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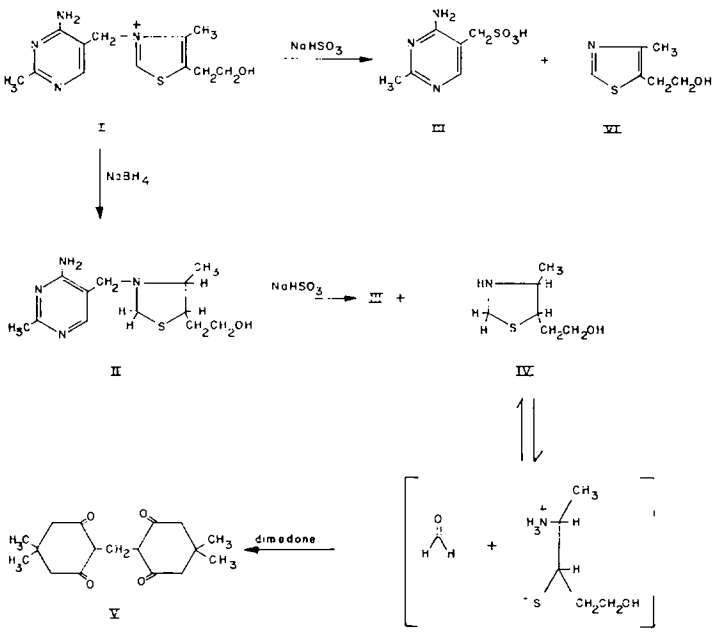
Thiamin biosynthesis. II*. A novel biosynthetic transformation of the S-methyl moiety of methionine

In the biosynthetic pathways which have been uncovered in the past one or two decades, methionine (as *S*-adenosylmethionine) is generally observed to function as a methyl group donor. With respect to the biosynthesis of thiamin (I), however, evidence[1,2] has been accumulated which suggests that methionine may be incorporated as a unit into the thiazole ring system and that the methyl carbon of methionine may become the C-2 of the thiazole ring. We have uncovered further evidence, based upon degradation studies which supports this conclusion that the methyl group of methionine becomes the C-2 of the thiazole ring.



The degradation scheme developed in our laboratories is similar to that recently reported by LINNETT AND WALKER[3]. Thiamin (I) was reduced to the tetrahydro derivative (II) by the action of cold aqueous sodium borohydride at a pH of 7.5–8.0. Bisulfite cleavage of II gave the pyrimidinesulfonic acid (III) and 4-methyl-5-(β-hydroxyethyl)-thiazolidine (IV)[4]. C-2 of the thiazolidine ring was isolated as the dimedone derivative of formaldehyde (V) when IV was warmed (80°) with an aqueous ethanol solution of dimedone and a catalytic amount of piperidine. The yield of V based on thiamine was generally 15–20%. All structures were substantiated by comparison with the literature and by nuclear magnetic resonance spectroscopy. Degradation of [*thiazole*-2-^{14}C]thiamin gave V with at least 80% of the activity of the original thiamine.

In contrast to previous experiments[2], we exposed *Bacillus subtilis* (ATCC 6633)

* Paper No. I in this series; see ref. 2.

to the isotopic label for an extended duration of time. Since we had previously demonstrated that [*Me*-^{14}C]methionine is effectively incorporated into thiamin using the technique of "pulse" labeling[2], we could be reasonably sure that our results would not be significantly distorted due to metabolic randomization. This would be especially true if the degradation showed a specific localization of the isotope.

Growth conditions for cultures of *B. subtilis* and the procedures for the isolation and purification of the thiamin were the same as described previously[2]. In Expt. 1 (Table I), Compound II was converted to its dihydrobromide which was recrystallized to constant specific activity (± 10 %) from ethanol or ethanol–ether. The pyrimidine (III) was isolated from the bisulfite cleavage and its specific activity determined after purification. This value was then subtracted from the value for the specific activity of the dihydrobromide of II to give the specific activity of the thiazole (VI). In Expt. 2, the sample was divided into three portions. One portion was subjected to the bisulfite cleavage, and the thiazole was purified by chromatography and its specific activity determined. The remaining two portions were each degraded according to the above scheme. In all degradations, the dimedone derivative (V) was recrystallized to constant specific activity (± 5 %) from ethanol–water.

