Selective inhibition of uracil tRNA methylases of *E. coli* by ethionine

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ABSTRACT

L-ethionine has been found to inhibit uracil tRNA methylating enzymes in vitro under conditions where methylation of other tRNA bases is unaffected. No selective inhibitor for uracil tRNA methylases has been identified previously. 15 mM L-ethionine or 30 mM D,L-ethionine caused about 40% inhibition of tRNA methylation catalyzed by enzyme extracts from *E. coli* B or *E. coli* M3S (mixtures of methylases for uracil, guanine, cytosine, and adenine) but did not inhibit the activity of preparations from an *E. coli* mutant that lacks uracil tRNA methylase. Analysis of the $^{14}\text{CH}_3$ bases in methyl-deficient *E. coli* tRNA after its in vitro methylation with *E. coli* B enzymes in the presence or absence of ethionine showed that ethionine inhibited $^{14}\text{CH}_3$ transfer to uracil in tRNA, but did not diminish significantly the $^{14}\text{CH}_3$ transfer to other tRNA bases. Under similar conditions 0.6 mM S-adenosylethionine and 0.2 mM ethylthioadenosine inhibited the overall tRNA base methylating activity of *E. coli* B preparations about 50% but neither of these ethionine metabolites preferentially inhibited uracil methylation. Ethionine was not competitive with S-adenosyl methionine. Uracil methylation was not inhibited by alanine, valine, or ethionine sulfoxide. It is suggested that the thymine deficiency that we found earlier in tRNA from ethionine-treated *E. coli* B cells, resulted from base specific inhibition by the amino acid, ethionine, of uracil tRNA methylation in vivo.

INTRODUCTION

Ethionine, an amino acid analog of methionine, has been shown to cause production of incompletely methylated tRNA in rat liver and in *E. coli* (1-8). The presence of hypomethylated tRNA indicates that ethionine and/or its metabolic products interfere with normal tRNA methylation in vivo. In the *E. coli* system the inhibition was seen to be quite base selective, since the methyl deficiency appeared primarily in the uracil moieties of the tRNA (1). Very little is known about how tRNA methylating enzymes are regulated in vivo, even though changes in tRNA methylases have been found to occur in neoplasia and in a number of developing and differentiating systems (9-11). Various compounds related to adenine have been found to inhibit guanine tRNA methylating enzymes preferentially, but no selective inhibitor for uracil tRNA methylating enzymes has been chemically identified, to date (12-16). This report describes the results of experiments undertaken to determine which substances might be responsible for the observed intracellular in-

hibition of methyl group transfer to uracil in tRNA during incubation of cells with ethionine. We have investigated this question by the combined use of methylated base analysis of tRNA after in vitro methylation, and of a specific assay for uracil tRNA methylating enzymes of E. coli, which we have described earlier (17). The effects of ethionine and some of its derivatives and of several amino acids have been studied.

MATERIALS AND METHODS

Chemicals. $^{14}\text{CH}_3$ -S-adenosylmethionine ($^{14}\text{CH}_3$ -Adomet), specific activity 52-55 mc/mM, was purchased from International Chemical and Nuclear Corp. Chemicals were purchased from the following sources: S-adenosyl-L-ethionine (Ado-eth), as the iodide, and methylthioadenosine (MTA) from Sigma Chem. Co.; ethionine sulfoxide from Nutritional Biochem. Co.; D,L-alanine, D,L-methionine, and D,L-ethionine from Calbiochem; L-ethionine from Schwarz-Mann. Ethylthioadenosine (ETA) was prepared from Adoeth by the methods described by Parks (18) except that Adoeth was heated in acetic acid instead of hydrochloric acid. Paper chromatography for the identification and determination of purity of Adomet, Adoeth, ETA and ethionine was conducted according to the procedures of Parks (18) using the two solvent systems: butanol, acetic acid: H_2O (60:15:25) and ethanol: acetic acid H_2O (65:1:34).

