Biochem. J. (1978) **176**, 1–7
Printed in Great Britain

The Biosynthetic Pathway of New Polyamines in *Caldariella acidophila*

By MARIO DE ROSA,* SALVATORE DE ROSA,* AGATA GAMBACORTA,* MARIA CARTENÌ-FARINA† and VINCENZO ZAPPIA†‡
**Laboratory for the Chemistry of Molecules of Biological Interest, C.N.R. Arco Felice, Naples, Italy, and*
†Department of Biochemistry, Second Chair, First Medical School, University of Naples, Naples, Italy

(*Received* 25 *November* 1977)

1. Spermidine and sym-nor-spermine (1,11-diamino-4,8-diazaundecane) were identified as the major components of the polyamine pool in *Caldariella acidophila*, an extreme thermoacidophilic bacterium. A minor component, a new polyamine, sym-nor-spermidine (1,7-diamino-4-azaheptane) was isolated and characterized. 2. To elucidate the biosynthetic pathway, labelled methionine, putrescine, spermidine and spermine were fed to *Caldariella acidophila*. Incubation of the bacterium in the presence of putrescine or spermidine labelled in the tetramethylene moiety gave unlabelled sym-nor-spermidine and sym-nor-spermine, whereas the radioactivity of propylamine-labelled methionine or spermidine was incorporated into these molecules. No radioactivity was recovered in the polyamines pool when spermine was fed to *Caldariella acidophila*. 3. *S*-Adenosylmethionine and *S*-(5′-adenosyl)-3-methylthiopropylamine were identified as intermediates of the biosynthetic pathway; the cellular contents of the two sulphonium compounds, measured with a new isotope-dilution technique, are 60 and 15nmol/g wet wt. of cells respectively. 4. The above results are indicative of a new pathway characterized by three propylamine-transfer reactions, decarboxylated *S*-adenosylmethionine being the common donor of the propylamine moiety. The reactions yielding sym-nor-spermidine and sym-nor-spermine are reported for the first time. 5. The probable intermediates related to the catabolism of the tetramethylene moiety of spermidine, γ-aminobutyraldehyde, γ-aminobutyric acid or Δ^1-pyrroline were not detectable. Experiments with [3-*aminopropyl*-3(n)-^{3}H]spermidine trihydrochloride plus [*tetramethylene*-1,4-^{14}C]spermidine trihydrochloride gave rise to an amount of labelled CO_2 equivalent to the spermidine catabolized.

Although the functions of polyamines are at present unknown, it is generally thought that they are mainly involved in interactions with nucleic acids (Cohen, 1971; Bachrach, 1973; Cohen & Fukuma, 1977): a binding of polyamines to the phosphate oxygen atoms, in the narrow grooves of DNA (Tsuboi, 1964; Liquori *et al.*, 1967) as well as in the helical loops of tRNA (Cohen *et al.*, 1969; Cohen 1970) has been proposed. This linkage results, among other effects, in a protection of DNA against thermal denaturation (Tabor, 1962; Hirschman *et al.*, 1967; Glasser & Gabbay, 1968). The increased thermal resistance of some bacterial species could be in part ascribed to such interaction, and the study of the occurrence of polyamines in thermophiles could contribute to the elucidation of the basic biochemical mechanisms responsible for thermophily. It is noteworthy, in this respect, that the pattern of polyamines in bacteria is closely related to their optimal growth temperature: the normally occurring polyamines in mesophilic bacteria are generally putrescine and spermidine, whereas spermine is the most abundant component in the moderate thermophile *Bacillus stearothermophilus* (Stevens & Morrison, 1968). Oshima (1975) has reported the presence of spermine and sym-nor-spermine, a new tetraamine, in some obligate thermophiles, and studies from our laboratories demonstrated the presence of sym-nor-spermidine, a new triamine, in the extreme thermophile *Caldariella acidophila* (De Rosa *et al.*, 1976*a*). Recently sym-nor-spermidine and sym-nor-spermine have been detected in the brine shrimp (*Penaeus setiferus*) (Stillway & Walle, 1977) and in *Scenedesmus acutus* (Rolle *et al.*, 1977).

‡ To whom reprint requests should be addressed, at Istituto di Chimica Biologica, Università di Napoli, Via Costantinopoli 16, 80138 Napoli, Italy.

In the present paper the biogenic relationships between spermidine and sym-nor-spermine have been examined and a new pathway of polyamine biosynthesis has been proposed. Preliminary reports of this work have already been made (De Rosa *et al.*, 1976*b*).

