, such as the stachydrines, exist as cycling structures. In general, the most commonly encountered QAs, with the exception of choline, are related structurally to the amino acids.

(a) Quaternary amines:



(b) Related compounds:

Figure 7.1. Structures of quaternary amines and related compounds of ecological significance

The derivatives of glycine, alanine and proline are well documented (Wyn Jones and Storey, 1981). These particular QAs are known generically as betaines, with the parent amino acids serving as a modifier (thus, glycine betaine, β-alanine betaine, etc.). Most of the QAs are dipolar, as are amino acids, but are electrically neutral at all pH values greater than 3–4 since the trimethylamino function is permanently charged. This feature of the betaines accounts in part for their role in osmoregulation (Section 5). Although most of the biologically important QAs have been described for some time, H. Truper and co-workers (pers. commun.) have recently reported the structure of a new and unusual molecule, ectoin (Figure 7.1), accumulated by an *Ectothiorhodospira* spp.; this unique QA apparently functions in osmoregulation. Other reported QAs include the betaine of taurine, and a variety of toxins and alkaloids that are widely distributed but usually present in only low concentrations (e.g. Fattorusson and Piattelli, 1980; Kanno *et al.*, 1984). Though interesting, these other amines will be ignored and further discussion will largely center on glycine betaine (GBT).

7.3 METHODS FOR THE ANALYSIS OF QUATERNARY AMINES

Because quaternary amines show only limited reactivity, specific analytical methods have been considerably more restricted than those for primary amines (Table 7.1). A number of methods have been recently developed that take advantage of the resolving power and sensitivity of gas and liquid chromatography, but again the options available to the analyst are few relative to those for other nitrogenous compounds. Further, it is only the HPLC methods that offer any significant convenience or flexibility. Unfortunately, the lack of a specific fluorimetric technique has limited the application of HPLC methods to samples with relatively high concentrations of QAs. Improvements in derivatization techniques and the application of other methods (e.g. ^{13}C NMR) promise to eliminate barriers that currently hinder ecological studies; however, for the moment, analysis of QA pools *in situ* remains difficult at best.

7.3.1 Ion exchange chromatography

The permanent positive charge residing on the trimethylamino group of most QAs is one convenient aspect of their chemistry that has been exploited for a number of methods, including HPLC. This charge allows QAs to be separated readily from most other nitrogenous compounds by simple ion exchange chromatography. Very commonly, tissues (or sediments) are extracted with alcoholic solutions, deionized water or various solvents (Barnes and Blackstock, 1974). QAs are subsequently purified and concentrated by passing the extracts through a strong anion exchanger (e.g. Dowex AG1, OH^- form) and then a weak cation exchanger (e.g. Amberlite IRC-50, H^+ form). The use of a strong anion and weak cation exchanger removes amino acids and most other anions and cations.

Table 7.1. Summary of analytical methods used for the determination of quaternary amines

Method	Procedure	References
A. Spectrophotometric	Analysis as Reinecke precipitate after dissolution in acetone	Strack and Schwaneberg, 1936; Focht *et al.*, 1956
	Analysis of Dragendorff complex	Wyn Jones and Storey, 1975
	Analysis of potassium triiodide complex	Wall *et al.*, 1969
	Analysis of *p*-dibromoacetophenone ester	Gorham *et al.*, 1982
B. Chromatographic	Pyrolysis GC	Hitz and Hanson, 1980
	HPLC–ion exchange	Gorham, 1984
	HPLC–ion pair (octane sulfonic acid) reverse phase	Warren and Pierce, 1983
	HPLC–amino-bonded silica	Vialle *et al.*, 1981
	TLC–silica gel and cellulosic phases	Blunden *et al.*, 1981; 1982
C. Other	Picrate analysis of TMA and TMAO	Barnes and Blackstock, 1974
	GC analysis of methylamines	King *et al.*, 1983; Lee and Olsen, 1984
	Ion exchange purification and concentration of QAs	Barnes and Blackstock, 1974

Separation schemes using strong cation exchangers (e.g. Dowex AG50, H^+ or Na^+ form) are also useful if individual QAs are to be purified (Charest and Dunn, 1984). Thus, ion exchange techniques provide a simple procedure for preprocessing samples for further analysis by one of several methods.

7.3.2 Spectrophotometry

One of the oldest and most widely used methods for the quantitation of QAs is based on the formation of precipitates by ammonium tetrathiocyanodiammonochromate or Reinecke salt (Strack and Schwanebert, 1936; Barnes and Blackstock, 1974). Reinecke salt precipitates primary, secondary, tertiary and quaternary amines, and can be used specifically for QAs after purification by ion exchange chromatography (Focht *et al.*, 1956; Carruthers *et al.*, 1960). Absorbance of the red reineckate precipitates is measured at 520 nm after washing and dissolution in acetone. Major drawbacks of this method include its tedious nature, lack of sensitivity, lack of specificity and relative inaccuracy. More recent spectrophotometric techniques have used reagents with

greater specificity and somewhat more sensitivity. Storey and Wyn Jones (1975) have described the use of Dragendroff's reagent (potassium iodide and bismuth suboxynitrate) to visualize QAs on TLC plates; the developed spots were removed from the plates and the absorbance of the QA complexes measured for a quantitative assay. The major advantage of this technique is the combination of TLC as a means to separate QAs, with Dragendroff's reagent to quantitate the individual compounds. The periodide method (Wall *et al.*, 1960; Storey and Wyn Jones, 1977) involves the non-specific formation of a potassium triiodide complex with QAs; absorbance of the complex is measured at 365 mm after extraction in α-1,2-dichloroethane. The method is relatively accurate but corrections must be made if GBT concentrations only, and not total QAs, are desired. This method has been used extensively for both bacterial and plant studies (e.g. Storey and Wyn Jones, 1977; Storey *et al.*, 1977; Cavalieri and Huang, 1981; Briens and Lahrer, 1982; Le Rudulier *et al.*, 1983; Bennert and Schmidt, 1984; Galinski and Truper, 1982; Kusel *et al.*, 1984; Popp *et al.*, 1984). An alternative procedure, that is reported as more highly specific and sensitive for GBT (Gorham *et al.*, 1982; Grieve and Mass, 1984), involves solvent partitioning of tissue extracts, purification by ion exchange chromatography, and then esterification with 2,4-dibromoacetophenone. Absorbance of the GBT ester is measured at 262 nm. Though an improvement in some respects, this technique requires a significant amount of sample handling and analysis time. It is not well suited for the routine analysis of large numbers of samples or environmental samples. Thus, like the other spectrophotometric methods, the esterification procedure has provided important insights about the distribution and biology of QAs but will probably be of limited use in ecological work.

7.3.3 Chromatographic methods

The spectrophotometric techniques are currently being replaced by several chromatographic methods which offer numerous advantages including more rapid analysis times, greater sensitivity and specificity and increased flexibility. Until recently, however, paper and thin-layer chromatography were the only procedures in common use. Separations of QAs by TLC have been employed extensively for the analysis of plant and animal tissue extracts (Robertson, 1961; Beers, 1967; Lahrer and Hamelin, 1975; Storey and Wyn Jones, 1975, 1977; Blunden *et al.*, 1981, 1982; Cavalieri and Huang, 1981; Briens and Lahrer, 1982; Hanson and Rhodes, 1983). In general the TLC separations are adequate for most purposes; in fact, Blunden *et al.* (1981) have evaluated nine different solvent systems used on silica gel and cellulose plates, and shown that the resolution for the more common QAs is adequate for both qualitative and quantitative needs. But in spite of its advantages, TLC is still time-consuming and too insensitive for ecological needs.

A pyrolysis gas chromatrographic procedure has been described by Hitz and

Hanson (1980) This method appears specific for GBT and published conditions allowed determination of as little as 2 nmol. The major pyrolysis product, trimethylamine, was formed in yields of 42–51%, which are thought to be maximal. The technique could be readily applied to a variety of sample types and may be especially suitable for tissue extracts purified by ion exchange chromatography. Ecological samples (e.g. porewaters or sediment extracts) could also be assayed effectively, though some concentration might be necessary. The analysis time is rapid and sample handling minimal. The major limitation, other than the requirement for a pyrolysis gas chromatograph, is that concentrations for only one QA, GBT, are obtained.

HPLC provides the most sensitive and versatile methods for the assay of both tissues and natural samples. Published reports have described the use of amino-bonded silica and silica phases (Vialle *et al.*, 1981), ion-pair reverse phase chromatography (Warren and Pierce, 1982); ion exchange chromatography (Gorham, 1984), and columns designed for carbohydrate analysis (Linden and Lawhead, 1975, Guy *et al.*, 1984). All have proven successful to a greater or lesser extent, depending on the needs of the analyst. In this author's laboratory, both ion-pair and ion exchange techniques have been evaluated for various types of samples, including porewaters. The scheme followed in the analyses is indicated in Figure 7.2. Of the two methods, the most reliable has been ion exchange chromatography using a mobile phase of 0.01 M KH_2PO_4 at a pH of 4.6 and a

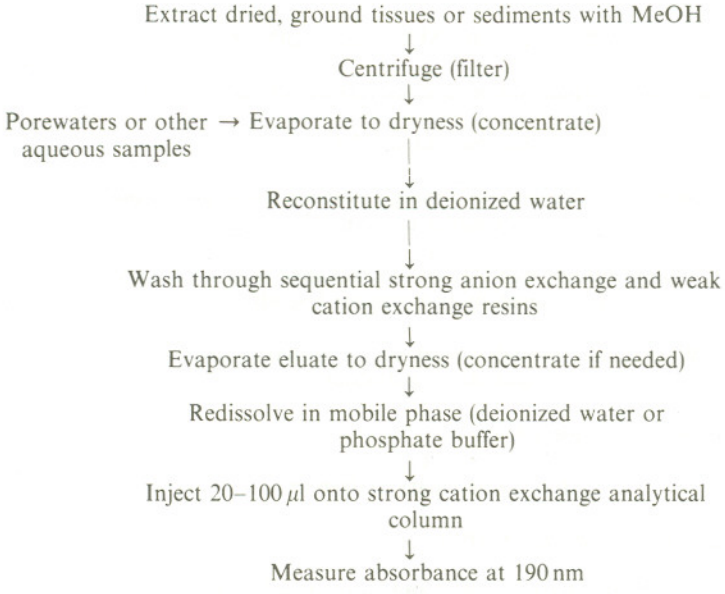


Figure 7.2. Analytical scheme for HPLC analysis of GBT and related quaternary amines

25 cm × 4.7 mm ID column containing a strong cation exchange resin. GBT, as well as a number of other significant QAs, are resolved in approximately 20 min. The effective lower limit of detection for GBT using a UV monitor at 190 nm is about 200 pmol (equivalent to the injection of 20 μl of 10 μmol/1 GBT). Tissue extracts can be injected directly after purification by ion exchange. Analysis of environmental samples requires a 10–100-fold concentration to insure adequate sensitivity. Greater sensitivity may be achieved through the formation of either benzyl esters (detected by UV absorption) or fluorescent coumarine derivatives. Both are currently being examined by the author for use with natural samples.