RNA. Transfer RNA (tRNA) was prepared from the following E. coli strains by a modification of the method of Fleissner and Borek (19): methionine-starved E. coli K12W6(A), a gift of Dr. T. Staudt, E. coli K12-25_{trm} and E. coli 3-IB_{trm}, gifts of Dr. M.L. Gefter. The tRNA synthesized by E. coli K12W6(A) during methionine starvation (St tRNA) is uniformly deficient in methyl groups at all sites (19,20) and thus can act as an acceptor for methyl group transfer catalyzed by all the different base specific tRNA methylases of E. coli. The tRNA from the trm- mutants of E. coli is methyl-deficient only at sites which normally contain thymine (21,22) and so this tRNA is a specific substrate for uracil tRNA methyltransferases of E. coli (T-tRNA).

Enzyme Extracts. Frozen cells of E. coli B, E. coli M3S (a gift of Dr. M. Lieb) or E. coli 3-IB_{trm} that had been harvested in mid-logarithmic growth phase were disrupted by grinding in the cold with three times their weight of wet alumina A-305 and then extracted at 0° with 0.01 M tris, 0.01 M MgCl_2 , 0.005 mercaptoethanol buffer, pH 8, containing 5 $\mu\text{g/ml}$ DNase. The supernatant fluid obtained from centrifugation at 800g at 4° for 20 min was centrifuged at 100,000xg for 70 min at 4°. The supernatant from this second centrifugation contains most of the tRNA methylating enzyme activity of the cells (S100 prep). The S-100 preps from E. coli B and E. coli

M3S contain tRNA methylating enzymes for transfer of methyl groups to uracil, guanine, cytosine and adenine in tRNA. The major activity is uracil tRNA methylase (about 60% for *E. coli* B and 80% for *E. coli* M3S (17). The S-100 preps from *E. coli* 3-IB_{trm-} contain the tRNA methylases for guanine, cytosine and adenine, but have only traces of uracil tRNA methylase (17,21).

tRNA methylase Assay. The assay measures the enzymatic transfer of $^{14}\text{CH}_3$ from $^{14}\text{CH}_3$ -S-adenosylmethionine ($^{14}\text{CH}_3$ -Adomet) to tRNA. In these experiments each incubation tube contained the following materials in a final buffer concentration of 0.01 M Tris, 0.01 M MgCl_2 , 0.005 M B-mercaptoethanol, pH 8.0: 100 μg tRNA, 0.1–0.15 μCi $^{14}\text{CH}_3$ -Adomet (unless otherwise indicated), S-100 prep equivalent to 23–235 μg of protein, and inhibitors as indicated, in a final volume of 0.45–0.55 ml. All experiments included simultaneously incubated control samples in which substrate RNA was omitted from the incubation mixture. The samples were incubated for 40–50 min at 35° . The reaction was stopped by the addition of 0.2 ml of 1.5 M hydroxylamine, pH 7.5, followed by incubation for 10 minutes at 35° . The samples then were chilled and the RNA was precipitated in the presence of carrier RNA. The precipitates were washed as described previously (17). Radioactivity was measured by liquid scintillation counting. Results are expressed as the difference between $^{14}\text{CH}_3$ incorporated into samples that contained the complete incubation mixture and samples without added RNA substrate.

Base Specificity of $^{14}\text{CH}_3$ Methyl Transfer. The incubation mixture contained about 250 μg of tRNA. The quantities of all other components of the assay mixture were increased proportionately. After incubation, the RNA was precipitated without added carrier RNA, washed by the same methods used for assays and then hydrolyzed in 0.4 N NaOH at 37° for 18 hr. The resultant nucleotides were separated by a combination of paper electrophoresis and chromatography as described earlier (17). The $^{14}\text{CH}_3$ -methylated base nucleotides were measured by scintillation counting of radioactivity.

RESULTS

Inhibition of Uracil tRNA Methylation. Figure 1 summarizes the results of several experiments which demonstrate that D,L-ethionine inhibits the *in vitro* methylation of uracil in tRNA, catalyzed by enzymes from *E. coli*. The substrate for methyl group acceptance in these experiments was thymine-deficient tRNA from a *trm-*mutant of *E. coli*. With homologous enzymes, this tRNA acts as a specific acceptor for methyl groups on uracil moieties in tRNA.

As can be seen in Figure 2, it is the L-isomer of ethionine that is primarily responsible for the inhibition.