Experimental

Chemicals

[1,4(n)-^{3}H]Putrescine dihydrochloride (sp. radioactivity 12.3Ci/mmol), L-[G-^{3}H]methionine (sp.

radioactivity 250 mCi/mmol), [*tetramethylene*-1,4-^{14}C]spermidine trihydrochloride (sp. radioactivity 122 mCi/mmol) and *S*-adenosyl-L-[*methyl*-^{14}C]methionine (sp. radioactivity 60 mCi/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K.; [3-*aminopropyl*-3(n)-^{3}H]spermidine trihydrochloride (sp. radioactivity 423 mCi/mmol) and [3-*aminopropyl*-3(n)-^{3}H]spermine tetrahydrochloride (sp. radioactivity 1.06 Ci/mmol) were obtained from New England Nuclear Corp., Dreieichenhain, Germany. The radiochemical purity of the above compounds was checked by t.l.c. and high-voltage electrophoresis.

1,7-Diamino-4-azaheptane was obtained from Koch–Light Laboratories, Colnbrook, Bucks., U.K., and 1,11-diamino-4,8-diazaundecane from Eastman Kodak, Rochester, NY, U.S.A.; putrescine, spermidine and spermine were from Sigma Chemical Co., St. Louis, MO, U.S.A.; Dowex 50W resin was obtained from Bio-Rad Laboratories, Richmond, CA, U.S.A.; trifluoroacetic anhydride was from Pierce Chemical Co., Rockford, IL, U.S.A., 1,4-bis-(5-phenyloxazol-2-yl)benzene (POPOP), 2,5-diphenyloxazole (PPO) and Cab-O-Sil thixotropic gel powder were from Packard Instruments Co., Downers Grove, IL, U.S.A.; glass beads (200 mesh) were from Fisher Co. NJ, U.S.A.; SE-30 and Chromosorb W (silicone-treated, 80–100 mesh) was from Carlo Erba, Milano, Italy; yeast extract and casamino acids were from Difco Laboratories, Detroit, MI, U.S.A.; cellulose F and silica-gel F-254 t.l.c. plates were from Merck, Darmstadt, Germany.

All other chemicals were the purest available grades from standard commercial sources.

Δ^1-Pyrroline was synthesized from ornithine and *N*-bromosuccinimide (Chappelle & Luck, 1957). *S*-Adenosyl-L-methionine was prepared from cultures of *Saccharomyces cerevisiae* (Schlenk & De Palma, 1957) and isolated by ion-exchange chromatography (Shapiro & Ehninger, 1966). To minimize decomposition, the sulphonium compound was stored at −20°C in 0.1 M-HCl.

Preparation of S-(5′-adenosyl)-3-methylthiopropylamine

S-(5′-Adenosyl)-3-methylthiopropylamine was obtained by enzymic decarboxylation of *S*-adenosylmethione by using *S*-adenosyl-L-methione decarboxylase purified from *Escherichia coli* as described by Wickner *et al.* (1970). After incubation, the mixture was deproteinized and *S*-(5′-adenosyl)-3-methylthiopropylamine was isolated by a new procedure developed in our laboratory (Galletti *et al.*, 1975; Zappia *et al.*, 1977*a*,*b*). The method involves acid hydrolysis at 100°C resulting in a selective and quantitative cleavage of residual *S*-adenosyl-L-methionine into homoserine and 5′-methylthioadenosine. *S*-(5′-Adenosyl)-3-methylthiopropylamine which is stable in these conditions, was then purified to high purity by chromatography on Dowex-50. The purity of sulphonium compound was checked by t.l.c. on silica gel with ethanol/acetic acid/water (64:1:35, by vol.) or butanol/acetic acid/water (12:3:5, by vol.), and by high-voltage electrophoresis (Zappia *et al.*, 1969). *S*-(5′-Adenosyl)-3-[*methyl*-^{14}C]thiopropylamine was prepared by the above method from *S*-adenosyl[*methyl*-^{14}C]methionine. The u.v. quenching for purine compounds, the ninhydrin reaction for amino acids and the chloroplatinate spray (Toennies & Kolb, 1951) for sulphur-containing compounds were used to detect *S*-(5′-adenosyl)-3-methylthiopropylamine and its decomposition products.

Micro-organisms and culture conditions

C. acidophila, strain MT-4, was isolated from an acidic hot spring in Agnano, Naples (De Rosa *et al.*, 1975). The bacteria were grown at 87°C in 25-litre batches in a Terzano fermentor with slow mechanical agitation and an aeration flux of 2.5–3 litre/min. The culture medium contained (per litre) 1 g of yeast extract, 1 g of casamino acid, 3.1 g of KH_2PO_4, 2.5 g of $(NH_4)_2SO_4$, 0.20 g of $MgSO_4,7H_2O$ and 0.25 g of $CaCl_2$, H_2O. The pH was adjusted to 3.0 with 0.1 M-H_2SO_4. The culture vessel was inoculated by adding 3 litres of a 12 h broth culture. The doubling time of the micro-organism in these conditions was 7.5 h.