7.3.4 Radiotracer methods

Although radiotracers are generally powerful tools, there have been only limited reports of the use of radiolabeled QAs in either physiological or ecological studies (Rafaeli-Eshkol and Avi-Dor, 1968; Hitz and Hanson, 1980; Hanson and Rhodes, 1983; Charest and Dunn, 1984; Guy *et al.*, 1984; King, 1985). The reason for this may be that radiotracers such as [^{14}C]GBT are not routinely available

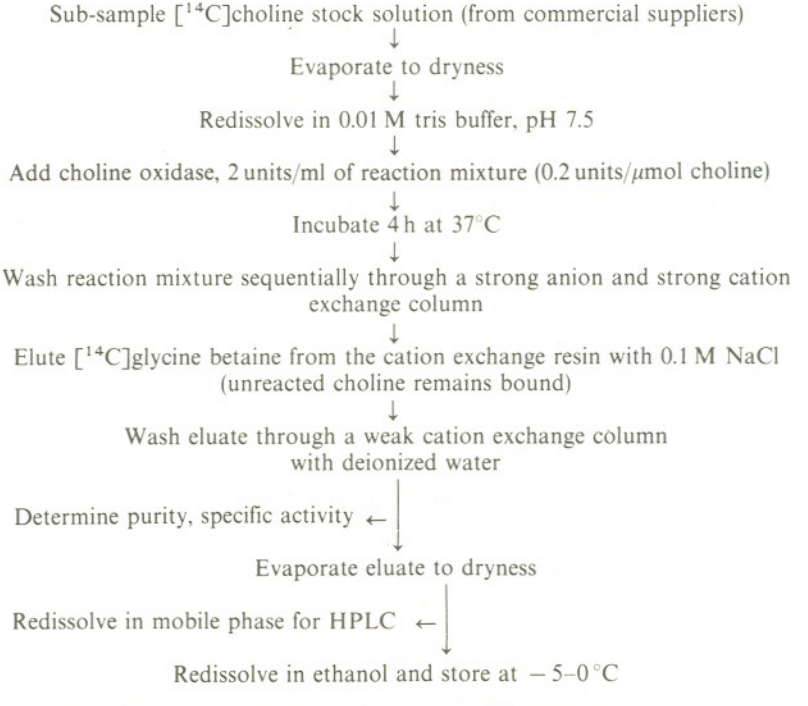


Figure 7.3. Scheme for synthesis of [^{14}C]glycine betaine

from commercial sources, and the expense of a custom synthesis is often prohibitive. Recently, King (1985, 1987) described a simple, inexpensive method for the synthesis of specifically labeled GBT (Figure 7.3). Radiolabeled choline, which can be obtained from the major radionuclide suppliers, serves as a precursor for radiolabled GBT. Stock solutions of labeled choline, with or without added carrier, are oxidized using the enzyme choline oxidase (also routinely available). This enzyme produces GBT efficiently, with only choline or GBT aldehyde as possible by-products. Radiolabeled GBT can be separated easily from both choline and GBT aldehyde using ion exchange resins (Charest and Dunn, 1984). Yields of [^{14}C-methyl]GBT are about 80% with a chemical purity > 95% as indicated by HPLC and TLC. Use of [^{14}C-methyl]GBT formed in this manner for analysis of sediment metabolism has verified earlier reports that were based on stable GBT at relatively high concentrations (King, 1985). Greater access to radiolabeled GBT should significantly enhance physiological and ecological studies of GBT metabolism.

7.3.5 Other techniques

The methods referenced above are generally applicable to a variety of QAs and in some cases tertiary amines (e.g. TMA) as well. For example, ion exchange methods (Charest and Dunn, 1984) have been used to separate choline, GBT and TMA. TMA can also be separated and identified using TLC. In addition, though, there are several methods that have been used specifically for TMA and TMAO, both of which are related metabolically to the QAs GBT and choline, and both of which may play an important role in the nitrogen cycle of some marine ecosystems. TMA and TMAO have been estimated by a number of methods, some of which are summarized by Hebard *et al.* (1982). Of the spectrophotometric methods, the use of picrate as a chromogen for TMA (and TMAO after reduction by titanous chloride) appears the most reliable (Barnes and Blackstock, 1974). Gas chromatography offers the most sensitive and accurate estimations, however. Several GC techniques have been described for the analysis of TMA (e.g. King *et al.*, 1983; Lee and Olsen, 1984; Glob and Sørensen, in press) that are appropriate for micromolar concentrations and thus suitable for natural samples. The most significant problems associated with these methods are the losses of TMA that occur by adsorption to glass or metal surfaces; adsorption appears particularly troublesome at very low (sub-micromolar) concentrations (Lee and Olson, 1984). Otherwise, the GC analysis of TMA is rapid, precise and can be tailored to numerous sample types. Gas chromatography is also well suited for the analysis of dimethylsulfoniopropionate (DMSP), an osmoticum that is accumulated analogously to some QAs. DMSP is readily hydrolyzed in a basic solution resulting in the formation of dimethylsulfide (DMS) and acrylic acid; this hydrolysis occurs by the Hoffman elimination as discussed above (White, 1982). The DMS produced by the elimination can be effectively analyzed

by GC with flame ionization or flame photometric detection. DMSP can also be analyzed by ion exchange HPLC as described above for GBT (Gorham, 1984).

7.4 DISTRIBUTION OF QUATERNARY AMINES AND RELATED COMPOUNDS

Quaternary amines, TMAO and TMA are widely distributed in the marine biota (Yancey *et al.*, 1982). The data base for these compounds is considerable and includes analyses of bacteria, algae, higher plants, invertebrates and vertebrates. The impetus for most of the survey work is due to the importance of QAs in osmoregulation and the role that TMAO and TMA play in the spoilage of commercially important fish and shellfish. The extent of the data is sufficient to identify QAs, TMAO and TMA as major components of the non-protein nitrogen content of many organisms. Thus it is evident that discussion of the dynamics of organic nitrogen in marine systems can no longer be strictly limited to amino acid pools. It is also evident, though, that the distribution and patterns of metabolism of QAs and related compounds may be less predictable than that of proteins or amino acids due to significant variations among species, within species and as a function of season and salinity. Therefore detailed ecosystem models of organic nitrogen may have to await more specific studies of QA dynamics.

7.4.1 Bacteria and cyanobacteria

QAs such as choline and carnitine are ubiquitous due to their role in lipid metabolism. However, these compounds are usually present in relatively small concentrations and do not contribute significantly to pools of organic nitrogen. Other QAs have been observed in bacteria, but too few studies have been conducted for any definitive conclusions. At present, though, it appears that halotolerant and halophilic bacteria contain relatively lower concentrations of QAs than their eukaryotic counterparts (Yancey *et al.*, 1982). The best-characterized halophilic bacteria—which belong to the genera *Halobacterium, Halococcus* and *Halomonas*—accumulate potassium ions or, in some cases, amino acids, for osmoregulation. Some non-halophilic and halotolerant bacteria also accumulate inorganic ions and amino acids (Tempest *et al.*, 1970; Measures, 1975; Vreeland *et al.*, 1983), but again, QAs are not as widely observed as they are for halotolerant (e.g. marine) eukaryotes. The major exceptions to this rule thus far are the halophilic photosynthetic bacteria of the genus *Ectothiorhodospira*, which accumulate up to 800 μmol/gdw of GBT (Table 7.2 and Galinski and Truper, 1982); a new and unusual QA, ectoin (Figure 7.1), has also been observed in high concentrations from *Ectothiorhodospira* spp. (H. G. Truper, personal communication). A variety of other bacteria are reported to utilize QAs in osmoregulation if provided as an exogenous solute (Rafaeli-Eshkol and Avi-Dor,

Table 7.2. Concentrations of methylated amines in various bacteria, cyanobacteria and algae; TMA, TMAO, GBT, PBT and QA refer to trimethylamine, trimethylamine oxide, glycine betaine, proline betaine and total quaternary amines respectively

Organism	Concentration	Salinity	References
Bacteria			
Ectothiorhodospira halochloris	0.9–1.6 (GBT)	0.5–1.0 M NaCl	Galinski and Truper, 1982
Rhizobium meliloti	70–300 (GBT)	0–0.65 M NaCl	Le Rudielier *et al.*, 1983
	350–980 (PBT)		
Cyanobacteria			
Aphanothece halophytica	0.3–0.9 (ttl QA)	0.35–2.1 M NaCl	Reed *et al.*, 1984
Coccochloris elabens	0.5–1.3 (ttl QA)	0.7–2.8 M NaCl	Reed *et al.*, 1984
Dactylococcopsis salina	0.6–1.5 (ttl QA)	0.7–2.8 M NaCl	Reed *et al.*, 1984
Synechocystis DUN52	0.5–1.6 (ttl QA)	0.35–2.8 M NaCl	Reed *et al.*, 1984
Algae	684.5 (TMA)	—	Fujiwara-Arasaki
Porphyra suborbiculata	743.0 (TMAO)	—	and Mino (1972)
	37.2 (TMA)	—	Fujiwara-Arasaki
Porphyra tenera	40.4 (TMAO)	—	and Mino (1972)
	1152.5 (TMA)	—	Fujiwara-Arasaki
Codium fragile	732.5 (TMAO)	—	and Mino (1972)
	18.8 (TMA)	—	Fujiwara-Arasaki
Codium divaricatum	12.0 (TMAO)	—	and Mino (1972)

The reported salinity values refer to the NaCl content of the growth media. Concentrations for bacteria and cyanobacteria are given in mol/kg cytoplasmic water with the exception of *R. meliloti*, where QAs are reported as μmol/g dry weight protein. Concentrations for algae are in μmol/g dry weight of tissue.