Fig. 1. Inhibition by D,L-ethionine of uracil tRNA methylating enzymes. Each incubation tube contained 100 μ g of thymine-deficient tRNA from an *E. coli* trm-mutant (T-tRNA) which is a specific acceptor for CH_3 groups on tRNA uracil moieties, S-100 preps from the indicated strains of *E. coli* (containing 250–300 μ g of protein), 0.10 μCi $^{14}\text{CH}_3$ -Adomet, and D,L-Adomet, and D,L-ethionine as indicated. After incubation the tRNA was isolated and incorporation of radioactivity was measured. Each point represents the average of values obtained from two or three separate samples.

Base Specificity of the Inhibition by Ethionine. Data are presented in Fig. 2 which show that the inhibition of uracil tRNA methylation catalyzed by an S-100 prep from *E. coli* M3S is dependent on the concentration of L-ethionine. This amino acid at a concentration sufficient to cause 35% inhibition of uracil methylation has virtually no effect on methylation of the other tRNA bases. Uracil methylation was measured in the same way as described for Figure 1, using thymine-deficient tRNA from *E. coli* 3-1b_{trm} as the specific substrate. Methylation of the other tRNA bases was assayed using, an enzyme extract of *E. coli* 3-1b_{trm} which contains tRNA methylating enzymes specific for adenine, cytosine and guanine but only trace amounts of the enzymes that methylate tRNA uracil (21) and, as substrate, St tRNA which can accept methyl groups at all the base moieties that can be recognized by *E. coli* enzymes.

The base selectivity of ethionine inhibition of uracil methylation was further demonstrated by determination of the $^{14}\text{CH}_3$ -methylated base content of St tRNA after *in vitro* methylation catalyzed by *E. coli* B enzymes in the presence of 4 μM $^{14}\text{CH}_3$ -Adomet with or without the addition of 30 mM D,L-ethionine. The results of this experiment are shown in Table 1. $^{14}\text{CH}_3$ methyl group transfer to uracil in tRNA was inhibited about 40 percent by ethionine, while methyl-group transfer to the other tRNA bases was virtually unaffected.

Fig. 2. Effect of L-ethionine on methylation of tRNA bases. Methylation of adenine, guanine, and cytosine were assayed by measuring $^{14}\text{CH}_3$ transfer to uniformly methyl-deficient tRNA from *E. coli* K12W6 (St tRNA) catalyzed by S-100 preps from *E. coli* 3-IB₁₀₀, which lacks uracil tRNA methylating enzymes. Methylation of uracil was measured as described for Fig. 1, using S-100 preps from *E. coli* M3S, L-ethionine concentrations were varied as indicated.

Table 1. Selective Inhibition of *E. coli* Uracil tRNA Methylating Enzymes by Ethionine.

Sample	$^{14}\text{CH}_3$ transferred to 100 μg St tRNA (cpm)			
	Total	MeA+MeC	MeG	MeU
Control	3635	360 $\pm$ 50	876 $\pm$ 45	2400 $\pm$ 95
Plus ethionine	2520	345 $\pm$ 55	770 $\pm$ 65	1475 $\pm$ 130

MeA, methyladenylic acid; MeC, methylcytidylic acid; MeG, methylguanylic acid, MeU, ribothymidylic acid.

Effects of Other Amino Acids. Experiments were carried out to see whether the inhibition of uracil tRNA methylation by ethionine was a non-specific effect which could be caused by similar concentrations of other amino acids. At 18 mM, D,L-alanine, D,L-valine, D,L-methionine and D,L-norleucine had no effect on the reaction. As shown in Fig. 1, this concentration of D,L-ethionine caused significant inhibition. When the concentration of amino acids was raised to 30 mM, methionine

and norleucine caused some inhibition (15 $\pm$ 5%). None of the amino acids cited above inhibited the methylation of tRNA bases other than uracil.

Effect of Assay Components on Ethionine Inhibition of Uracil tRNA Methylation In an attempt to learn something about the nature of the inhibition of uracil tRNA methylation caused by ethionine, the effects of varying the concentrations of components of the incubation mixture other than ethionine were tested. The results of varying the concentration of Adomet, in a range where the level of Adomet was rate limiting, indicate that the inhibition by ethionine is not competitive with Adomet. (Table 2).