The labelled precursors, [1,4(n)-^{3}H]putrescine dihydrochloride (0.35 mCi), L-[G-^{3}H]methionine (0.25 mCi), [*tetramethylene*-1,4-^{14}C]spermidine trihydrochloride (0.25 mCi), [3-*aminopropyl*-3(n)-^{3}H]spermidine trihydrochloride (0.25 mCi) or [3-*aminopropyl*-3(n)-^{3}H]spermine tetrahydrochloride (0.25 mCi) were added to the culture (25 litres) at the beginning of the exponential phase. The cells were then harvested in the late-exponential phase (25 h incubation) by continuous-flow centrifugation in an Alfa–Laval model LAB 102 B-25 separator.

E. coli. A wild-type strain of *E. coli* W was grown in a 100-litre fermentor at 37°C, in a salt medium (Vogel & Bonnei, 1956) containing 0.2% dextrose. The cells were harvested in late-exponential phase by continuous-flow centrifugation and sonicated for 10 min at maximum power and 2°C with a Bronson Sonic J-174 instrument. The supernatant after centrifugation at 15000 g for 10 min was used as a source of *S*-adenosylmethionine decarboxylase.

Extraction of polyamines and adenosylsulphonium compounds

Polyamines and sulphonium compounds were extracted from the cells as described by De Rosa *et al.* (1976*a*). Wet cells (5 g) were washed with 20 ml of

0.01 M-Tris/HCl buffer, pH 7.4, and collected by centrifugation at 16000*g* for 20 min. The pellet was ground with 13 g of glass beads and 5 ml of 1 M-$HClO_4$ in the stainless-steel chamber of a Sorvall Omnimixer at full speed for 20 min. The beads were removed by centrifugation at 650*g* for 10 min, washed with 2 × 8 ml of 1 M-$HClO_4$ and centrifuged again for 10 min. The three supernatants were combined and the extraction was performed at 2°C for 48 h with mechanical stirring. The high-speed (35000*g* for 40 min) supernatant of the extraction mixture, adjusted to pH 3.0 with dilute NaOH, was directly chromatographed on a column (18 cm × 2 cm) of Dowex 50 (H^+ form) previously equilibrated with 0.1 M-HCl. The column was washed with 200 ml of 0.1 M-sodium phosphate buffer/0.7 M-NaCl, pH 8, and then with 250 ml of 2.5 M-HCl. Polyamines and sulphonium compounds were co-eluted with 100 ml of 6 M-HCl.

Quantification of S-adenosyl-L-methionine and S-(5′-adenosyl)-3-methylthiopropylamine by isotope dilution

The quantitative determination of the cellular contents of *S*-adenosylmethionine and *S*-(5′-adenosyl)-3-methylthiopropylamine was performed either with a partially modified isotope-dilution technique (Salvatore *et al.*, 1971; Zappia *et al.*, 1977*a*) or with the enzymic assay of Baldessarini & Kopin (1963), specific for *S*-adenosylmethionine. *S*-(5′-Adenosyl)-3-[*methyl*-^{14}C]thiopropylamine (1.3×10^6 d.p.m.) was added to 10 g of wet cells before acid extraction. The two sulphonium compounds were then isolated as described above. The 6 M-HCl eluate from a Dowex 50 column was evaporated to dryness under reduced pressure and the residue was dissolved in 3 ml of water; from the specific radioactivity of *S*-(5′-adenosyl)-3-[*methyl*-^{14}C]thiopropylamine recovered the original amounts of both sulphonium compounds were determined. Quantitative determination of the two adenosylsulphonium compounds was carried out in a Zeiss spectrophotometer model PMQII, by using the molar absorbance of 15400 (Zappia *et al.*, 1969). To measure the relative amounts of *S*-(5′-adenosyl)-3-methylthiopropylamine, the mixture was hydrolysed at pH 4.5 at 100°C for 1 h. A selective degradation of *S*-adenosylmethionine is performed in these conditions (Zappia *et al.*, 1977*a*,*b*). The reaction mixture was then purified by a Dowex 50 (H^+ form) column (7 cm × 2 cm) previously equilibrated with 0.1 M-HCl. The hydrolytic products of *S*-adenosylmethionine were eluted with 115 ml of 2 M-HCl and *S*-(5′-adenosyl)-3-methylthiopropylamine was eluted with 85 ml of 6 M-HCl. The strongly acidic eluate was evaporated to dryness under reduced pressure. From the specific radioactivity of *S*-(5′-adenosyl)-3-methylthiopropylamine the concentration of the sulphonium compound in the acid extract was calculated. The relative amount of *S*-adenosylmethionine was calculated by difference.