1968; Le Rudulier *et al.*, 1983, 1984). The addition of GBT, γ-butyrobetaine and stachydrine have been noted to increase the salinity tolerance of *Salmonella typhimurium, Klebsiella pneumoniae* and *Rhizobium meliloti*, even though these bacteria apparently do not produce QAs (Le Rudulier and Bouillard, 1983; Le Rudulier *et al.*, 1983, 1984). Thus, while QAs may serve as osmotica, they are probably not accumulated under normal circumstances. Broader surveys of the bacterial populations in a variety of marine and hypersaline environments are obviously needed to establish the general significance of QAs.

Another group of prokaryotes, the cyanobacteria, also appear to accumulate QAs to a limited extent. Mackay *et al.* (1983) have suggested that osmoregulatory solutes can be used as a taxonomic tool to distinguish 'truly' marine cyanobacteria from non-marine and hypersaline forms. The truly marine forms are

hypothesized to accumulate glucosylglycerol, while those characteristic of hypersaline environments accumulate inorganic ions, QAs or both. Reed *et al.* (1984) have confirmed this notion in part by demonstrating that four cyanobacteria isolated from various hypersaline environments accumulate high concentrations of GBT (Table 7.2). Again, this pattern is more restricted than in the eukaryotic marine biota, which accumulate QAs at much lower salinities. However, surveys of the marine cyanobacteria are insufficient as yet to make firm generalizations.

7.4.2 Plants and algae

There are several reports which document the presence of QAs in various marine algae (e.g. Fujiwara-Arasaki and Mino, 1972; Blunden *et. al.*, 1981, 1982 1983). The patterns observed by Blunden *et al.* (1981, 1982) have indicated that QAs may be of some taxonomic value; 4-hydroxystachydrine appeared characteristic of *Gigartina* and *Ceramium* spp.; stachydrine characteristic of *Chaetomorpha* and *Bryopsis* spp.; GBT associated with *Cladophora* spp., and DMSP with the Ulvaceae. Blunden *et al.* (1982) did not report QA concentrations, however, so it is difficult to determine the significance of the various pool sizes to nitrogen metabolism. It has been known for some time, though, that DMSP is present in significant concentrations in *Ulva lactuca* and *Enteromorpha* spp., where it serves as an osmoticum (and Dickson *et al.*, 1980; White, 1982). Similar situations may exist for QAs in other species. Fujiwara-Arasaki and Mino (1972) have reported concentrations of TMA and TMAO in a number of red, green and brown macroalgae (Table 7.2). These compounds are most abundant in the reds, then the browns and greens. The observed concentrations suggest a possible role in osmoregulation, and that a significant fraction of the organic nitrogen may occur as tertiary or quaternary amines. The data, though limited, are indicative of the potential ecological importance of QAs in macroalage. Similar data are unavailable for unicellular or planktonic algae so it is difficult to extrapolate generally to marine systems. There are several observations which document the presence of DMSP in dinoflagellates of the genus *Phaeocystis* (e.g. Ackman *et al.*, 1966; Barnard *et al.*, 1984) but there are no broad-based comparative data on amines. If patterns from the cyanobacteria (Mackay *et al.*, 1983) can be extrapolated to the phytoplankton as a whole, it would appear that QAs may be somewhat more limited than in the macroalgae or in algae from hypersaline systems. Surveys of phytoplankton will be necessary to resolve this issue.

In contrast to the algae, there is a substantial data base on the distribution of QAs in higher plants. Many halophytes and glycophytes from a number of different families are known to accumulate a variety of QAs, with GBT being the most common (Table 7.3; Wyn Jones and Storey, 1981). Of interest here are those plants that occur in nearshore marine systems (e.g. saltmarshes and seagrass meadows). The genera *Aster, Atriplex, Avicennia, Beta, Chenopodium, Juncus,*

Table 7.3. Examples of concentrations of glycine betaine (GBT) and related osmoregulatory solutes in varied coastal and saltmarsh halophytes

Genus and species	Tissue	GBT (μmol/g dry weight)	References*
Aster tripolium	sh	164	(1)
Avicennia maritima	lv	265	(1)
Beta maritima	lv	195	(1)
Erigeron bonariensis	sh	68	(1)
Halimone portulacoides	sh	238	(1)
Juncus maritmus	lv	37 (TMOC)	(2)
	rhi	29 (TMOC)	(2)
	rt	39 (TMOC)	(2)
Limonium carolinianum	sh	2	(3)
L. vulgare	sh	50 (BABT)	(1)
Orbione sibirica	sh	153	(1)
Salicornia europea	sh	(45)	(1)
Salicornia spp.	sh	181–310	(3)
Spartina alterniflora	lv	160	(4)
	lv	80–212	(3)
	rt	44	(3)
Sp. cynosuroides	lv	5	(3)
Sp. patens	lv	27	(3)
Sp. townsendii	lv	141 (TMOC)	(2)
	rt	90 (TMOC)	(2)
Sporobolus virginicus	sh	101	(1)
Zostera marina	lv	12 (DMSP)	(5)

* (1) = Wyn Jones and Story, 1981; (2) = Briens and Lahrer, 1982; (3) = King, unpublished data, (4) = Cavalieri and Huang, 1981; (5) = White, 1982.

DMSP refers to dimethylsulfoniopropionate; BABT refers to β-alanine betaine; and (TMOC) refers to total methylated onium compounds. Numbers in parentheses are in μmol/g fresh weight.

Limonium, Posidonia, Salicornia, Scirpus, Spartina and Sueda, among others, contain significant amounts of QAs that are used in osmoregulation (Lahrer and Hamelin, 1975; Storey and Wyn Jones, 1975; Lahrer *et al.*, 1977; Storey *et al.*, 1977; Cavalieri and Huang, 1981; Wyn Jones and Storey, 1981; Briens and Lahrer, 1982; Cavalieri, 1983; Allaway *et al.*, 1984; Bennert and Schmidt, 1984; Guy *et al.*, 1984; Popp *et al.*, 1984; King, 1985). The seagrass, *Zostera marina*, is not reported to contain QAs but it is known to contain DMSP, an analogous compound (White, 1982). For some of these plants the concentrations of QAs are correlated with water stress, measured either as soil water (matric potential), soil or water salinity, leaf water potential, or tissue water osmotic potentials (e.g. Storey and Wyn Jones, 1975; Storey *et al.*, 1977; Cavalieri and Huang, 1981; Bradford and Hsaio, 1982; Cavalieri, 1983; Bennert and Schmidt, 1984; Guy *et al.*, 1984; Morgan, 1984). Cavalieri and Huang (1981) and Cavalieri (1983) have also demonstrated that the concentration of GBT in *Spartina alterniflora* is

controlled in part by salinity, as well as the availability of nitrogen; when salinity-stressed plants were fertilized with ammonium nitrate, both tissue GBT concentrations and growth were enhanced. These results indicate that edaphic factors exert a significant influence on the standing stocks of QAs within halophytes, and that nitrogen regimes within any given system may markedly effect plant productivity as well as osmoregulation. In coastal environments subject to eutrophication, then, at least some of the increased nitrogen may be consumed for the synthesis of osmotica, with the remainder supporting growth. This pattern could have important, unpredictable implications for the speciation and turnover of nitrogen.

An additional aspect of QA distribution that should be mentioned concerns the levels found in different tissues within a given species. Typically, the concentrations in roots are much lower than in leaves or stems (Storey and Wyn Jones, 1975; Briens and Lahrer, 1982; Cavalieri, 1983); conversely, polyols that are used in osmoregulation seem to be more concentrated in the roots than in leaves. Given the high root:shoot ratios observed for plants such as *S. alterniflora*, the actual standing stocks of QAs above and below ground may be similar, while there may be markedly higher polyol levels below ground. These patterns will have a significant impact on the pathways and dynamics of carbon and nitrogen mineralization in the rooted zone of coastal ecosystems.

7.4.3 Animals

An even more extensive literature exists for QAs in higher animals than exists for plants. This is due in part to the greater historical interest in higher animal osmoregulation. But even allowing for the more limited plant literature, it is apparent that QAs and related compounds are more widespread throughout the marine fauna (see Table 7.4 and Beers, 1967; Gilles, 1979; Hebard *et al.*, 1982; Yancey *et al.*, 1982; Bishop *et al.*, 1983; and Somero and Bowlus, 1983, for pertinent summaries). Various QAs or other methyl amines have been observed in coelenterates, mollusks, echinoderms, crustacea, elasmobranchs and teleost fish. Reported concentrations range from trace levels to significant components of the total nitrogen (Warren and Pierce, 1983; Yancey *et al.*, 1982). The concentration in a given species is a function of the ambient salinity, since QAs and methyl amines serve as osmotica. This is perhaps of greatest significance to the biota of coastal systems, where salinities are most subject to wide fluctuations. As with plants, then, edaphic factors are important determinants of animal nitrogen content and speciation. Of additional ecological significance are reports that at least some organisms (e.g. mollusks and teleost fish) depend on their diet for a source of QAs (Hebard *et al.*, 1982; Bishop *et al.*, 1981); biosynthetic pathways in such organisms are either limited or absent. Fluctuations in salinity would therefore appear to necessitate changes in the availability of dietary amines, and since cellular QAs decline during periods of exposure to lower

Table 7.4. Examples of the distribution and concentrations of quaternary amines and related compounds in various invertebrates and vertebrates

Organism	Concentration* GBT	Concentration* TMAO	References
A. Mollusca			
Geukensia demissus	186.5[a]		(1)
Littorina irrorata	271.7[a]		(1)
Mytilus edulis		17.1[b]	(2)
Sepia officinalis		105.4[b]	(2)
Pectin grandis		56.4–60[b]	(2)
B. Echinodermata			
Strongylocentrotus droebachiensis	10.2–15.2[b]		(3)
Cucumaria punctata	5.1–10.2[b]		(3)
C. frondosa		54.3–64.1[b]	(2)
Asteria vulgaris		14.3[b]	(2)
Stichopus japonicus (arsenobetaine)	41.6[c]		(4)
C. Arthropoda			
Uca pugnax	143.8[a]		(1)
Homarus americanus	> 15.2[b]		(3)
Nephrops norvegicus		80.0–85.0[b]	(2)
Paleomonetes vulgaris	10.2–15.2[b]		(3)
Squilla mantis		67.6–72.1[b]	(2)
Limulus polyphemus	601.7[a]		(5)
Cancer cancer		18.1–18.6[b]	(2)
D. Chordata			
Carcharhinus japonicus		131.3[b]	(2)
Raja hollandi		181.4[b]	(2)
Squalus acanthius		123.3[b]	(2)
Clupea harengus		52.9[b]	(2)
Gadus morhua		13.3–144.0[b]	(2)
Hippoglossoides dubius		14.7– 80.0[b]	(2)
Limanda herzensteini (arsenobetaine)	181[c]		(4)

[a] = μmol/g dry weight; [b] = μmol/g fresh weight; [c] = nmol/g dry weight.
(1) = King, 1985; (2) = Hebard *et al.*, (3) = Beers, 1967; (4) = Shiomi *et al.*, 1983; (5) = Warren and Pierce, 1982.

salinities (e.g. Dall, 1971; Warren and Pierce, 1982), organisms deficient in biosynthetic capability may lose nitrogen. This has not yet been substantiated, but deserves attention given the implication for nitrogen mineralization.

