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# Betaine-Homocysteine Transmethylase in *Pseudomonas denitrificans*, a Vitamin $B_{12}$ Overproducer

RAYMOND F. WHITE, LOUIS KAPLAN, AND JEROME BIRNBAUM

*Fermentation Research Department, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065*

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A pantothenate-methionine auxotroph (J741) of *Pseudomonas denitrificans* was isolated whose growth requirement for methionine could not be satisfied by known precursors of the amino acid, including homocysteine. However, some "methyl rich" compounds such as betaine and dimethylacetothetin (DMT) could satisfy the requirement. *S*-Methyl-methionine and *S*-adenosylmethionine were ineffective. Extracts were found to contain an enzyme, betaine-homocysteine transmethylase (BHTase), that uses betaine or DMT as a methyl donor and homocysteine as an acceptor to produce methionine. Growth of J741 in methionine leads to a total repression of the BHTase, whereas the use of DMT leads to a three- to sixfold stimulation of enzyme synthesis compared to betaine-grown cells. The pantothenate requirement is unrelated to the methionine auxotrophy, since the growth of other single auxotrophic mutants as well as revertants of J741 still have their methionine requirement satisfied by betaine or DMT. Another methionine auxotroph that could not use betaine for growth was devoid of BHTase activity.

Microorganisms possess two mechanisms for the methylation of homocysteine to yield methionine (23). These reactions are mediated by two enzymes which differ with respect to the folate derivative required for activity. One of these enzymes requires vitamin $B_{12}$ (cobalamin-dependent) for activity.

Lago and Demain (12) found that the vitamin $B_{12}$ overproducer, *Pseudomonas denitrificans*, uses the cobalamin-dependent pathway for methionine synthesis. This was suggested from studies with a mutant of *P. denitrificans* which had an alternate requirement for $B_{12}$ or methionine. Recently, we isolated another methionine auxotroph of this organism. This mutant is blocked in the conversion of homocysteine to methionine. It was found that the growth requirement for this mutant could be satisfied by the methyl-rich compounds, betaine and dimethylacetothetin (DMT). Evidence is presented in this paper to show that *P. denitrificans* contains an enzyme that transfers a methyl group from betaine or DMT to homocysteine to form methionine. This is the first demonstration of this enzyme, betaine-homocysteine transmethylase (BHTase; betaine: L-homocysteine *S*-methyltransferase; EC 2.1.1.5) in a microorganism. This enzyme has been observed previously only in animal tissues (8-11).

## MATERIALS AND METHODS

**Strains.** Most of the work reported here was performed with a mutant of *P. denitrificans* designated J741. This culture was a double auxotroph requiring pantothenic acid and L-methionine for growth. The mutant was selected from a wild-type strain, 2436, after mutagenesis with *N*-methyl-*N*-nitroso-*N'*-nitroguanidine (MNG). Strain M13 was a pantothenate revertant of J741 (also induced by MNG) that retained its methionine requirement. Mutant 2196, described previously (12), is blocked in the biosynthesis of vitamin $B_{12}$ and has an alternate requirement for methionine or vitamin $B_{12}$. Strain 2196 ($B_{12}$-R) is a spontaneous revertant of 2196 that has the wild-type phenotype. Mutant 2202 is a methionine auxotroph similar to strain J741 but lacking the requirement for pantothenic acid. Wild-type 2436 was maintained on a complex agar medium containing in grams per liter: beet molasses, 60; brewers' yeast, 1.0; Sheffield N-Z-amine, 1.0; $(NH_4)_2HPO_4$, 2.0; $ZnSO_4 \cdot 7H_2O$, 0.02; $MnSO_4 \cdot 1H_2O$, 0.2; $MgSO_4 \cdot 7H_2O$, 1.0; $Na_2MO_4 \cdot H_2O$, 0.005; and agar (Difco), 25. The *p*H was adjusted to 7.4 before autoclaving. The

remaining strains were kept on slants containing the medium described by Lago and Demain (12) plus 20 μg of L-methionine per ml and 5 μg of calcium pantothenate per ml. This is referred to below as L-D medium.

