Table 1. ISOTOPE ENRICHMENT DURING MICROBIOLOGICAL METABOLISM OF SULPHUR COMPOUNDS

Organism	Starting compound	Range of isotopic enrichment			
		Intermediate		End product	
		Compound	$\delta^{34}S$*	Compound	$\delta^{34}S$*
T. concretivorus	H_2S	polythionate	+0·6 to +19·0	$SO_4^{=}$	−10·6 to −18·0
	S°	—	—	$SO_4^{=}$	−0·1 to +1·4
Chromatium sp.	H_2S	polythionate	+8·4 to +11·2	$SO_4^{=}$	−2·9 to +0·9
	S°	—	—	$SO_4^{=}$	+0·3 to +0·4
S. cerevisiae	$SO_3^{=}$	—	—	H_2S	−34·0 to −41·0
	S°	—	—	H_2S	−0·6 to +0·3

* $\delta^{34}S$ refers to the enrichment (or depletion) of sulphur-34 in parts per thousand relative to the $^{34}S/^{32}S$ ratio of the starting compound.

had been stopped earlier is suggested by the accumulation of an intermediate, most likely a polythionate, which was markedly enriched in ^{34}S (Table 1). The same was observed for *T. concretivorus*.

It is generally believed that elemental sulphur is an intermediate in the chemosynthetic and photosynthetic oxidation of sulphide to sulphate and it is possible that it is also an intermediate in the reduction of sulphite. The question then arises as to why no fractionation occurred during either elemental sulphur oxidation or reduction. Since there is no reason to believe that the same enzymes or the same rate-limiting processes are involved in the oxidation or reduction *per se*, it is suggested that the explanation for the lack of fractionation in both processes lies in the feature common to both, the manner of transport of the elemental sulphur across the cell boundary.

It was observed that *Chromatium* sp. grown on elemental sulphur contained sulphur globules within the cells which disappeared on standing in a sulphur-free medium. This, together with the observed accumulation of sulphur in vacuoles of elemental sulphur-grown *Thiobacilli*[2], suggests that the sulphur was transported into the cell without a change of valence. The absence of significant isotopic fractionation during sulphur metabolism further indicates that groups of atoms are transferred across the cell membrane, eliminating any kinetic isotope effect. The atomic groups involved could be single or multiple molecules of sulphur chains; but the evidence does not permit speculation on the size. It also suggests that all, or the major part, of the sulphur entering the cell is metabolized to sulphate without an equilibrium existing between external and internal free sulphur.

The experimental results thus support the concept of movement of elemental sulphur *per se* across the cell boundary but cast no light on the actual mechanism of transport. The suggestion of Vishniac and Santer[3] that the sulphur is first reduced to sulphide seems unlikely in view of the significant fractionation observed with sulphide as a substrate.

This research was supported by a grant from the Petroleum Research Fund administered by the American Chemical Society.

I. R. KAPLAN
S. C. RITTENBERG

Department of Bacteriology,
University of Southern California,
Los Angeles.

[1] Umbreit, W. W., Vogel, H. R., and Vogler, K. G., *J. Bacteriol.*, **43**, 141 (1942).
[2] Knaysi, G., *J. Bacteriol.*, **46**, 451 (1943).
[3] Vishniac, W., and Santer, M., *Bacteriol. Rev.*, **21**, 195 (1957).
[4] Kaplan, I. R., Ph.D. thesis (Univ. South. Calif., 1962).
[5] Parker, C. D., *Nature*, **159**, 439 (1947).

Tetramethylpyrazine: a *Bacillus subtilis* Growth Factor

WE have found[1] that a strain of *Bacillus subtilis* produced tetramethylpyrazine in synthesized media containing asparagine as nitrogen source.

The strain did not show satisfactory growth in media containing any amino-acid, except asparagine and proline, etc., as nitrogen source.

From the fact, it seems that the amino-acids asparagine and proline participate in the biological synthesis of tetramethylpyrazine, and the compound is a growth factor for the strain. In fact, growth of the strain and *Bacillus subtilis PCL* was considerably accelerated when teramethylpyrazine was added in culture media containing amino-acid, such as alanine, histidine, lysine, tryptophan and glycine, etc. The results obtained are given in Table 1.

Table 1. EFFECTS OF SUBSTANCES ON GROWTH OF THE *Bacillus subtilis* STRAIN AND *Bacillus subtilis PCL*

Substance as nitrogen source	*BS* factor	Degree of growth			
		B. subtilis strain		*B. subtilis PCL*	
		20 hr.	35 hr.	20 hr.	35 hr.
Asparagine	−	+ +	+ + +	+	+ +
	+	+ +	+ + +	+ +	+ + +
Proline	−	+	+ +	+	+ +
	+	+	+ +	+	+ +
Alanine	−	−	+	−	−
	+	+	+ +	−	±
Histidine	−	+	+	−	±
	+	+	+ +	+ +	+ +
Cystine	−	−	+	−	−
	+	±	+ +	±	+
Lysine	−	−	−	−	−
	+	−	+	±	+ +
Phenylalanine	−	−	−	−	−
	+	−	+	−	+
Glutamine	−	+	+ +	±	+
	+	+	+ +	+	+ + +
Tryptophan	−	−	−	−	−
	+	−	+	−	+
Glycine	−	−	−	−	−
	+	+ +	+ +	±	+ +
Arginine	−	+ +	+ + +	−	+
	+	+ +	+ + +	+	+ +
Tyrosine	−	−	+	−	−
	+	−	+ +	−	+
Leucine	−	−	−	−	−
	+	+	+	+	+
Methionine	−	−	−	−	−
	+	−	−	−	−
BS factor	+	−	−	−	−

As shown in the table, it might well be said that tetramethylpyrazine is a growth factor for the *Bacillus subtilis* strain and *Bacillus subtilis PCL*. We therefore suggest the name of *BS* factor (*Bacillus subtilis* factor) for tetramethylpyrazine.

From these results tetramethylpyrazine may be accepted as a growth factor for *Bacillus subtilis*.

TAKUO KOSUGE
HIROKO KAMIYA

Shizuoka College of Pharmacy,
Shizuoka, Japan.

[1] Kosuge, T., and Kamiya, H., *Nature*, **193**, 776 (1962).

Oxidation of Anthranilic Acid by a Species of *Achromobacter* isolated from Soil

STUDIES of the microbial decomposition of anthranilic acid have been confined mostly to work with various strains of *Pseudomonas* spp.[1-3] which oxidize anthranilic acid to catechol by an unknown pathway. The role of salicylic acid in this conversion is not clear since reported experiments based on the technique of

© 1962 Nature Publishing Group