Of further ecological importance is the distribution of QAs within the functional groups of the biota of any particular system. If QA pools occur primarily in pelagic organisms, the pathways of metabolism and rates of turnover may be considerably different than if pools are primarily in benthic populations. To date there have been no detailed studies of QA distribution, so that generalizations are speculative at best. It is noteworthy, though, that QAs are more common in invertebrates, many of which are benthic in nature; in contrast, TMA and TMAO are more prevalent in elasmobranchs and teleosts. Thus the distribution and mineralization of methyl amines may differ considerably from QAs such as GBT. The impact of such differences on the nitrogen cycle of coastal ecosystems is unclear, however.

It is appropriate at this point to discuss the distribution of arsenobetaine. Several recent studies have documented the presence of high concentrations of organic arsenic in marine mollusks, echinoderms, crustacea and fish (Edmonds *et al.*, 1977; Edmonds and Francesconi, 1981; Edmonds *et al.*, 1982; Shiomi *et al.*, 1983; Maher, 1984). This arsenic exists as arsenobetaine (Figure 7.1), an analog of GBT. Arsenobetaine is presumably acquired from dietary sources and is accumulated along with GBT. It is not known if arsenobetaine serves any osmoregulatory function, if it is excreted, or if it is toxic. It is evident, however, that arsenobetaine can occur at high concentrations, and that the dynamics of this form of arsenic may mimic those of GBT. Of greatest concern is the potential biomagnification of arsenic in commercially important species used for human consumption (e.g. lobster). Of somewhat more academic interest is the possibility that arsenic biogeochemistry may be related to that of nitrogen (see Section 7.5.2).

7.4.4 Sediments

To date there have only been a few very limited analyses of QAs or related compounds in the water column or in sediments. Thus one can only offer a bit of speculative discussion. Oremland *et al.* (1982) measured TMA in slurries of saltmarsh sediments amended with fresh *Spartina foliosa* leaves; relatively high concentrations of TMA were observed (Table 7.5), suggesting that this amine might be a significant form of nitrogen in such systems. However, since the genus *Spartina* contains high concentrations of GBT (Table 7.4), it is possible that the observed values for TMA resulted from mineralization of the added plant material. Due to the potential importance of GBT input from *Spartina*, and the possible relationships with methanogenesis (Section 7.5.2), further study of saltmarshes should prove fruitful. King *et al.* (1983) and Sørensen and Glob (1987) have measured TMA concentrations in porewaters from surface sediments

Table 7.5. Distribution of glycine betaine and trimethylamine in various marine sediments (dissolved concentrations given in μmol/l; extractable and bound concentrations given in nmol/g dry weight)

Site	GBT	TMA	References
A. Dissolved			
1. San Francisco Bay saltmarsh slurries	—	0–1000	Oremland *et al.*, 1982
2. Lowes Cove, ME intertidal zone	—	2.2–2.4	King *et al.*, 1983
3. Buzzards Bay, MA	—	0.4–29	Lee and Olsen, 1984
4. East tropical North Pacific Ocean	—	0–0.14	Lee and Olsen, 1984
B. Extractable and bound			
1. Buzzards Bay, MA	—	93	Lee and Olsen, 1984
2. ETNP	—	0.5–7	Lee and Olsen, 1984
3. West End Salt Pond St Croix, USVI	0.1–84*	5–238	King, in press
4. Belle Barauch saltmarsh, SC	13–356	12–403	King, unpublished

* μmol/gdw.

of an intertidal mudflat. The observed values (Table 7.5) were lower than those reported by Oremland *et al.* (1982), possibly reflecting different experimental conditions and a more limited source of QAs. Lee and Olson (1984) have measured both DMA and TMA concentrations in subtidal sediments from Buzzards Bay and the eastern tropical North Pacific Ocean off the coast of Mexico. They observed higher DMA and TMA concentrations in surface sediments of Buzzards Bay than reported by King *et al.* (1983); lower values were observed from the North Pacific site (Table 7.5). In both sites the amines decreased with depth, DMA concentrations exceeded those of TMA, and there were significant pools of amines in exchangeable and fixed forms. It is of interest to note that neither King *et al.* (1983) nor Lee and Olson (1984) have observed monomethylamine, and that King *et al.* did not observe measurable concentrations of DMA. Differences among sites may be the result of differences in active metabolic pathways and amine sources. Comparisons among sites might well profit from some knowledge of the structure and biomass of benthic populations, so that sources as well as sediment pool sizes can be examined.

Data on pools of QAs in sediments are even more scarce than those for methyl amines. King (in press) has recently measured total extractable pools of GBT in sediments from a saltmarsh and from a hypersaline pond (salinity = 180). GBT concentrations were quite high in the surface sediments of the hypersaline pond, which consisted of an algal mat dominated by *Spirulina subsalsa*. In the upper 5 mm, GBT accounted for 18% of the total sediment

nitrogen. At deeper depths, GBT rapidly disappeared, and contributed only trival amounts to total nitrogen. TMA was also observed in the pond sediments. Concentrations decreased exponentially with depth and paralleled the distribution of GBT; maximum values at the surface were about 200 nmol/g dry weight. In the saltmarsh sediments examined, GBT was only observed in the upper 2 cm where concentrations were about 5 μmol/gdw. The upper 2 cm is the depth containing the highest amount of plant roots (Gallagher, 1974; Howes *et al.*, 1984) which are presumably the major GBT source.

Future studies must examine the distribution of QAs, such as GBT, and other amines in a greater variety of sediment. It will be necessary to determine the presence of exchangeable and fixed or tightly adsorbed pools, as well as free dissolved pools. The presence of these varied pools, along with questions about the state of the dissolved pool, will be critical for understanding rate measurements and amine dynamics. Again, ancillary data on benthic populations or other amine sources wil be important in understanding temporal and spatial patterns of distribution.

7.5 PHYSIOLOGY AND METABOLISM OF QUATERNARY AMINES AND RELATED COMPOUNDS

7.5.1 Laboratory studies

While quaternary amines have been recognized as significant components of some tissues for a number of years (Guggenheim, 1958), the function of these compounds has been clarified only recently. Earlier discussions postulated roles in nitrogen storage or methylation (Cromwell and Rennie, 1954; Cantoni, 1960). Both of these notions have been largely abandoned in view of the results from studies on osmoregulation in microorganisms, plants and animals. Within the past 20 years the role of QAs as compatible solutes has been firmly established (Yancey *et al.*, 1982). For reviews of some of the fundamental concepts involved in osmoregulation, the reader is referred to the following: Borowitzka (1981) and Griffin (1981) for treatments of water relations in cells; Borowitzka (1981), Hochachka and Somero (1984) and Somero and Bowlus (1984) for the biochemical basis of solute regulation; Griffin and Luard (1979) for water relations in microorganisms; Bradford and Hsiao (1982) and Morgan (1984) for water relations in higher plants; and Gilles (1979) for general aspects of osmoregulation in animals.

For the sake of brevity it will simply be noted here that QAs, amines, along with other organic solutes, are accumulated to partially offset the osmotic potential that arises from lower intracellular than extracellular sodium concentrations. Sodium is maintained at relatively low concentrations intracellularly due to its cytotoxicity. The accumulation of organic solutes provides a mechanism for controlling water fluxes across the cell membrane in the presence of rather

substantial sodium gradients. The properties that all of the organic compatible solutes share are: a negative or zero net charge at physiological pH; little or no perturbing effects on enzymatic activity or protein conformation; little or no perturbing effects on intermediary metabolism when present at high concentrations. These properties are found in only a relatively small number of polyhydric alcohols, amino acids and QAs; it is the combination of these characteristics which determines the significance of any given solute in osmoregulation.

The major QAs, GBT and TMAO, are both derived from choline in bacterial, plant and animal systems (Watts and Watts, 1974; Wyn Jones and Storey, 1981; Hochachka and Somero, 1984; Le Rudulier *et al.*, 1984), though in at least some of the fishes GBT and TMAO may be acquired form the diet (Hebard *et al.*, 1982). GBT is apparently formed by choline oxidase (Le Rudulier *et al.*, 1984) while TMAO may be formed from choline-derived TMA by the enzyme, TMA monooxygenase (Colby *et al.*, 1979; Anthony, 1982; Hebard *et al.*, 1982). Regulation of the synthesis of GBT and TMAO is not currently well known, though it is clear that tissue concentrations are a function of salinity or water stresses. It is interesting to note that GBT accumulates and disappears relatively slowly in *Spartina alterniflora* as salinity is increased or decreased respectively (Cavalieri, 1983); similarly, decreases in ambient salinity are accompanied by relatively slow decreases in tissue GBT in *Limulus polyphemus* (Warren and Pierce, 1982). These patterns are due to the presence of other compatible solutes, especially amino acids, that provide the primary short-term acclimation responses. The fate of GBT-nitrogen during downshifts in GBT concentration is not clear. Losses of fixed nitrogen would seem wasteful, but there is no evidence for storage. This is an area in which further study is warranted; analysis of intertidal or estuarine organisms that are exposed to varying salinity would be especially helpful.

In addition to the synthetic and degradative pathways briefly summarized above, there are several other aspects of QA metabolism that are important. For example, methyl amines can serve as sole energy sources for a variety of methylotrophic bacteria and yeasts. These chemoautotrophs couple methyl amine oxidation to CO_2 fixation. Metabolism occurs by successive demethylation resulting in the formation of formaldehyde and ammonia. Formaldehyde is further oxidized to CO_2 with the concomitant reduction of NAD^+ (Colby *et al.*, 1979). TMAO can also support methylotrophic growth in some bacteria (Colby *et al.*, 1979; Anthony, 1982) but, in addition, TMAO can serve as an electron-acceptor during respiratory metabolism by a number of facultative anaerobes (Ishimoto and Shimokawa, 1978; Strom *et al.*, 1979; Easter *et al.*, 1982). TMAO supports growth under both microaerophilic and anaerobic conditions with TMA as the end-product. The enzyme system, TMAO reductase, shares a number of features in common with nitrate reductase, but in general its characterization is incomplete (Easter *et al.*, 1982; Stryvold and Strom, 1984).