**Growth and $B_{12}$ production medium.** For growth studies, the organisms were transferred to L-D medium (40 ml per 250-ml Erlenmeyer flask) containing the optimal levels of the required nutrilites. All additions to the L-D medium were made from filter-sterilized concentrated stock solutions. Incubation was for 48 hr at 20 C on a rotary shaker. Cells were removed by centrifugation, washed once with 0.039 M $Na_2HPO_4$-$KH_2PO_4$ (*p*H 7.4) buffer, and inoculated into a fresh flask of L-D medium. Flasks were incubated as above, and growth measurements were made on a Bausch and Lomb Spectronic 20 at 600 nm. Samples were diluted in buffer to make up for the nonlinearity of the instrument at high cell densities. An optical density of 1.0 is equivalent to 0.68 mg of dry cells per ml as determined by a calibrated curve relating dry weight to optical density.

For the measurement of vitamin $B_{12}$ production capacity, the inoculum of the various strains, as grown above, was transferred to flasks containing 40 ml of the following medium (SV-12): 3% sucrose, 1% betaine, 0.5% sodium glutamate, 0.5% $(NH_4)_2HPO_4$, 0.1% $MgSO_4 \cdot 7H_2O$, 0.09% KCl, 0.01% L-methionine, 0.003% $FeSO_4 \cdot 7H_2O$, 0.0025% 5,6-dimethylbenzimidazole, 0.002% $MnSO_4 \cdot H_2O$, 0.002% $ZnSO_4 \cdot 7H_2O$, 0.0016% $Co(NO_3)_2 \cdot 6H_2O$, and 0.002% $Na_2MoO_4 \cdot 2H_2O$ (*p*H 6.8 before autoclaving). Sucrose was sterilized separately and added to the sterile medium from a concentrated stock solution. Incubation was at 28 C for 5 days on a rotary shaker.

**Preparation of extracts.** Cells were harvested by centrifugation, washed three times with 0.039 M sodium-potassium-phosphate buffer (*p*H 7.4), and suspended in the same buffer. The final suspension of cells represented about a tenfold concentration as compared to that found in the growth medium. The cells were ruptured with an Aminco French pressure cell at 14,000 psi; cellular debris was removed by centrifugation (12,000 *g* for 10 min), and the resulting cell-free preparation was used for enzyme assay. All the steps described above were carried out at 0 to 4 C. Since the enzyme activity of the extract was quite labile, fresh preparations were made for each experiment.

**Assays.** BHTase was determined by established procedures (22) by using the nitroprusside reaction for measurement of the methionine formed in the reaction mixture. One unit of enzyme activity (*Enzyme Nomenclature*, Elsevier Publishing Co., 1965) was defined as that amount which catalyzed the formation of 1 μmole of methionine per min. The reaction product was shown to be identical to authentic methionine by paper chromatography in three solvent systems (1-butanol-acetic acid-water [4:1:5, v/v]; isopropyl alcohol-formate-water [20:1:5, v/v]; and 1-butanol-pyridine-water [12:3:5, v/v]). Protein was determined by the method of Lowry et al. (13). Crystalline bovine serum albumin served as the standard. Vitamin $B_{12}$ was determined by microbiological assay with *Lactobacillus lactis* Dorner (ATCC 10697). Crystalline cyanocobalamin was used as a standard. Whole broth samples were prepared for assay by autoclaving for 3 min with 2.25% $NaNO_2$-0.1% KCN at *p*H 3 to 4.

## RESULTS

Table 1 shows the growth response of *P. denitrificans* J741 to the compounds structurally or biosynthetically related to methionine and betaine. Homocysteine or any of the early biosynthetic precursors of homocysteine could not satisfy the methionine requirement of the mutant. These data, along with the fact that the organism was still capable of vitamin $B_{12}$ production, suggested that the lesion was in the apoenzyme moiety of the cobalamin-dependent methionine synthetase. The growth requirement for methionine by J741 could be satisfied by methyl-rich compounds such as choline, betaine, and DMT. Other compounds related to betaine (glycine, *N*-methylglycine, *N*-dimethylglycine) and the higher homologue of DMT, dimethyl-β-propiothetin, were inactive. Also inactive were *S*-methyl-DL-methionine and *S*-adenosyl-L-methionine. A titration of the response to the active methionine substitutes is shown in Fig. 1. Though the slope of the dose-response curve was different for each of