Radioactivity measurements

Radioactivity was measured in a Tri-Carb liquid-scintillation spectrometer (Packard, model 3380), equipped with an absolute radioactivity analyser; 10 ml of Bray's (1960) solution was added to 0.5 ml of aqueous samples; the quenching was corrected by external standardization.

To assay the radioactivity incorporated into whole cells, 1 ml of culture was rapidly filtered on a Millipore filtration apparatus. The filter was then washed twice with 2 ml of water, dried and counted for radioactivity in the scintillation solution in the presence of Cab-O-Sil (0.1 g). The radioactivity measurements on paper and t.l.c. plates were performed by an Actigraph III scanner model 1002 (Nuclear–Chicago).

$^{14}CO_2$ trapped as $Ba^{14}CO_3$ was measured in the scintillation solution in presence of Cab-O-Sil.

The radioactivity of γ-aminobutyric acid was measured after separation by high-voltage electrophoresis or t.l.c. cellulose. Radioactive Δ^1-pyrroline was assayed by the method of Okuyama & Kobayashi (1961) modified by Tryding & Willert (1968).

G.l.c. of radioactive materials

The distribution of radioactivity in the polyamines pool was determined by g.l.c. of the radioactive trifluoroacetyl derivatives. The dried samples of polyamines were dissolved in equal portions (1.0 ml) of acetonitrile and trifluoroacetic anhydride; an appropriate amount of spermine as internal standard was also added. The derivative formation was accomplished by placing the capped sample tube in an oil bath at 100°C for 5 min. The solvent and the reactants were then removed by evaporation with N_2 at room temperature. For the radio-g.l.c. measurements a Carlo Erba instrument model GV, equipped with a flame-ionization detector coupled with a Nuclear–Chicago flow counter model 4988 was utilized. The analyses were performed by using a column (2 m × 8 mm) packed with 1% SE-30 on Chromosorb W (silicone-treated, 80–100 mesh), with a programmed temperature from 150 to 250°C at 5°C/min and helium flow of 1.1 ml/min. The composition of trifluoroacetylated polyamine samples was determined by integration of the peak areas.

Results

Cellular concentrations of sulphonium compounds

Cellular concentration of *S*-adenosylmethionine and *S*-(5′-adenosyl)-3-methylthiopropylamine in *C.*

Table 1. *Incorporation of labelled precursors by C. acidophila into whole cells and into polyamine pool*
The labelled precursors, [1,4-(n)-^{3}H]putrescine dihydrochloride (0.35 mCi), L-[G-^{3}H]methionine (0.25 mCi), [*tetramethylene*-1,4-^{14}C]spermidine trihydrochloride (0.25 mCi) or [3-*aminopropyl*-3-(n)-^{3}H]spermidine trihydrochloride (0.25 mCi), were added to the culture (25 litres) at the beginning of the exponential phase. The cells were then harvested in the late-exponential phase (25 h incubation) by continuous flow centrifugation.

	Cells			Polyamine pool*		
Precursor	Yield (g)	10^{-7} × Total radioactivity (d.p.m.)	Percentage of recovered radioactivity*	Yield (mg)	10^{-7} × Total radioactivity (d.p.m.)	Percentage of recovered radioactivity†
[1,4(n)-^{3}H]Putrescine dihydrochloride (3.1 × 10^{8} d.p.m.; sp. radioactivity 12.3 Ci/mmol)	15.0	0.70	2.3	54	0.37	52.9
L-[G-^{3}H]Methionine (1.0 × 10^{9} d.p.m.; sp. radioactivity 250 mCi/mmol)	15.1	4.10	4.0	50	0.46	11.2
[*tetramethylene*-1,4-^{14}C]-Spermidine trihydrochloride (4.0 × 10^{8} d.p.m.; sp. radioactivity 122 mCi/mmol)	16.0	13.80	34.5	59	13.67	97.6
[3-*aminopropyl*-3-(n)-^{3}H]-Spermidine trihydrochloride (7.2 × 10^{8} d.p.m.; sp. radioactivity 423 mCi/mmol)	15.4	64.00	88.9	50	63.61	99.4

* Indicates the amount of polyamines recovered in the 6 M-HCl eluate from Dowex 50 (see the Experimental section).
† Percentage of radioactivity recovered in the total polyamine pool; 100 refers to the total radioactivity recovered in the whole cells.

acidophila harvested in the exponential phase of growth were measured by the above isotopic-dilution technique. The concentrations of *S*-adenosylmethionine and its decarboxylated product were 60 and 15 nmol/g wet wt. of cells respectively. The presence of the two sulphonium compounds in very low amounts supports their intermediary role in the pathway under investigation.