TMAO is also degraded by a poorly known system present in many fishes and invertebrates; this system results in the formation of DMA and formaldehyde as major end-products (Hebard *et al.*, 1982).

TMA and other simple amines have recently been reported to support the growth of some of the methanogenic bacteria (Hippe *et al.*, 1979; Patterson and Hespell, 1979; Konig and Stetter, 1982; Sowers and Ferry, 1983). The methyl amines are used to form methane, CO_2, and ammonia. The utilization of methyl amines as methane precursors appears limited to both marine and freshwater isolates of the genera *Methanosarcina* and *Methanococcus* (King, 1984). TMA metabolism apparently involves a sepcific TMA:HS-coenzyme methyltransferase that requires activation by a reducing agent, presumably produced *in vitro* by partial oxidation of TMA methyl groups (Naumann *et al.*, 1984). The capacity to use methyl amines has been hypothesized to account for the coexistance of sulfate-reducing and methane-producing bacteria in marine sediments, since methanogens compete effectively for these substrates (Oremland *et al.*, 1982; King *et al.*, 1983, 1984a,b).

The more complex QAs (e.g. GBT and choline) are also metabolized anaerobically. The fermentation of choline by members of the genera *Clostridium* and *Desulfovibrio* is well known (Hayward and Stadtman, 1959; Senez and Pascal, 1961; Bradbeer, 1965; Postgate, 1979; Fiebig and Gottschalk, 1984). End-products are TMA and either acetate or ethanol, depending on the availability of hydrogen 'sinks'. In the clostridia, choline fermentation occurs via the Stickland reaction, in which the oxidation of one organic is coupled to the reduction of a second; choline serves as the electron-acceptor in the pair. A similar reaction has been described for the metabolism of GBT by *Clostridium sporogenes* (Naumann *et al.*, 1983). Two other pathways have been described for anaerobic GBT metabolism. Müller *et al.* (1981) reported on the fermentation of GBT to N,N-dimethylglycine, acetate and butyrate by *Eubacterium limosum*, and Moller *et al.* (1984) reported on the production of TMA, *N,N*-dimethylglycine, acetate and CO_2 by the newly discovered genus, *Sporomusa*. A significant feature of each of these pathways is the formation of end-products which in turn are substrates for other organisms. In particular, the production of TMA or other methyl amines forms the basis of a mutualism between methanogens and fermentors that results in the complete mineralization of at least two important QAs.

At present, relatively little is known about the metabolism of other amines, such as β-alanine betaine, trigonelline, homarine or the stachydrines. Neither the pathways for synthesis nor degradation have been well documented. Such studies are obviously areas for fruitful and valuable future work. Similarly, relatively little is known about the metabolism of DMSP and related sulfonium compounds or about arsenobetaine. Given the current emphases of a number of different research groups on the topics of methane production, marine toxicology and the atmospheric (and biospheric) sulfure cycle, among others, it is imperative

that basic physiological studies be carried out to clarify the types of processes that may be important in structuring the dynamics of QAs.

7.5.2 *In situ* studies

While significant strides have been made in the organismal physiology and metabolism of QAs, a great deal remains to learned about their metabolism *in situ*. Studies at the organismal level have provided a framework for guiding the ecological research. For example, what is known about the distribution of QAs within the marine biota suggests that research efforts should be directed towards coastal systems, such as marshes, hypersaline environments and habitats where the biomass of benthic populations is high. In these systems, QAs may account for a relatively large fraction of the total nitrogen, and therefore have a significant impact on nitrogen dynamics. Data from physiological studies suggest that salinity and oxygen regimes must be considered in designing and interpreting field data. Shifts in salinity may result in dramatic changes in QA pool sizes, while the presence or absence of oxygen will determine the extent of methylotrophic versus fermentative and methanogenic metabolism. Existing data on the microbiology of QAs also suggest that multiple pathways may be involved in metabolism; thus investigations must consider the dynamics of varied populations and their end-products.

Most of the ecological studies of quaternary and methyl amines to date have focused on anaerobic metabolism in saltmarsh or other intertidal sediments. Oremland *et al.* (1982) and Oremland and Polcin (1982) have described the utilization of TMA in sediments from a saltmarsh and a hypersaline lake. These authors have shown that TMA is rapidly converted to methane and CO_2, and that metabolism of TMA could account for a major fraction of saltmarsh methanogenesis. Oremland and co-workers also indicated that at relatively high concentrations, TMA (and several other simple methylated compounds) was used primarily by methanogenic bacteria; the observed patterns were consistent with the concept of 'non-competitive' substrates providing for the survival and activity of methanogens in an otherwise unfavorable marine environment. Later studies by King *et al.* (1983) and Winfrey and Ward (1983) documented the potential importance of methanogenesis from methyl amines at natural concentrations. Winfrey and Ward (1983) found that methyl amine was converted to both methane and CO_2 in anoxic intertidal sediments. However, ratios of $^{14}CH_4/^{14}CO_2$ indicated that at least a portion of the methyl amine was converted to CO_2 by a non-methanogenic process. King *et al.* (1983) have also reported that MA and TMA were converted to methane and CO_2 (Figure 7.4; Table 7.6). The use of inhibitors of methanogenesis and sulfate reduction suggested that sulfate reducers oxidized a fraction of the TMA present, but that metabolism was dominated by methanogens. An analysis of rates of methane

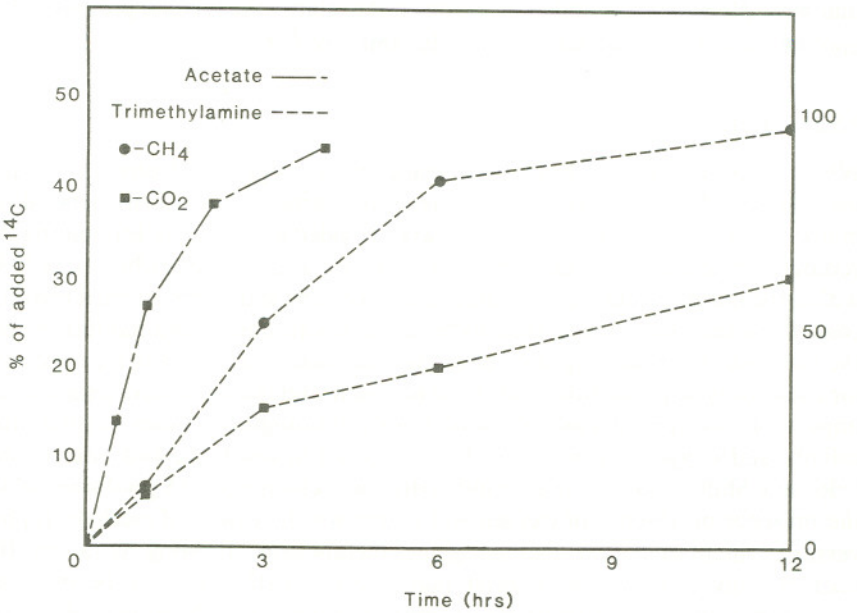


Figure 7.4. Production of $^{14}CO_2$ (■———■) from [2-^{14}C]acetate (right scale) and production of $^{14}CO_2$ (■———■) and $^{14}CH_4$ (●———●) from [^{14}C] TMA (left scale) in sediment slurries. (Redrawn from King *et al.*, 1983.)

Table 7.6. Summary of methane precursor metabolism in intertidal surface sediments from Lowes Cove—ME, and effects of inhibitors of sulfate reduction (sodium molybdate) and methanogenesis (bromoethanesulfonic acid—BES)

Substrate	Inhibitor	$^{14}CO_2/^{14}CH_4$	Percentage of total CH_4 production
Acetate	None	< 0.001	0.5
Methanol		0.19	2.4
Trimethylamine		2.04	61.3
Acetate	Na_2MoO_4	0.4	
Methanol	(20 mM)	1.8	
Trimethylamine		2.8	
Acetate	BES (27 mM)	—	
Methanol		0.2	
Trimethylamine		—	

From King *et al.*, 1983.

production and TMA turnover at *in situ* concentrations also indicated that TMA was a major methane precursor, accounting for up to 61% of total methanogenesis; MA appeared unimportant (Table 7.6). Recently, Giani *et al.* (1985) described the stimulation of methanogenesis in sediments from the Solar Lake by methylamines. Their data suggest that these compounds may be common methane precursors in a number of saline environments. This would be consistent with the isolation of methyl amine degrading methanogens from a variety of subtidal sediments and hypersaline systems (e.g. Konig and Stetter, 1982; Sowers and Ferry, 1983; Paterek and Smith, 1985).

Of course, the significance of TMA or other methyl amines to methanogenesis *in situ* depends on its availability. King (1984) has examined patterns of GBT and choline fermentation in anoxic intertidal sediments and observed that these substrates stimulate both methanogenesis and sulfate reduction simultaneously (Figures 7.5 and 7.6). The use of inhibitors of methanogenesis and sulfate reduction showed that GBT and choline were fermented to TMA and acetate; TMA was subsequently used by methanogens while acetate was oxidized by sulfate-reducers. GBT was fermented at a somewhat slower rate than choline and methane yields were lower than for either choline or TMA. In addition, neither GBT nor choline were fermented initially by sulfate-reducers; this contrasts with a

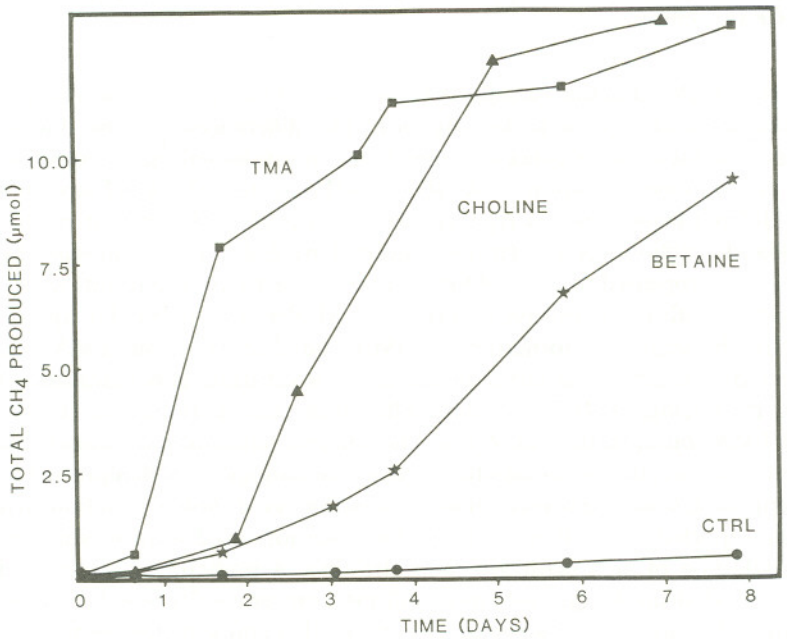


Figure 7.5. Production of CH_4 from TMA, choline and glycine betaine added at 1 mM concentrations in sediment slurries. CRTL, control