TABLE 1. *Growth response of P. denitrificans J741 (Pan⁻, Met⁻) to compounds structurally or biosynthetically related to methionine and betaine*

| Compound[a] | Optical density at 660 nm |
|---|---|
| None | 0.03 |
| L-Methionine | 3.2 |
| L-Homoserine | 0.06 |
| L-Serine | 0.05 |
| L-Cysteine | 0.05 |
| DL-Homocysteine | 0.04 |
| Cyanocobalamin | 0.09 |
| Tetrahydrofolic acid | 0.03 |
| *S*-Adenosyl-L-methionine | 0.06 |
| *S*-Methyl-DL-methionine | 0.06 |
| Betaine | 2.2 |
| Choline | 2.8 |
| Glycine | 0.06 |
| *N*-Methylglycine | 0.06 |
| *N*-Dimethylglycine | 0.05 |
| Dimethylacetothetin | 3.5 |
| Dimethylpropiothetin | 0.04 |

[a] Calcium pantothenate was added at a concentration of 0.02 mM to all flasks. Test compounds were included in L-D medium at 0.13 mM, except for *S*-methyl-DL-methionine and DL-homocysteine which were at 0.26 mM. Growth was measured after 48 hr.

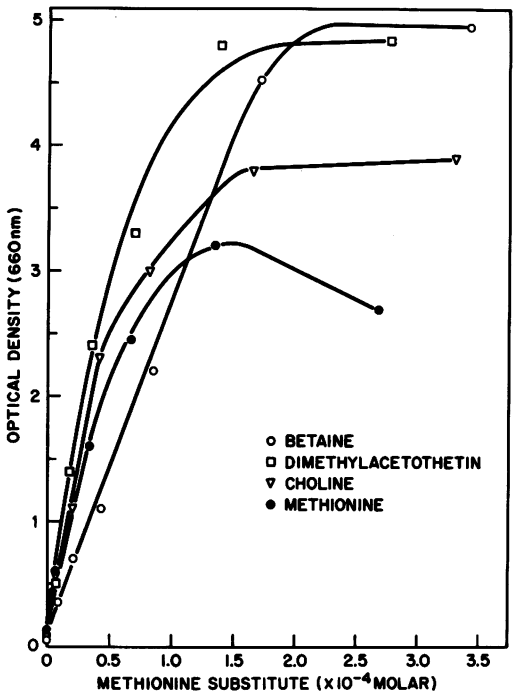


FIG. 1. *Growth response of P. denitrificans J741 to methionine substitutes. All compounds were added to L-D medium from concentrated filter-sterilized stock solutions. Incubation was for 48 hr.*

the compounds, maximal growth was obtained at approximately $1.5 \times 10^{-4}$ M for all the compounds.

These results suggest that, in addition to the cobalamin-dependent methionine synthetase, *P. denitrificans* contains a second mechanism for the methylation of homocysteine to yield methionine. Several organisms contain the enzyme *S*-adenosylmethionine homocysteine transmethylase (SAHTase) (18, 19). The methyl donor in this reaction is either *S*-methylmethionine or *S*-adenosylmethionine. Since neither of these materials could be used by J741 in place of methionine (Table 1), it was felt that this was not the methyl transfer reaction operative in *P. denitrificans*. It was not possible to demonstrate activity of the SAHTase in extracts of *P. denitrificans*.