Table 2. Inhibition by Ethionine of Uracil tRNA Methylation at Various S-adenosylmethionine Concentrations.

Adomet Conc. ($\times 10^{-6}$ M)	$^{14}\text{CH}_3$ transferred to 100 ug T-tRNA (cpm)		
	Control	Plus ethionine	Percent Inhibition
1.2	945 $\pm$ 90	870 $\pm$ 95	29
2.6	2940 $\pm$ 105	1930 $\pm$ 60	34
5.2	6870 $\pm$ 105	4905 $\pm$ 205	28
8.2	9665 $\pm$ 1000	6750 $\pm$ 530	30

Enzyme extracts from *E. coli* M35 containing 230 ug protein were used. L-ethionine concentration was 15 mM.

The effects of varying the enzyme concentration are shown in Figure 3. The concentrations used ranged from linear dependence on enzyme level to enzyme saturation levels. The percent inhibition by ethionine showed no direct dependence on enzyme concentration.

Inhibition was measured also over a large range of tRNA substrate concentrations. No change in the extent of inhibition by a given amount of ethionine was seen when the RNA concentration was varied from rate limiting levels to a 10-fold excess.

Effects of Ethionine Metabolites and Related Substances. In view of the high concentrations of ethionine required to cause inhibition of uracil methylation, it seemed important to test metabolites of ethionine and potential trace contaminants of ethionine preparations for their ability to cause selective inhibition of this reaction. The effect of S-adenosylethionine (Adoeth), ethylthioadenosine (ETA) which is derived from Adoeth, and methylthioadenosine (MTA) a derivative of Adomet that is normally present in small amounts in *E. coli*, on the *E. coli* B enzymes that catalyze the combined methylation of all species of tRNA bases was measured. The results are shown in Figure 4. The substrate used here was St tRNA. It is seen that this group of enzymes is more sensitive to ETA than to either Adoeth or MTA.

We measured the ability of ETA and MTA to inhibit uracil tRNA methylation

Fig. 3. Inhibition by ethionine of uracil tRNA methylation at various enzyme concentrations. Incubation conditions were the same as described for Fig. 1 except that the amount of S-100 enzyme prep from *E. coli* M35 were varied as indicated.

using the specific assay for this reaction (17). In the presence of 4.1×10^{-6} M Adomet, the enzymatic methylation of tRNA that accepts methyl groups only on uracil moieties was inhibited 50 percent by 1.4×10^{-4} M ETA, but was unaffected by 7×10^{-4} M MTA. As shown in Figure 4, 7×10^{-4} M MTA caused significant inhibition of tRNA methylation (about 30 percent) when St tRNA, which can accept methyl groups on other bases in addition to uracil served as substrate MTA thus appears to have a relatively small effect on uracil tRNA methylases as compared with its ability to inhibit the enzymes that catalyze methylation of other tRNA bases. The relative effect of ETA on methylation of the various tRNA bases was investigated by assaying the $^{14}\text{CH}_3$ -methylated bases present in St tRNA after its methylation *in vitro* catalyzed by *E. coli* B enzymes in the presence and in the absence of ETA. In experiments where a total of 4,800 cpm as $^{14}\text{CH}_3$ were transferred to St tRNA from 6.7×10^{-6} M $^{14}\text{CH}_3$ -Adomet in control samples, the addition of 1.6×10^{-4} M ETA reduced the $^{14}\text{CH}_3$ transfer to the same quantity of tRNA to 2,950 cpm. In the

Fig. 4. Inhibition of *E. coli* tRNA methylating enzymes by ethylthioadenosine, methylthioadenosine and S-adenosylethionine. The substrate for tRNA methylation was St tRNA from *E. coli* K12W6. The S-100 preps were from *E. coli* B. These assays measured the combined methylation of all tRNA base sites recognized by the enzymes.

samples with ETA, uracil methylation was inhibited by 18%, guanine methylation by 69%, and the combined methylation of adenine and cytosine was inhibited 58%. Uracil methylation was thus seen to be less susceptible to inhibition by ETA than methylation of guanine, adenine and cytosine.