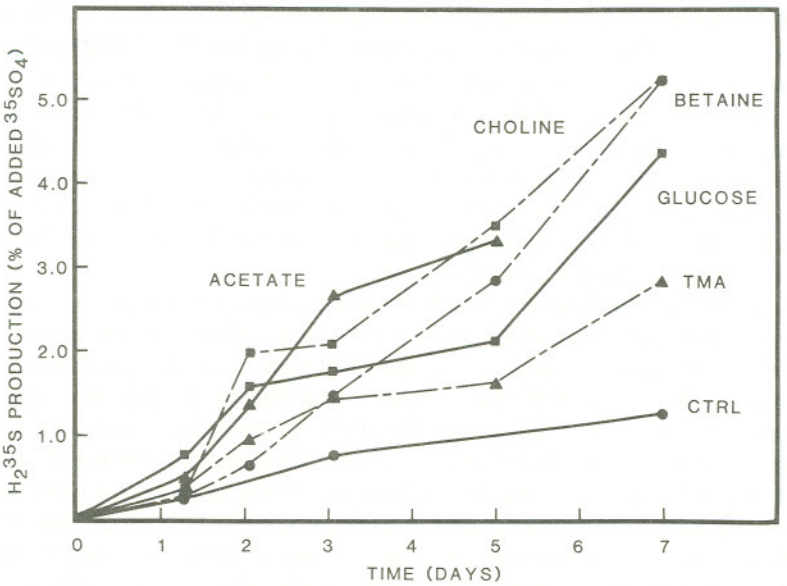


Figure 7.6. Reduction of tracer $^{35}SO_4^{2-}$ in sediment slurries containing 1 mM glucose, acetate, choline, glycine betaine or TMA, or no added substrates. CRTL, control

report by Fiebig and Gottschalk (1983) who noted that *Desulfovibrio* dominated choline fermenters in a sludge digestor. The differences may be due to the presence of high sulfate concentrations in the intertidal sediments, which would not favor choline utilization by *Desulfovibrio* (Postgate, 1979). GBT fermentation also differed from previous reports (Naumann *et al.*, 1983) in that neither the Stickland reaction nor the pathway utilized by *Eubacterium limosum* (Müller *et al.*, 1981) appeared to account for the observed activity. The results were more consistent with the pathways described by Moller *et al.* (1984) for *Sporomusa*. Finally, the sequential formation of DMA and then MA from TMA was not observed in sediments at either *in situ* or 1 mM substrate concentrations (King *et al.*, 1983; King, 1984). This pattern differs from that of Hippe *et al.* (1979), who used TMA concentrations at 50 mM. The different patterns may indicate that all the enzymes involved in amine utilization by methanogens are derepressed *in situ*. Derepression would be consistent with the pathways of choline metabolism in the rumen reported by Neill *et al.* (1978); these authors used a tracer study rather than addition of high substrate concentrations. A preliminary tracer study of GBT metabolism (King, 1985) has confirmed earlier results based on substrate addition. Postulated pathways for QA and methyl amine metabolism in marine sediments are summarized in Figure 7.7.

At this point a few comments about the metabolism of arsenobetaine are in

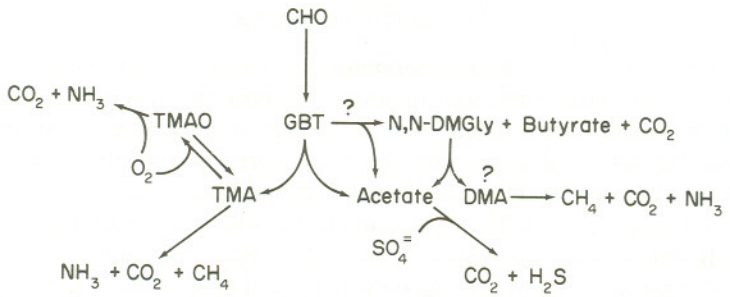


Figure 7.7. Pathways for the formation and metabolism of various methylated amines

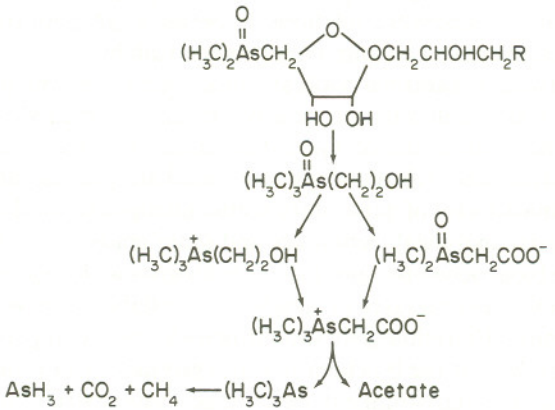


Figure 7.8. Possible pathways for the formation and subsequent metabolism of arseno-betaine

order. Arsenobetaine is an arsenic-containing analog of GBT (Figure 7.1) accumulated by a number of marine organisms (see Section 7.4.3). Edmonds *et al.* (1982) have suggested that arsenobetaine originates from anaerobic fermentation of an arsenofuranoside common in brown macroalgae. Apparently the sugar fermentation results in the formation of dimethyloxyarsylethanol, which is subsequently methylated and oxidized to arsenobetaine (Figure 7.8). Little is known about the dynamics or fate of arsenobetaine other than the fact that it is accumulated to levels that can be toxic to humans. It is interesting to speculate that arsenobetaine may be fermented similarly to GBT, with trimethylarsine as a product; trimethylarsine might be further metabolized to the highly toxic and volatile arsine (Figure 7.8). Thus, at least a portion of the biogeochemistry of arsenic may be related to that of nitrogen, and to a unique class in particular.

7.6 CONCLUSIONS

Quaternary amines and related compounds are a widely distributed component of many marine organisms, and in some instances they account for a major fraction of total cellular nitrogen. The physiology of these compounds has been examined for several decades with the most intensive and illustrative studies occurring in the past 10–15 years. However, the ecological significance of QAs is only now being explored. The limited data available indicate that QAs may be especially important in nearshore or coastal systems that contain large populations of organisms that osmoregulate with QAs, that contain rooted plants (e.g. saltmarshes or seagrass beds), or that are exposed to high salinities (e.g. salt ponds, hypersaline lagoons, etc.). Such regions clearly contain some of the most productive marine environments known. Thus, it is imperative that more complete analyses of nitrogen dynamics, including QAs, be undertaken. The pertinent questions concern the relationship between QA pool sizes in the biota and edaphic variables such as salinity, nitrogen availability and seasonality; the relationship between organismal pools and dissolved and bound pools within the sediment and water column; the relative extent of methylotrophic versus heterotrophic mineralization of QAs; the extent of anaerobic metabolism, pathways involved and relationships with methanogenesis; and finally, the significance of mass fluxes of quaternary amino nitrogen to total nitrogen fluxes. The advent of new analytical techniques and more ready access to radiotracers should provide biogeochemists and marine ecologists with appropriate tools for determining pool sizes and rates of metabolism; collaboration with microbiologists and organismal physiologists should privide for a comprehensive understanding of the role that the biochemistry and physiology of osmoregulation or adaptation to a saline environment have on ecosystem structure and activity.

REFERENCES

Ackman, R. G., Tocher, C. S., and McLachlan, J. (1966). Occurrence of dimethylpropiothetin in marine phytoplankton. *J. Fish. Res. Bd. Canada*, **23**, 357–64.

Allaway, W. G., Pitman, M. G., Storey, R., Tyerman, S., and Ashford, A. E. (1984). Water relations of Coral Cay vegetation on the Great Barrier Reef: water potentials and osmotic content. *Aust. J. Bot.*, **32**, 449–64.

Allinger, N. L., Cava, N. P., DeJongh, D. C., Johnson, C. R., Lebel, N. A., and Stevens, C. L. (1971). *Organic Chemistry*. Worth, New York.

Andreae, M. O., and Barnard, W. R. (1984). The marine chemistry of dimethylsulfide. *Mar. Chem.*, **14**, 267–79.

Anthony, C. (1982). *The Biochemistry of Methylotrophs*. Academic Press, New York.

Barnard, W. R., Andreae, M. O., and Iverson, R. L. (1984). Dimethylsulfide and *Phaeocystis poucheti* in the southeastern Bering Sea. *Cont. Shelf Res.*, **3**, 103–13.

Barnes, H., and Blackstock, J. (1974). The separation and estimation of free amino acids, trimethylamine oxide and betaine in tissues and body fluids of marine invertebrates. *J. Exp. Mar. Biol. Ecol.*, **16**, 29–45.

Beers, J. R. (1967). The species distribution of some naturally-occurring quaternary ammonium compounds. *Comp. Biochem. Physiol.*, **21**, 11–21.

Bennert, H. W., and Schmidt, B. (1984). On the osmoregulation in *Atriplex hymenelytra* (Torr.) Wats. (Chenopodiaceae). *Oecol. (Berl.)*, **62**, 80–4.

Bishop, S. H., Greenwalt, D. E., and Burcham, J. M. (1981). Amino acid cycling in the ribbed mussel tissues subjected to hyperosmotic shock. *J. Exp. Zool.*, **215**, 277–87.

Blunden, G., El Barouni, M. M., Gordon, S. M., McLean, W. F. H., and Rogers, D. J. (1981). Extraction, purification and characterization of Dragendorff-positive compounds from some British marine algae. *Bot. Mar.*, **24**, 451–6.

Blunden, G., Gordon, S. M., McLean, W. F. H., and Guiry, M. D. (1982). The distribution and possible taxonomic significance of quaternary ammonium and other Dragendorff-positive compounds in some genera of marine algae. *Bot. Mar.*, **25**, 563–7.

Blunden, G., Gordon, S. M., McLean, W. F. H., and Keysell, G. R. (1983). β-Stachydrine, a novel betaine from *Griffithsia floculosa*. *Phytochemistry*, **22**, 293.