An additional methyl transfer reaction has been found in mammalian liver (8–11). This reaction uses betaine, DMT, or dimethyl-β-propiothetin as methyl donor and homocysteine as acceptor, and produces L-methionine. The enzyme has been termed betaine- (or thetin-) homocysteine transmethylase (BHTase). Since it was observed that two of the three possible methyl donors in this reaction satisfied the methionine requirement of strain J741, extracts of organisms were examined for BHTase activity (Fig. 2). The extracts showed activity which remained linear for about 90 to 100 min. The product of the reaction was identified as methionine by paper chromatography in three different solvent systems. No methionine was formed when betaine, DMT, or homocysteine were omitted from the reaction mixture. Though choline was a growth substrate for the organism (Table 1), it was inactive as a methyl donor in the cell extracts. This can be explained by the fact that choline is slowly converted to betaine by whole cells of *P. denitrificans*, but not by cell extracts (White and Birnbaum, *unpublished*). Dimethyl-β-propiothetin that could not support growth (Table 1) also could not act as a methyl donor in the extract. The addition of methionine or dimethylglycine (at concentrations produced by the extracts) to the reaction mixture had no effect on enzyme activity, indicating a lack of feedback inhibition. Figure 3 shows the effect of temperature and *p*H on the activity of J741 extracts. For this experiment, J741 was grown in L-D medium with betaine and DMT. Extracts were prepared and tested at different temperatures and hydrogen ion concentrations, with the corresponding growth

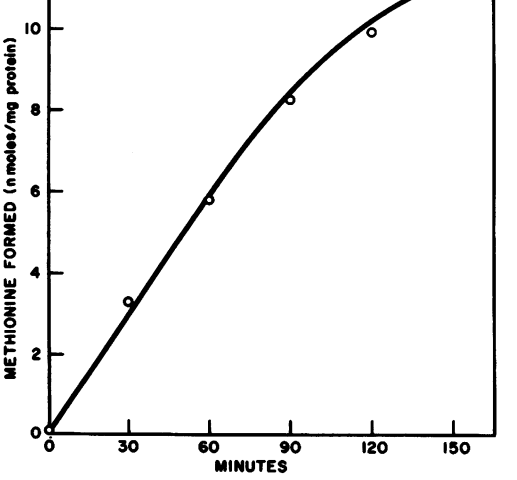


FIG. 2. *Betaine-homocysteine transmethylase activity of extracts of P. denitrificans J741. Reaction mixture contained: 2 μmoles of betaine, 2 μmoles of* DL*-homocysteine (both in 0.039 M* $Na_2HPO_4$-$KH_2PO_4$ *buffer, pH7.4), and 2 ml of cell extract in a total volume of 3 ml. Incubation was at 37 C. The reaction was stopped with 0.5 ml of 30% trichloroacetic acid. Samples were removed and assayed for methionine.*

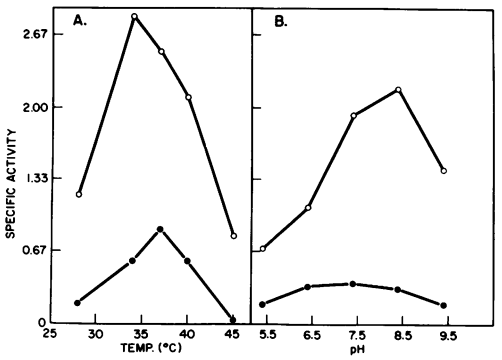


FIG. 3. *Effect of temperature and pH on the betaine-homocysteine transmethylase in extracts of P. denitrificans J741. Symbols: ●, cells grown with DMT and assayed with DMT as methyl donor; ○, cells grown with betaine and assayed with betaine. Different pH values were obtained by varying the proportions of $Na_2HPO_4$ and $KH_2PO_4$ to give 0.039 M. Incubation time was 3 hr. Other conditions were the same as for Fig. 2.*

substrate as methyl donor. Extracts obtained from cells grown with DMT and tested for BHTase with DMT as the substrate had three to six times the activity of extracts from the betaine system. The optimal temperature for the reaction was 34 C for the DMT-grown cells and 37 C for those grown and assayed with betaine as a methyl donor. The optimal *p*H for DMT cells was 8.4; there was no sharp *p*H optimum for the betaine-grown cells. These results suggested that either growth with DMT depressed BHTase synthesis, or that both types of cells had a similar content of BHTase but that betaine was a poorer substrate for the cell-free preparation than was DMT. To answer this, J741 was grown with L-methionine, betaine, or DMT, and then each cell type was tested for BHTase activity for both substrates. The growth substrate had a pronounced effect on the synthesis of the transmethylase (Table 2). Methionine completely repressed the synthesis of the enzyme, whereas DMT stimulated production about fivefold. Although DMT is superior in the synthesis of BHTase, betaine is equally as effective as DMT as a substrate for the enzyme.