Incorporation of labelled precursors into polyamines

In five separate experiments, growing cultures of *C. acidophila* were incubated with [1,4(n)-^{3}H]putrescine dihydrochloride, L-[G-^{3}H]methionine, [*tetramethylene*-1,4-^{14}C]spermidine trihydrochloride, [3-*aminopropyl*-3(n)-^{3}H]spermidine trihydrochloride and [3-*aminopropyl*-3(n)-^{3}H]spermine tetrahydrochloride. The polyamines recovered from harvested cells were purified and their trifluoroacetyl derivatives resolved by t.l.c., into sym-nor-spermidine, spermidine and sym-nor-spermine as described in the Experimental section. The amount of the three polyamines was comparable in all experiments mentioned above.

The results reported in Table 1 indicate the amount of radioactivity incorporated in the whole cells as well as in the polyamine pool. The similarity of cell yields in the reported experiments ensures the comparability of the results of incorporation of the different precursors. The recovery of cellular radioactivity in the experiments where putrescine and methionine were used as precursors was very low compared with the high values of incorporation of spermidine. The total cellular radioactivity recovered from spermidine labelled in the tetramethylene moiety is significantly lower compared with that from spermidine labelled in the aminopropyl moiety, indicating a possible degradation of tetramethylene moiety of spermidine into a catabolite excreted from the cells.

Only 50% of putrescine and 11% of methionine incorporated in the cells is recovered in the polyamine pool, suggesting an alternative metabolic pathway utilizing these molecules. On the other hand spermidine is quantitatively recovered in the polyamine pool.

Table 2 illustrates the distribution of radioactivity from the various precursors into the three polyamines detected in the *C. acidophila*. The radioactivity of putrescine and that of spermidine labelled in the tetramethylene moiety is recovered only as spermidine. Conversely the radioactivity of methionine as well as that of spermidine labelled in the propylamine moiety is recovered in sym-nor-spermidine, sper-

Table 2. *Distribution of radioactivity from labelled precursors into the polyamines of C. acidophila*
For experimental conditions see Table 1. Values in parentheses indicate the specific radioactivity (d.p.m./mmol) of the resolved polyamines.

Precursor	10^{-6} × Total radioactivity in the resolved trifluoroacetylated polyamines by g.l.c. (d.p.m.)					
	sym-Nor-spermidine		Spermidine		sym-Nor-spermine	
[1,4(n)-^{3}H]Putrescine dihydrochloride*	0	(0)	3.65	(19)	0	(0)
L-[G-^{3}H]Methionine	0.18	(6)	1.93	(10)	2.05	(17)
[*tetramethylene*-1,4-^{14}C]Spermidine trihydrochloride	0	(0)	130	(630)	0	(0)
[3-*aminopropyl*-3(n)-^{3}H]Spermidine trihydrochloride	43	(1400)	434	(2200)	123	(1000)

* No radioactivity was recovered at the retention time of putrescine.

Table 3. *Incorporation of ^{14}C and/or ^{3}H into polyamines and CO_2 from [tetramethylene-1,4-^{14}C]spermidine plus [3-aminopropyl-3(n)-^{3}H]spermidine*
The labelled precursors, [*tetramethylene*-1,4-^{14}C]spermidine trihydrochloride (9×10^6 d.p.m.) plus [3-*aminopropyl*-3(n)-^{3}H]spermidine trihydrochloride (2.4×10^7 d.p.m.) were added to the culture (25 litres) at the beginning of the exponential phase. The cells were then harvested in the late-exponential phase (25 h incubation) by continuous-flow centrifugation. Abbreviation: n.d., not detected.

Compound	10^{-6} × Total radioactivity (d.p.m.)		Distribution of radioactivity (%)* in:	
	^{3}H	^{14}C	^{3}H	^{14}C
Sym-nor-spermidine	1.4	n.d.	7	—
Spermidine	14	3.4	72	77
Sym-nor-spermine	4.0	n.d.	21	—
CO_2	—	1.0	—	23
γ-Aminobutyric acid	—	n.d.	—	n.d.

* Percentage of the total radioactivity recovered in the polyamines for ^{3}H and total radioactivity recovered in the polyamine and CO_2 for ^{14}C.

midine and sym-nor-spermine. The differences in specific radioactivity are indicative of diversified turnover rates and/or possible cellular compartmentation. No radioactivity was detectable in the three polyamines in parallel experiments with labelled spermine.