The inhibition of tRNA methylation by 7×10^{-4} M Adoeth in the presence of 2.8×10^{-6} M Adomet was found to be 68%. Uracil methylation was inhibited 68%, guanine methylation 62% and adenine plus cytosine methylation 84%. Adoeth was reported earlier by Moore to be a preferential inhibitor of tRNA adenine and cytosine methylation (14). We obtained similar results, using rat liver enzymes (23).

DISCUSSION.

We have demonstrated recently that incubation of *E. coli* B cells with D,L-ethionine resulted in production of incompletely methylated tRNA that was recognized as a substrate by homologous tRNA methylating enzymes(1). This indicated that tRNA methylation was inhibited *in vivo* in these cells. Ethionine has

been found to cause analogous effects in animals. As first shown by Rajalakshmi (2) and subsequently by us and by other investigators, when D,L-ethionine was given to rats or mice, incompletely methylated tRNA could be isolated from the livers of these animals (3-8). In spite of the apparent similarities in the effects of ethionine on tRNA methylation in vivo in animals and in bacteria, we have found that there are also certain significant differences in effect. In each case we assayed the base specificity of the in vivo inhibition of tRNA methylation by determining the ability of tRNA from ethionine-treated organisms to accept methyl groups in vitro by the action of homologous enzymes. In rat liver tRNA, the principal methyl-deficiency was found to be on guanine moieties (23), while in E. coli tRNA the major methyl-deficiency occurred on uracil moieties, with only a slight inhibition of guanine methylation apparent (1). This indicated that the base specificity of inhibition in the two species was different. It is possible that these differences are due to species specific differences in the methylating enzymes. However, it seems more likely that species-specific differences in the intermediary metabolism of ethionine are responsible, since it is known from earlier work that the base-specific tRNA methylases differ in their sensitivity to various inhibitors. For example, methylation of adenine and cytosine are preferentially inhibited by Adoeth (14,23) and by Poly I-Poly C (25). A number of compounds related to adenine and adenosine are preferential inhibitors of guanine tRNA methylating enzymes (12-16, 24,26). Included amongst these inhibitors of guanine tRNA methylases are cytokinins which are either N⁶-substituted adenosine analogs or ureidopurine derivatives (13,15,26) and adenosine analogs which are cytotoxic to animal cells in culture (12-14). Some of the purine analogs that inhibit guanine tRNA methylases are ineffective against uracil tRNA methylases, while some of these analogs such as tubercidin (7-deazaadenosine) and isopropylureidopurine, although they are able to inhibit uracil tRNA methylation, more strongly inhibit guanine methylation (15). Until now, no selective inhibitor for uracil tRNA methylation has been identified chemically. When we found that preferential inhibition of uracil tRNA methylation occurred in vivo in E. coli B cells incubated in 5 mg/ml D,L-ethionine, (1), it appeared possible to us that this base selective inhibition was due either to ethionine itself or to contaminants or metabolites commonly associated with this amino acid.

The results of our in vitro experiments indicate that uracil tRNA methylation by E. coli enzymes can be inhibited by L-ethionine under conditions in which methylation of other tRNA bases is unaffected. Ethionine sulfoxide, a potential contaminant of ethionine preparations and a common metabolite of ethionine in rats (27) was not detectable by paper chromatography in the ethionine used for either the in vitro or the in vivo experiments, and did not, under our assay conditions, inhibit the

in vitro methylation. Neither Adoeth, nor ETA was found to have the appropriate base specificity of inhibition necessary to explain the observed effect. It is interesting to note, however, that ETA is a more potent inhibitor of tRNA methylation than MTA, thus providing further evidence for the structural specificity requirements for inhibitors of the tRNA methylases. In earlier in vitro experiments we found that S-adenosylhomocysteine (SAH) inhibited all of the base specific tRNA methylases equally (23), so it appears unlikely that SAH was responsible for the selective inhibition of uracil tRNA methylation in vivo.