Table 3 shows the growth response to betaine and DMT, $B_{12}$ overproduction, and BHTase activity of several mutants of *P. denitrificans* and a wild-type strain. The wild-type strain (2436) was independent of methionine and methyl donor compounds, overproduced $B_{12}$, and contained BHTase. J741, the double auxotroph, and its pantothenate revertant, M13, had identical BHTase activity. Since the revertant no longer required pantothenate, it appears that this locus had no role in the growth response to betaine or in the level of BHTase. Mutant 2202 is another methionine auxotroph that had no other nutritional requirements, responded to betaine or DMT, and produced BHTase. Though 2202 had one-sixth the level of BHTase, it grew as rapidly and to the same cell density as J741 on either methionine, betaine, or DMT. This indicated that even the low level of BHTase in 2202 was not limiting for growth. Strain 2196 has an alternate requirement for $B_{12}$ or methionine and could not use betaine or DMT as a methionine source. Extracts of this organism had no detectable BHTase. A methionine revertant of 2196 (2196 $B_{12}$-R) was isolated, and this strain could again overproduce $B_{12}$ but still had no BHTase activity. Apparently, 2196 was a double mutant with a lesion in $B_{12}$ synthesis and in BHTase synthesis. However, these mutations were completely independent of each other.

High levels of betaine are required for overproduction of vitamin $B_{12}$ by *P. denitrificans* (6). The principal function of betaine is not as a direct precursor but rather as a regulatory effector in corrin and porphyrin syntheses (7, 21). Since DMT was an alternate substrate for BHTase, this compound was tested as a substitute for betaine in the vitamin $B_{12}$ fermentation (Table 4). DMT could not replace betaine in vitamin $B_{12}$ oversynthesis. Choline, which can be slowly converted to betaine, was only partially effective as a substitute for betaine.

## DISCUSSION

We believe that this is the first demonstration of BHTase in a microorganism. Previously, this enzyme had been demonstrated only in pigeon (Sloane et al., Fed. Proc. 14:282, 1955) and mammalian liver (8–11) and rat kidney (8).

TABLE 2. *Effect of growth substrate on the level of BHTase*

| Addition to growth medium[a] | Specific activity (milliunits/mg of protein) | |
|---|---|---|
| | Betaine as substrate | Dimethyl acetothetin as substrate |
| L-Methionine | 0 | 0 |
| Betaine | 0.4 | 0.4 |
| Dimethylacetothetin | 2.0 | 2.0 |

[a] Additives were at 0.13 mM. Calcium pantothenate was included in all flasks at 0.02 mM. Enzyme assays were performed as described in Fig. 2. Incubation time was 3 hr.

TABLE 3. *Growth requirements and BHTase levels of mutants of P. denitrificans*

| Strain | Phenotype | Growth on: | | | | $B_{12}$ overproduction | Specific activity BHTase[b] |
|---|---|---|---|---|---|---|---|
| | | Methionine | $B_{12}$ | Betaine | DMT | | |
| 2436 | Wild type | I[a] | I | I | I | Yes | 0.50 |
| J741 | Pan$^-$, Met$^-$ | + | − | + | + | Yes | 1.59 |
| M13 (J741 panto R) | Met$^-$ | + | − | + | + | Yes | 1.59 |
| 2196 | Met$^-$/$B_{12}^-$, BHTase$^-$ | + | + | − | − | No | 0 |
| 2196 ($B_{12}$-R) | BHTase$^-$ | I | I | I | I | Yes | 0 |
| 2202 | Met$^-$ | + | − | + | + | Yes | 0.26 |

[a] Symbols: I, independent (i.e., wild type) growth; +, growth in 48 hr; −, no growth in 48 hr.