Degradation of double-labelled spermidine

To investigate in more detail the metabolism of the tetramethylene moiety of spermidine, experiments with double-labelled spermidine were devised. The results reported in Table 3 indicate the conversion of the ^{3}H-labelled aminopropyl moiety into sym-nor-spermidine and sym-nor-spermine, whereas the ^{14}C-tetramethylene-labelled moiety is oxidized to CO_2. It is noteworthy that the percentage of radioactivity recovered as $^{14}CO_2$ equals that recovered as ^{3}H in sym-nor-spermidine plus sym-nor-spermine. No radioactive intermediates, such as γ-aminobutyric acid and Δ^1-pyrroline, were detected.

Washed-cell experiments

The presence of possible intermediates of the catabolism of the spermidine tetramethylene moiety was also investigated by using cultures of *C. acidophila* supplemented with spermidine.

The micro-organism was grown in the standard media plus 1% (w/v) spermidine trihydrochloride and collected in the middle of the exponential phase. The cells were washed and then suspended in water and the pH was adjusted to pH 4 with dilute HCl; the suspension contained 0.1 g wet wt. of cells/ml. Spermidine trihydrochloride (1.2 mg) was added to 6 ml of suspension and the solution was incubated at 85 °C for 45 h. Samples were taken at different times and the cell debris (16000 *g* for 20 min) was removed by centrifugation; 50 μl of the supernatant was analysed by t.l.c. for the presence of γ-aminobutyraldehyde, γ-aminobutyric acid, 1,3-diaminopropane, aminopropionaldehyde, β-alanine and putrescine. No ninhydrin-positive intermediate was detectable;

Δ^1-pyrroline, assayed by a colorimetric method (Holmsted *et al.*, 1961), was also absent.

Discussion

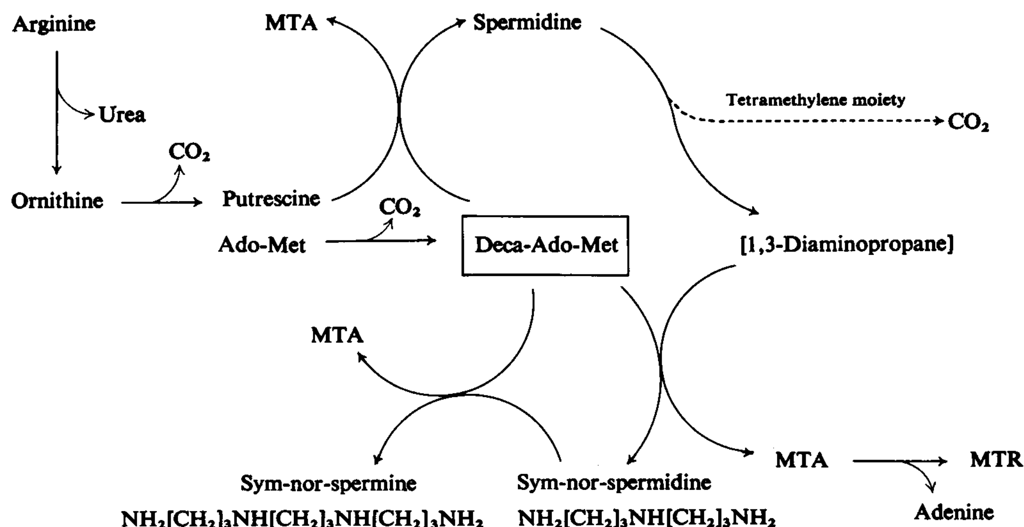
The high contents of polyamines as well as their distribution pattern in *C. acidophila* are probably related to the thermophilic properties of this micro-organism. The presence of sym-nor-spermidine and sym-nor-spermine is peculiar to extreme thermophiles (De Rosa *et al.*, 1976*a*; Oshima, 1975) and their carbon-chain length and molecular structure could facilitate specific interactions with nucleic acids, in turn responsible for thermostability. A stabilizing effect of the new polyamines on homologous DNA, according to the DNA–polyamine-interaction model proposed by Liquori *et al.* (1967), deserves further investigation. It is noteworthy in this respect that sym-nor-spermine modifies the 'melting' profile of heterologous DNA from *Acetobacter aerogenes* and from calf thymus (Stevens, 1967).

The charge distribution of sym-nor-spermidine and sym-nor-spermine could also contribute *in vivo* to the stabilization of the double-helix arrangements. It is noteworthy in this respect that the G + C content of DNA from *C. acidophila* is only 42% (De Rosa *et al.*, 1975) and does not account for its stability at the increased growth temperature (87 °C).