Borowitzka, L. J. (1981). Solute accumulation and regulation of cell water activity. In: Paleg, and Aspinall, (eds), *The Physiology and Biochemistry of Drought Resistance in Plants*, pp. 97–130. Academic Press, New York.

Bradbeer, C. (1965). The clostridial fermentations of choline and ethanolamine. I. Preparation and properties of cell-free extracts. *J. Biol. Chem.* **240**, 4669–74.

Bradford, K. J., and Hsiao, T. C. (1982). Physiological responses to moderate water stress. In: Lange, O. L., Nobel, P. S., Osmond, C. B., and Ziegler, H. (eds), *Encyclopedia of Plant Physiology*, new series, vol. 12B, pp. 264–324. Springer-Verlag, Berlin.

Briens, M., and Lahrer, F. (1982). Osmoregulation in halophytic higher plants: a comparative study of soluble carbohydrates, polyols, betaines and free proline. *Plant Cell Environ.*, **5**, 287–92.

Cantoni, G. L. (1960). Onium compounds and their biological significance. In: Florkin, M., and Mason, H. S. (eds), *Comparative-Biochemistry*, pp. 181–241. Academic Press, New York.

Carruthers, A., Oldfield, J. F. T., and Teague, H. J. (1960). The removal of interfering ions in the determination of betaine in sugarbeet juice and plant material. *Analyst*, **85**, 272–5.

Cavalieri, A. J. (1983). Proline and glycinebetaine accumulation by *Spartina alterniflora* Loisel. in response to NaCl and nitrogen in a controlled environment. *Oecol. (Berl.)*, **57**, 20–4.

Cavalieri, A. J., and Huang, A. H. C. (1981). Accumulation of proline and glycinebetaine in *Spartina alterniflora* Loisel. in response to NaCl and nitrogen in the marsh. *Oecol. (Berl.)*, **49**, 224–8.

Charest, R., and Dunn, A. (1984). Chromatographic separation of choline, trimethylamine, trimethylamine oxide and betaine from tissues of marine fish. *Analyt. Biochem.*, **136**, 421–4.

Colby, J., Dalton, H., and Whittenbury, R. (1979). Biological and biochemical aspects of growth on C_1 compounds. *Ann. Rev. Microbiol.*, **33**, 481–518.

Cromwell, B. T., and Rennie, S. D. (1954). The biosynthesis and metabolism of betaines in plants. II. The biosynthesis of betaine in higher plants. *Biochem. J.*, **58**, 318–22.

Dall, W. (1971). The role of homarine in decapod crustacea. *Comp. Biol. Physiol.*, **39B**, 31–44.

Dickson, D. M., Wyn Jones, R. G., and Davenport, J. (1980). Steady state osmotic adaptation in *Ulva lactuca*. *Planta*, **150**, 158–65.

Easter, M. C., Gibson, D. M., and Ward, F. B. (1982). A conductance method for the assay and study of bacterial trimethylamine oxide reduction. *J. Appl. Bacteriol.*, **52**, 357–65.

Edmonds, J. S., and Francesconi, K. A. (1981). Arseno-sugars from brown kelp (*Eklonia radiata*) as intermediates in cycling of arsenic in a marine ecosystem. *Nature*, **289**, 602–4.

Edmonds, J. S., Francesconi, K. A., Cannon, J. R., Raston, C. L., Skelton, B. W., and White, A. H. (1977). Isolation, crystal structure and synthesis of arseno betaine, the arsenical constituent of the western rock lobster *Panulirus longipes cygnus*, George. *Tetrahedron Lett.*, **18**, 1543–6.

Edmonds, J. S., Francesconi, K. A., and Hansen, J. S. (1982). Dimethyloxyarsylethanol from anaerobic decomposition of brown kelp (*Eklonia radiata*): a likely precursor of arsenobetaine in marine fauna. *Experientia*, **38**, 643–4.

Fattorusson, E., and Piattelli, M. (1980). Amino acids from marine algae. In: Scheuer, P. J. (ed.), *Marine Natural Products: chemical and biological perspectives*, pp. 95–140. Academic Press, New York.

Fiebig, K., and Gottschalk, G. (1983). Methanogenesis from choline by a coculture of *Desulfovibrio* sp. and *Methanosarcina barkeri*. *Appl. Environ. Microbiol.*, **45**, 161–8.

Focht, R. L., Schmidt, F. H., and Dowling, B.B. (1956). Colorimetric determination of betaine in glutamate process end liquor. *Agr. Food Chem.*, **4**, 546–8.

Fujiwana-Arasaki, T., and Mino, N. (1972). The distribution of trimethylamine and trimethylamine oxide in marine algae. In: *Proc. 7th Int. Seaweed Symp., Sapporo, Japan*, pp. 506–10.

Galinski, E. A., and Truper, H. G. (1982). Betaine, a compatible solute in the extremely halophilic phototrophic bacterium *Ectothiorhodospira halochloris*. *FEMS Microbiol. Lett.*, **13**, 357–60.

Gallagher, J. L. (1974). Sampling macro-organic matter profiles in salt marsh plant root zones. *Soil Sci. Soc. Amer. Proc.*, **38**, 154–5.

Giani, D., Giani, L., Cohen, Y., and Krumbein, W. E. (1985). Methanogenesis in the hypersaline Solar Lake (Sinai). *FEMS Microbiol. Lett.*, **25**, 219–24.

Gilles, R. (1979). Intracellular organic osmotic effectors. In: Gilles, R. (ed.), *Mechanisms of Osmoregulation in Animals: maintenance of cell volume*, pp. 111–56. John Wiley and Sons, New York.

Glob, E., and Sørensen, J. (1987). Determination of dissolved and exchangeable trimethylamine in sediments. *J. Microbiol. Methods*, in press.

Gorham, J. (1984). Separation of plant betaines and their sulphur analogues by cation exchange high performance liquid chromatography. *J. Chromatogr.*, **287**, 345–51.

Gorham, J., McDonnell, E., and Wyn Jones, R. G. (1982). Determination of betaines as ultraviolet-absorbing esters. *Analyt. Chem. Acta*, **138**, 277–83.

Grieve, C. M., and Maas, E. V. (1984). Betaine accumulation in salt-stressed sorghum. *Physiol. Plant*, **61**, 167–71.

Griffin, D. M. (1981). Water and microbial stress. In: Alexander, M. (ed.), *Advances in Microbial Ecology*, pp. 91–136. Plenum, New York.

Griffin, D. M., and Luard, E. J. (1979). Water stress and microbial ecology. In: Shilo, M. (ed.), *Strategies of Microbial Life in Extreme Environments*, pp. 49–64. Verlag Chemie, New York.

Guggenheim, M. (1958). Die biogenen amine in der pflanzenwelt. In: Ruhland, W. (ed.), *Encyclopedia of Plant Physiology*, vol. VIII, pp. 919–27. Springer Verlag, Berlin.

Guy, R. D., Warne, P. G., and Reid, D. M. (1984). Glycinebetaine content of halophytes: improved analysis by liquid chromatography and interpretations of results. *Physiol. Plant*, **61**, 195–202.

Hanson, A. D., and Rhodes, D. (1983). ^{14}C tracer evidence for synthesis of choline and betaine via phosphoryl base intermediates in salinized sugarbeet leaves. *Plant Physiol.*, **71**, 692–700.

Hayward, H. R., and Stadtman, T. C. (1959). Anaerobic degradation of choline. I. Fermentation of choline by an anaerobic cytochrome-producing bacterium, *Vibrio cholinicus* n. sp. *J. Bacteriol.*, **78**, 557–61.

Hebard, C. E., Flick, G. J., and Martin, R. E. (1982). Occurrence and significance of

trimethylamine oxide and its derivatives in fish and shellfish. In: Martin, R. E., Flick, G. J., Hebard, C. E., and Ward, D. R. (eds), *Chemistry and Biochemistry of Marine Food Products*, pp. 149–304. AVI, Westport, CT.

Hippe, H., Caspari, D., Fiebig, K., and Gottschalk, G. (1979). Utilization of trimethylamine and other *N*-methylated compounds for growth and methane formation by *Methanosarcina barkeri*. *Proc. Natl. Acad. Sci. USA*, **76**, 494–8.

Hitz, W. D., and Hanson, A. D. (1980). Determination of glycine betaine by pyrolysis–gas chromatography in cereals and grasses. *Phytochemistry*, **19**, 2371–4.

Hochachka, P. W., and Somero, G. N. (1984). *Biochemical Adaptation*. Princeton University Press, Princeton.

Howes, B. L., Dacey, J. W. H., and King, G. M. (1984). Carbon flow through oxygen and sulfate reduction pathways in salt marsh sediments. *Limnol. Oceanogr.*, **29**, 1039–51.

Ishimoto, M., and Shimokawa, O. (1978). Reduction of trimethylamine N-oxide by *Escherichia coli* as anaerobic respiration. *Z. Allg. Mikrobiol.*, **18**, 173–81.

King, G. M. (1984a). Metabolism of trimethylamine, choline, and glycine betaine by sulfate-reducing and methanogenic bacteria in marine sediments. *Appl. Environ. Microbiol.*, **48**, 719–25.

King, G. M. (1984b). Utilization of hydrogen, acetate, and 'non-competitive' substrates by methanogenic bacteria in marine sediments. *Geomicrobiol. J.*, **3**, 275–306.

King, G. M. (1985). Distribution and metabolism of quaternary amines in salt marshes. *Proc. First Symposium on Biospheric Research*, p. 29. NASA.

King, G. M. (1987). An enzymatic synthesis of specifically radiolabelled derivatives of the common osmolyte, glycine betaine. *J. Exp. Mar. Biol. Ecol.*, **107**, 145–154.

King, G. M., Klug, M. J., and Lovley, D. R. (1983). Metabolism of acetate, methanol, and methylated amines in intertidal sediments of Lowes Cove, Maine. *Appl., Environ. Microbiol.*, **45**, 1848–53.

Konig, H., and Stetter, K. O. (1982). Isolation and characterization of *Methanolobus tindarius*, sp. nov., a coccoid methanogen growing only on methanol and methylamines. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. *Hyg. Abt. 1 Orig. Reihe*, **C3**, 478–390

Konno, K., Shirahama, H., and Matsumoto, T. (1984). Clithioneine, an amino acid betaine from *Clitocybe acromelalga*. *Phytochemistry*, **23**, 1003–6.