[b] Milliunits per milligram of protein. To measure BHTase, cells were grown in L-D medium supplemented with 0.13 mM DMT and 0.02 mM pantothenic acid (except for 2196, which received 0.1 μg of cyanocobalamin per ml in place of DMT). To measure vitamin $B_{12}$ production capacity, SV-12 medium was used (see Materials and Methods) which was supplemented with 0.02 mM pantothenic (plus 0.1 μg of cyanocobalamin per ml in the case of mutant 2196).

TABLE 4. *Production of vitamin $B_{12}$ by P. denitrificans*

| Addition to basal production medium[a] | Vitamin $B_{12}$ production | |
|---|---|---|
| | Culture 2436 | Culture J741 |
| None | 0.5[b] | 0.5 |
| Betaine | 11.7 | 9.8 |
| Choline | 2.4 | 1.6 |
| Dimethylacetothetin | 0.5 | 0.5 |

[a] Additives were at 37 mM. Calcium pantothenate was added to all flasks at 0.02 mM. Basal production medium was SV-12 lacking betaine.

[b] Assayed as micrograms of cyanocobalamin per milliliter.

The *S*-methyl group of L-methionine contributes the six extra methyl groups on the corrin ring in the biosynthesis of vitamin $B_{12}$ (2, 3). Further, there is an absolute requirement for betaine for production of the vitamin by *P. denitrificans* in chemically defined media (6). The finding of BHTase in *P. denitrificans* suggested that this enzyme might have an important role in the synthesis of the vitamin. In spite of the fact that BHTase has betaine as its substrate and methionine as the product of its action, two observations reported here suggest that this enzyme is not of importance to corrin production. First, a mutant was isolated (2196 $B_{12}$-R) that did not have BHTase activity, but still overproduced vitamin $B_{12}$, and second, DMT, which is as good a substrate as betaine for the enzyme, cannot substitute for betaine in vitamin $B_{12}$ synthesis.

Most organisms studied in detail have more than one enzyme mechanism for the methylation of homocysteine to generate methionine. For example, *Escherichia coli* and *Aerobacter aerogenes* have the two folate-requiring enzymes and a nonfolate enzyme, SAHTase (1, 17, 23). *Candida albicans* and *Saccharomyces cerevisiae* have the cobalamin-independent folate enzyme and SAHTase (16, 20). *Salmonella typhimurium* has the two folate enzymes (5). Mammalian cells have the cobalamin-dependent methionine synthetase and the BHTase (8 to 11, 15). Apparently, *P. denitrificans* has the same enzyme types as the higher forms.

Attempts to demonstrate the SAHTase in *P. denitrificans* were unsuccessful. The methodology employed (16) was adequate, since it was possible to find activity of SAHTase in extracts of *C. albicans*, so we were confident that the enzyme could have been demonstrated if it were present in *P. denitrificans*.

The metabolic significance of these multiple mechanisms for methylation of homocysteine is largely unknown. Methionine is a unique amino acid in that it is required both as a building block for protein and as a source of methyl groups, via *S*-adenosylmethionine, in many biosynthetic sequences and in the methylation of transfer ribonucleic acid (14), deoxyribonucleic acid (M. Gold, and J. Hurwitz, Fed. Proc. 22:230, 1963), and lipids (4). Thus, the demand for methionine is high. It is tempting to speculate that the multiple mechanisms for methylation of homocysteine are required to keep up with the metabolic demand for methionine. During periods of rapid growth, no single pathway might suffice. Organisms with several pathways for biosynthesis of methionine may have a physiological advantage.