The data reported in the present paper are indicative of the biosynthetic pathway outlined in Scheme 1. The biosynthesis of spermidine closely resembles the pathway occurring in mesophilic micro-organisms (Tabor & Tabor, 1976) and mammalian tissues (Zappia *et al.*, 1977*b*) in that putrescine provides the 1,4-diaminobutane moiety and methionine donates the propylamine group. The intermediary role of *S*-adenosylmethionine and its decarboxylated product has also been indicated; the relative concentrations of the two sulphonium compounds, determined for the first time by a new procedure (Zappia *et al.*, 1977*a*), are of the same order of magnitude. Moreover the cellular contents of *S*-adenosylmethionine in *C. acidophila* are similar to those reported in eukaryotes (Salvatore *et al.*, 1971): this result was unexpected in view of the thermal lability of the molecule (Schlenk, 1977). The protection against hydrolysis at the elevated growth temperatures could be ascribed either to an interaction with a specific binding protein (Smith, 1976), or to a masking of the carboxy group of methionine, which is in turn responsible for the intramolecular hydrolytic reaction (Zappia *et al.*, 1977*a*).

The last steps of the biosynthesis of sym-nor-spermidine and sym-nor-spermine appear to be very unusual in that spermidine plays the role of precursor of the trimethylene moiety of these polyamines. In fact the triamine rapidly undergoes a propylamine transfer with *S*-(5′-adenosyl)-3-methylthiopropylamine, yielding 5′-methylthioadenosine and sym-nor-spermidine, which in turn reacts with a second molecule of sulphonium compound yielding sym-nor-spermine.

The role of 1,3-diaminopropane as a free intermediate in this chain of reactions can be hypothesized, although we were unable to detect appreciable



Scheme 1. *Biosynthetic pathway of polyamines in C. acidophila*

Abbreviations used: MTA, 5′-methylthioadenosine; MTR, methylthioribose; Ado-Met, *S*-adenosylmethionine; Deca-Ado-Met, *S*-(5′-adenosyl)-3-methylthiopropylamine.

amounts of this diamine in cellular extracts: 1,3-diaminopropane represents a catabolic product of spermidine oxidation in several bacterial species (Bachrach, 1973). On the other hand the tetramethylene moiety of spermidine is rapidly oxidized to CO_2, as reported in the experiments with double-labelled spermidine (see Table 3). The fast turnover of this C_4 fragment could also account for the low cellular recovery of radioactivity from labelled putrescine, although a limited permeability of the molecule cannot be ruled out. The former hypothesis is in agreement with the absence of detectable amounts of putrescine in the cells.

It is noteworthy that when labelled spermine was supplied as precursor, no radioactivity was recovered in the polyamine pool. This result supports the existence of a critical mechanism of membrane permeability.

The presence of some enzymes of the metabolic pathway has also been demonstrated in preliminary experiments (Zappia *et al.*, 1975, 1977*c*). Decarboxylation of ornithine and *S*-adenosylmethionine are considered to be the limiting steps of polyamine biosynthesis in eukaryotes (Tabor & Tabor, 1976) and the detection of the appropriate enzymes in *C. acidophila* is particularly relevant to the physiology of these micro-organisms.

We thank Dr. Marina Porcelli and Dr. Fulvio Della Ragione for the experiments on chromatographic resolution of the sulphonium compounds, and Dr. Antonella Alfano and Dr. Marina Camardella for their skilled assistance in some of the experiments. The technical assistance of Enrico Esposito and Salvatore Sodano is gratefully acknowledged. This work was supported by a grant from the Consiglio Nazionale delle Ricerche, Roma, Italy.

References

Bachrach, U. (1973) *Function of Naturally Occurring Polyamines*, Academic Press, New York

Baldessarini, R. J. & Kopin, I. J. (1963) *Anal. Biochem.* **6**, 289–292

Bray, G. A. (1960) *Anal. Biochem.* **1**, 270–273

Chappelle, E. V. & Luck, J. M. (1957) *J. Biol. Chem.* **229**, 171–179

Cohen, S. S. (1970) *Ann. N.Y. Acad. Sci.* **171**, 869–876

Cohen, S. S. (1971) *Introduction to the Polyamines*, Prentice-Hall, Englewood Cliffs

Cohen, S. S. & Fukuma, I. (1977) in *The Biochemistry of S-Adenosylmethionine* (Salvatore, F., Borek, E., Zappia, V., Williams-Ashman, H. G. & Schlenk, F., eds.), pp. 453–472, Columbia University Press, New York