Kusel, J. P., Fa, Y. H., and Demain, A. L. (1984). Betaine stimulation of vitamin B_{12} biosynthesis in Pseudomonas denitrificans may be mediated by an increase in activity of δ-aminolaevulinic acid synthase. *J. Gen. Microbiol.*, **130**, 835–41.

Lahrer, F., and Hamelin, J. (1975). L'acide β-trimethylaminopropionique des remeaux de *Limonium vulgare* Mill. *Phytochemistry*, **14**, 205–7.

Lahrer, F., Hamelin, J., and Stewart, G. R. (1977). L'acide dimethylsulfonium-3 propanoique de *Spartina anglica*. *Phytochemistry*, **16**, 2019–20.

Lee, C., and Olson, B. L. (1984). Dissolved, exchangeable and bound aliphatic amines in marine sediments: initial results. *Org. Geochem.*, **6**, 259–63.

Le Rudulier, D., and Bouillard, L. (1983). Glycine betaine, an osmotic effector in *Klebsiella pneumoniae* and other members of the Enterobacteriaceae. *Appl. Environ. Microbiol.*, **46**, 152–7.

Le Rudulier, D., Bernard, T., Pocard, J. -A., and Goas, G. (1983). Accroissement de l'osmotolerance chez *Rhizobium meliloti* par la glycine betaine et la proline proline betaine. *C. R. Acad. Sci. Paris*, **297**, 155–60.

Le Rudulier, D., Bernard, T., Goas, G., and Hamelin, J. (1984). Osmoregulation in *Klebsiella pneumoniae*: enhancement of anaerobic growth and nitrogen fixation under stress by proline betaine, α-butyrobetaine, and other related compounds. *Can. J. Microbiol.*, **30**, 299–305.

Le Rudulier, D., Strom, A. R., Dandekar, A. M., Smith, L. T., and Valentine, R. C. (1984). Molecular biology of osmoregulation. *Science*, **224**, 1064–8.

Linden, J. C., and Lawhead, C. L. (1975). Liquid chromatography of saccharides. *J. Chromatogr.*, **105**, 125–33.

Mackay, M. A., Norton, R. S., and Borowitzka, L. J. (1983). Marine blue-green algae have a unique osmoregulatory system. *Mar. Biol.*, **73**, 301–7.

Maher, W. A. (1984). Characteristic of arsenic in a marine mollusc and crustacean. *Mar. Biol. Lett.*, **5**, 47–53.

Measures, J. C. (1975). Role of amino acids in osmoregulation of non-halophilic bacteria. *Nature (Lond.)*, **257**, 398–400.

Miller, I. T., and Springall, H. D. (1966). *The Organic Chemistry of Nitrogen.* Clarendon Press, Oxford.

Moller, B., Ossmer, R., Howard, B. H., Gottschalk, G., and Hippe, H. (1984). *Sporomusa*, a new genus of gram-negative anaerobic bacteria including *Sporomusa sphaeroides* spec. nov. and *Sporomusa ovata* spec. nov. *Arch. Microbiol.*, **139**, 388–96.

Morgan, J. M. (1984). Osmoregulation and water stress in higher plants. *Ann. Rev. Plant Physiol.*, **35**, 299–319.

Muller, E., Fahlbusch, K., Walther, R., and Gottschalk, G. (1981). Formation of *N, N*-dimethylglycine, acetic acid, and butyric acid from betaine *Eubacterium limosum. Appl. Environ. Microbiol.*, **42**, 439–45.

Naumann, E., Hippe, H., and Gottschalk, G. (1983). Betaine: a new oxidant in the Stickland reaction and methanogenesis from betaine and L-alanine by a *Clostridium sporogenes–Methanosarcina barkeri* coculture. *Appl. Environ. Microbiol.*, **45**, 474–83.

Naumann, E., Fahlbusch, K., and Gottschalk, G. (1984). Presence of a trimethylamine:HS-Coenzyme M methyltransferase in *Methanosarcina barkeri. Arch. Microbiol.*, **138**, 79–83.

Neill, A. R., Grime, D. W., and Dawson, R. M. C. (1978). Conversion of choline methyl groups through trimethylamine into methane in the rumen. *Biochem. J.*, **170**, 529–35.

Oremland, R. S., and Polcin, S. (1982). Methanogenesis and sulfate reduction: competitive and non-competitive substrates in estuarine sediments. *Appl. Environ. Microbiol.*, **44**, 1270–6.

Oremland, R. S., Marsh, L. M., and Polcin, S. (1982). Methane production and simultaneous sulfate reduction in anoxic, salt marsh sediments. *Nature (Lond.)*, **296**, 143–5.

Paterek, J. R. and Smith, P. H. (1985). Isolation and characterization of a halophilic methanogen from Great Salt Lake. *Appl. Environ. Microbiol.*, **50**, 837–81.

Patterson, J. A., and Hespell, R. B. (1979). Trimethylamine and methylamine as growth substrates for rumen bacteria and *Methanosarcina barkeri. Curr. Microbiol.*, **3**, 79–83.

Popp, M., Lahrer, F., and Weigel, P. (1984). Chemical composition of Australian mangroves. III. Free amino acids, total methylated onium compounds and total nitrogen. *Z. Pflanzenphysiol. Bd.*, **114**, 15–25.

Postgate, J. R. (1979). The Sulfate-reducing Bacteria. Cambridge University Press, London.

Rafaeli-Eshkol, D., and Avi-Dor, Y. (1968). Studies on halotolerance in a moderately halophilic bacterium. Effect of betaine on salt resistance of the respiratory system. *Biochem. J.*, **109**, 687–91.

Reed, R. H., Chudek, J. A., Foster, R., and Stewart, W. D. P. (1984). Osmotic adjustment in cyanobacteria from hypersaline environments. *Arch. Microbiol.*, **138**, 333–7.

Robertson, J. D. (1961). Studies on the chemical composition of muscle tissue. II. The abdominal flexor muscle of the lobster, *Nephrops norvegicus* (L.). *J. Exp. Biol.*, **38**, 707–28.

Senez, J. C., and Pascal, M. C. (1961). Degradation de la choline par les bacteries sulfato-reductrices. Identification de *Desulfovibrio desulfuricans* et de *Vibrio cholinicus. Z. Allg. Mikrobiol.*, **1**, 142–9.

Shiomi, K., Shinagawa, A., Azuma, M., Yamakana, H., and Kikuchi, T. (1983). Purification and comparison of water-soluble arsenic compounds in a flatfish *Limanda herzensteini*, sea squirt *Halocynthia roretzi*, and sea cucumber *Stichopus japonicus*. *Comp. Biochem. Physiol.*, **74C**, 393–6.

Somero, G. N., and Bowlus, R. D. (1983). Osmolytes and metabolic endproducts of molluscs: the design of compatible solute systems. In: Wilbur, K. M. (ed.), *The Mollusca*, pp. 77–100. Academic Press, New York.

Sørensen, J., and Glob, E. (1987). Influence of benthic fauna on trimethylamine concentrations in coastal marine sediments. *Mar. Ecol. Progr. Ser.*, **39**, 15–21.

Sowers, K. R., and Ferry, J. G. (1983). Isolation and characterization of a methylotrophic marine methanogen, *Methanococcoides methylutens* gen. nov., sp. nov. *Appl. Environ. Microbiol.*, **45**, 684–90.

Storey, R., and Wyn Jones, R. G. (1975). Betaine and choline levels in plants and their relationship to NaCl stress. *Plant Sci. Lett.*, **4**, 161–8.

Storey, R., and Wyn Jones, R. G. (1977). Quaternary ammonium compounds in plants in relation to salt resistance. *Phytochemistry*, **16**, 447–53.

Storey, R., Ahmed, N., and Wyn Jones, R. G. (1977). Taxonomic and ecological aspects of the distribution of glycinebetaine and related compounds in plants. *Oecol. (Berl.)*, **27**, 319–32.

Strack, E., and Schwaneberg, H. (1936). Uber die bestimmung von basen aus tierischen geweben. *Z. Physiol. Chem.*, **245**, 11–18.

Strom, A. R., Olafsen, J. A., and Larsen, H. (1979). Trimethylamine oxide: a terminal electron acceptor in anaerobic respiration of bacteria. *J. Gen. Microbiol.*, **112**, 315–20.

Stryvold, O. B., and Strom, A. R. (1984). Dimethylsulphoxide and trimethylamine oxide respiration of *Proteus vulgaris*: evidence for a common terminal reductase system. *Arch. Microbiol.*, **140**, 74–8.

Tempest, D. W., Meers, J. L., and Brown, C. M. (1970). Influence of environment on the content and composition of microbial free amino acid pools. *J. Gen. Microbiol.*, **64**, 171–95.

Vialle, J., Kolosky, M., and Rocca, J. L. (1981). Determination of betaine in sugar and wine by liquid chromatography. *J. Chromatogr.*, **204**, 429–35.

Vreeland, R. H., Mierau, B. D., Litchfield, C. D., and Martin, E. L. (1983). Relationship of the internal solute composition to the salt tolerance of *Halomonas elongata*. *Can. J. Microbiol.*, **29**, 407–14.

Wall, J. S., Christian, D. D., Dimler, R. J., and Sentri, I. Q. (1960). Spectrophotometric determination of betaine and other quaternary nitrogen compounds as their periodides. *Analyt. Chem.*, **32**, 870–4.

Warren, M. K., and Pierce, S. K. (1982). Two cell volume regulatory systems in the *Limulus* myocardium: an interaction of ions and quaternary ammonium compounds. *Biol. Bull.*, **163**, 504–16.

Watts, R. L., and Watts, D. C. (1974). Nitrogen metabolism in fishes. In: Florkin, M., and Scheer, B. T. (eds), *Chemical Zoology*, vol. VIII, pp. 369–446. Academic Press, New York.

White, R. H. (1982). Analysis of dimethylsulfonium compounds in marine algae. *J. Mar. Res.*, **40**, 529–36.

Winfrey, M. R., and Ward, D. M. (1983). Substrates for sulfate reduction and methane production in intertidal sediments. *Appl. Environ. Microbiol.*, **45**, 193–9.

Wyn Jones, R. G., and Storey, R. (1981). Betaines. In: Paleg, L. G., and Aspinall, D. (eds), *The Physiology and Biochemistry of Drought Resistance in Plants*. Academic Press, New York.

Yancey, P. H., Clark, M. E., Hand, S. C., Bowlus, R. D., and Somero, G. N. (1982). Living with water stress: evolution of osmolyte systems. *Science*, **217**, 1214–22.