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## LITERATURE CITED


1. Babish, E., and S. K. Shapiro. 1966. Cystathionine as a precursor of methionine in *Escherichia coli* and *Aerobacter aerogenes*. J. Bacteriol. **92**:1331–1336.
2. Bray, R., and D. Shemin. 1958. The biosynthesis of porphyrin-like moiety of vitamin $B_{12}$. Biochim. Biophys. Acta **30**:647–648.
3. Bray, R. C., and D. Shemin. 1963. On the biosynthesis of vitamin $B_{12}$. The derivatization of the corrin structure from δ-aminolevulinic acid and the methyl group of methionine. J. Biol. Chem. **238**:1501–1508.
4. Bremer, J., and D. M. Greenberg. 1961. Methyl transfering enzyme system of microsomes in the biosynthesis of lecithin (phosphatidyl-choline). Biochim. Biophys. Acta **46**:205–216.
5. Cauthen, S. E., M. A. Foster, and D. D. Woods. 1966. Methionine synthesis by extracts of *Salmonella typhimurium*. Biochem. J. **98**:630–635.
6. Demain, A. L., H. J. Daniels, L. Schnable, and R. F. White. 1968. Specificity of the stimulatory effect of betaine on the vitamin $B_{12}$ fermentation. Nature (London) **220**:1324–1325.
7. Demain, A. L., and R. F. White. 1971. Porphyrin overproduction by *Pseudomonas denitrificans:* essentiality of betaine and stimulation by ethionine. J. Bacteriol. **107**:456–460.
8. Dubnoff, J. W., and H. Borsook. 1948. Dimethylthetin and dimethyl-β-propiothetin in methionine synthesis. J. Biol. Chem. **176**:789–796.
9. Ericson, L. E. 1960. Betaine-homocysteine-methyl-transferases. III. The methyl donor specificity of the transferase isolated. Acta Chem. Scand. **14**:2127–2134.
10. Kensler, C. J., and H. Langemann. 1954. Metabolism of choline and related compounds by hepatic tissue from several species including man. Proc. Soc. Exp. Biol. Med. **85**:364–367.
11. Klee, W. A. 1962. The synthesis of methionine by enzymic transmethylation. IX. Formation of subunits of thetin-homocysteine methylpherase by reaction with sodium dodecyl sulfate. Biochim. Biophys. Acta **59**:562–568.
12. Lago, B. D., and A. L. Demain. 1969. Alternate requirement for vitamin $B_{12}$-producing bacterium. J. Bacteriol. **99**:347–349.
13. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
14. Mandel, L. R., and E. Borek. 1961. Variability in the structure of ribonucleic acid. Biochem. Biophys. Res. Commun. **4**:14–18.
15. Mangum, J. H., and J. A. North. 1968. Vitamin $B_{12}$-dependent methionine biosynthesis in Hep-2 cells. Biochem. Biophys. Res. Commun. **32**:105–110.
16. Mardon, D. N. 1971. Methionine biosynthesis in *Candida albicans*. I. S-Adenosyl-L-methionine (or S-methyl-L-methionine):homocysteine methyltransferase in cell-free extracts from yeast-like cells. Can. J. Microbiol. **17**:795–802.
17. Morningstar, J. F., Jr., and R. I. Kisliuk. 1965. Interrelations between two pathways of methionine biosynthesis in *Aerobacter aerogenes*. J. Gen. Microbiol. **39**:48–51.
18. Shapiro, S. K. 1958. Adenosylmethionine-homocysteine transmethylase. Biochim. Biophys. Acta **29**:405–409.
19. Shapiro, S. K., and D. A. Yphantis. 1959. Assay of S-methylmethionine and S-adenosylmethionine homocysteine transmethylase. Biochim. Biophys. Acta **36**:241–244.
20. Shapiro, S. K., D. A. Yphantis, and A. Almenus. 1964. Biosynthesis of methionine in *Saccharomyces cerevisiae* partial purification and properties of S-adenosylmethionine:homocysteine methyl-transferase. J. Biol. Chem. **239**:1551–1556.
21. White, R. F., and A. L. Demain. 1971. Catabolism of betaine and its relationship to cobalamin overproduction. Biochim. Biophys. Acta **237**:112–119.
22. Williams, J. N., W. J. Monson, A. Suenivasan, L. S. Dietrich, A. E. Harper, and C. A. Elvehjem. 1953. Effects of a vitamin $B_{12}$ deficiency on liver enzymes in the rat. J. Biol. Chem. **202**:151–156.
23. Woods, D. D., M. A. Foster, and J. R. Guest. 1965. Cobalamin-dependent and -independent methyl transfer in methionine synthesis, p. 138–156. *In* S. K. Shapiro and F. Schlenk (ed.), Transmethylation and methionine biosynthesis. University of Chicago Press, Chicago.