Cohen, S. S., Morgan, S. & Streiel, E. (1969) *Proc. Natl. Acad. Sci. U.S.A.* **64**, 669–676

De Rosa, M., Gambacorta, A. & Bu'Lock, J. D. (1975) *J. Gen. Microbiol.* **86**, 156–164

De Rosa, M., De Rosa, S., Gambacorta, A., Carteni-Farina, M. & Zappia, V. (1976*a*) *Biochem. Biophys. Res. Commun.* **69**, 253–261

De Rosa, M., De Rosa, S., Gambacorta, A., Carteni-Farina, M. & Zappia, V. (1976*b*) *Abstr. IUB Meet. 10th, Hamburg*, abstr. no. 16-7-248

Galletti, P., Oliva, A., De Santis, A. & Zappia, V. (1975) *Abstr. FEBS Meet. 10th, Paris*, abstr. no. 1424

Glasser, R. & Gabbay, E. J. (1968) *Biopolymers* **6**, 243–254

Hirschman, S. Z., Leng, M. & Felsenfeld, G. (1967) *Biopolymers* **5**, 227–233

Holmsted, B., Learson, L. & Tham, R. (1961) *Biochim. Biophys. Acta* **48**, 182–186

Liquori, A. M., Costantino, L., Crescenzi, V., Elia, V., Giglio, E., Puliti, R., De Santis-Savino, M. & Vitagliano, V. (1967) *J. Mol. Biol.* **24**, 113–122

Okuyama, T. & Kobayashi, J. (1961) *Arch. Biochem. Biophys.* **95**, 242–250

Oshima, T. (1975) *Biochem. Biophys. Res. Commun.* **63**, 1093–1098

Rolle, I., Hobucher, H. E., Kneifel, H., Paschold, B., Riepe, W. & Soeder, C. J. (1977) *Anal. Biochem.* **77**, 103–109

Salvatore, F., Utili, R., Zappia, V. & Shapiro, S. K. (1971) *Anal. Biochem.* **41**, 16–22

Schlenk, F. (1977) in *The Biochemistry of S-Adenosylmethionine* (Salvatore, F., Borek, E., Zappia, V., Williams-Ashman, H. G. & Schlenk, F., eds.), pp. 3–17, Columbia University Press, New York

Schlenk, F. & De Palma, R. E. (1957) *J. Biol. Chem.* **229**, 1037–1050

Shapiro, S. R. & Ehninger, D. J. (1966) *Anal. Biochem.* **15**, 323–327

Smith, J. D. (1976) *Biochem. Biophys. Res. Commun.* **73**, 7–10

Stevens, L. (1967) *Biochem. J.* **103**, 811–815

Stevens, L. & Morrison, M. R. (1968) *Biochem. J.* **108**, 633–640

Stillway, L. W. & Walle, T. (1977) *Biochem. Biophys. Res. Commun.* **77**, 1103–1107

Tabor, H. (1962) *Biochemistry* **1**, 496–501

Tabor, H. & Tabor, C. W. (1976) *Annu. Rev. Biochem.* **45**, 285–306

Toennies, G. & Kolb, J. J. (1951) *Anal. Chem.* **23**, 823–828

Tryding, J. & Willert, K. D. (1968) *Scand. J. Clin. Lab. Invest.* **22**, 29–33

Tsuboi, M. (1964) *Bull. Chem. Soc. Jpn.* **37**, 1514–1522

Vogel, H. J. & Bonnei, D. M. (1956) *J. Biol. Chem.* **218**, 97–101

Wickner, R. B., Tabor, C. W. & Tabor, H. (1970) *J. Biol. Chem.* **245**, 2132–2139

Zappia, V., Zydek-Cwick, C. R. & Schlenk, F. (1969) *J. Biol. Chem.* **244**, 4499–4509

Zappia, V., Carteni-Farina, M., De Rosa, M. & Gambacorta, A. (1975) *Abstr. SIB Meet. 1st, Naples*, abstr. no. PC27

Zappia, V., Galletti, P., Oliva, A. & De Santis, A. (1977*a*) *Anal. Biochem.* **79**, 535–543

Zappia, V., Carteni-Farina, M. & Galletti, P. (1977*b*) in *The Biochemistry of S-Adenosylmethionine* (Salvatore, F., Borek, E., Zappia, V., Williams-Ashman, H. G. & Schlenk, F., eds.), pp. 473–492, Columbia University Press, New York

Zappia, V., Carteni-Farina, M., Oliva, A., De Rosa, M., De Rosa, S. & Gambacorta, A. (1977*c*) *Abstr. SIB Meet. 3rd, Siena*, abstr. no. C38