These results demonstrate that C-2 of the thiazole ring of thiamin can derive from the methyl carbon of methionine. In addition, they indirectly reinforce the earlier conclusion[2] that [*Me*-^{14}C, ^{3}H]methionine supplies tritium to the α-carbon of the β-hydroxyethyl side chain, a point of evidence which indicates the participation of the entire methionine molecule in the biosynthesis of the thiazole.

Using *Saccharomyces cerevisiae*, LINNETT AND WALKER[3] have found that both [2-^{14}C]glycine and [^{14}C]formate are more effective precursors of the thiazole moiety than is [*Me*-^{14}C]methionine. They have further shown that 74 % of the label from [2-^{14}C]glycine resides in the 2-position of the thiazole whilst nearly all of the label from [^{14}C]formate is in some other position(s). Even though their findings with respect to the effectiveness of formate as a precursor to the thiazole differ markedly with the reports of other investigators[1,2,5,6] and while the possibility of differing biosynthetic pathways does exist, we, nevertheless, believe that the results, obtained

TABLE I

THE DEGRADATION OF THIAMIN OBTAINED FROM CULTURES OF *Bacillus subtilis* (ATCC 6633) GROWN ON [*Me*-^{14}C]METHIONINE

All counting was performed using either a Nuclear Chicago Mark I or a Packard Tri-Carb liquid scintillation counter using a toluene fluor. Efficiencies were calculated from both channels ratio and the external standard methods.

Expt. No.	*Dose (mC)*	*[Me-^{14}C]Methionine specific activity (mC/mmole)*	*Specific activities (disint./min per μmole)*		*% ^{14}C at 2 position of thiazole*
			VI	*V*	
1A	0.5	40	34.1	32.8	96
1B			34.3	22.3	65*
2A	0.4	57	8.4	7.8	93
2B			8.4	7.8	93

* We cannot account for this anomalous value except to say it may be the result of an error in sample analysis.

in our hands and elsewhere, are not necessarily contradictory but may simply reflect the active participation of formate and glycine in numerous metabolic pathways coupled with the inherent differences in the organisms under investigation. Experiments are being conducted in this laboratory in order to resolve these apparent discrepancies as well as other problems of thiamine biosynthesis.

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Kinetics of the fluorescence response to microelectrophoretically introduced metabolites in the single living cell

Microfluorimetric studies have revealed the specific responses of mitochondrial and extramitochondrial pyridine nucleotides to microelectrophoretically introduced metabolites[1]. In these experiments, the fluorescence recordings corresponded to steady-state levels, as there was a 10–20-sec time lag, due to performance of cell manipulations under visible light and change from visible light to ultraviolet light. To study the kinetics of pyridine nucleotide oxidation–reduction, a Chance–Legallais microfluorimeter[2] supplemented with a beam splitter was developed for fluorescence recording synchronous with cell manipulations[3] (Fig. 1). The mirror used to reflect light towards the ocular was replaced by a beam splitter (type Filtraflex-DC, transmission 90 % below 570 mμ, less than 5 % over 650 mμ, courtesy of Balzers, Lichtenstein). Thus, red light (for cell and microinstrument visualization) is reflected towards the ocular, while the blue light emitted by fluorescent intracellular structures goes through to the photocathode[3].

Although a variety of cell types has been used successfully in the microfluorimeter, for both mitochondrial and extramitochondrial studies[1], the highest fluorescence levels so far recorded correspond to the glycolytic response of the EL2 giant[1], obtained by X-irradiation of ascites cells in culture. Mixtures containing fructose 1,6-diphosphate (Fru-1,6-P_2) and/or glucose 6-phosphate (Glc-6-P) were therefore introduced into the cytoplasm of EL2 giants by microelectrophoresis.