The inhibition observed with ethionine is not a general amino acid effect. Valine and alanine have little or no influence on the methylation of uracil. Methionine and norleucine, which are closely related to ethionine, inhibit the reaction, but are only about one half as effective as ethionine. Equally important in demonstrating the specificity of the effect of ethionine on tRNA uracil methylation, is the fact that ethionine has no effect on the methylation of adenine, guanine and cytosine.

If the preferential inhibition of ribothymidine synthesis seen in vivo was due to ethionine itself such an effect would be consistent with our earlier observations with ethionine-treated E. coli cells, particularly if the inhibition in vivo occurred under conditions similar to those required in vitro. It had seemed somewhat paradoxical that the in vitro activity of tRNA methylating enzyme extracts from ethionine-treated E. coli cells did not differ significantly from those of untreated cells, in specific activity, maximum capacity to transfer methyl groups or base specificity (1). In addition, enzyme preparations from ethionine-treated cells could carry out in vitro the methylation of the tRNA uracil sites that were not methylated in vivo by the enzymes of these same cells. If the inhibition in vivo like the inhibition in vitro required relatively high concentrations of ethionine, the dilution of ethionine that occurred during cell disruption and extraction would result in preparations that were very much like those from control cells. It may be pertinent that several groups of investigators have found that ethionine is an extremely poor substrate for E. coli S-adenosylmethionine synthetase (28,29,30). Results of preliminary experiments in this laboratory suggested that there was little or no build-up of Adoeth in E. coli B cells incubated with radioactive ethionine.

Although it is not possible, at this time, to ascribe the preferential inhibition of tRNA ribothymidine synthesis seen in vivo to ethionine positively, such a mechanism would be compatible with both in vitro and in vivo observations. Since no other base-selective inhibitors for uracil tRNA methylating enzymes other than L-ethionine have been identified to date, our observation may be useful as a guide to structural requirements, in the search for other inhibitors of these enzymes.

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REFERENCES.

1. Wainfan, E. and Maschio, F.A. (1975) *Ann. N.Y. Acad. Sci.* 255, 567-575.
2. Rajalakshmi, S. J. (1972) *Proc. Am. Assoc. Cancer Res.* 14, 39.
3. Wildenauer, D. and Gross, H.J. (1974), *Nucleic Acids Res.* 1, 279-388.
4. Kerr, S.J. (1975) *Cancer Res.* 35, 2969-2973.
5. Wainfan, E., Moller, M.L., Maschio, F.A., and Balis, M.E. (1975) *Cancer Res.* 35, 2830-2835.
6. Lu, L.W., Chiang, G.H. and Randerath, K. (1976) *Nucleic Acids Res.* 3, 2243-2253.
7. Friedman, S. (1977) *Nucleic Acids Res.* 4, 1853-1871.
8. Gross, H.J. (1975) *Ann. N.Y. Acad. Sci.* 255, 564-566.
9. Kerr, S.J. and Borek, E. (1973) *Adv. in Enz. Reg.*, Weber G., Ed. 2, 63-77.
10. Kerr, S.J. (1975) in "Post-Synthetic Modification of Macromolecules" Antoni, F. and Farago, A., Eds. Amer. Elsevier, Publ. pp88-98.
11. Craddock, V.M. (1970) *Nature* 228, 1264-1268.
12. Wainfan, E. and Borek, E. (1967) *Molec. Pharmacol.* 3, 595-598.
13. Wainfan, E. and Landsberg, B. (1973) *Biochem. Pharmacol.* 22, 493-500.
14. Moore, B.G. (1970) *Canad. J. Biochem.* 48, 702-705.
15. Wainfan, E., Chu, J. and Chheda, G.B. (1974) *Biochem. Pharmacol.* 23, 1-5.
16. Hurwitz, J., Gold, M. and Anders, M. (1964) *J. Biol. Chem.* 239, 3474-3482.
17. Wainfan, E. and Chu, J. (1973) *Anal. Biochem.* 55, 388-393.
18. Parks, L.W. (1958) *J. Biol. Chem.* 232, 169-176.
19. Fleissner, E. and Borek, E. (1966) *Proc. in Nucl. Acid Res.* 461-465. Harper and Row, N.Y., Pub.
20. Fleissner, E. and Borek, E. (1963) *Biochem.* 2, 1093-1100.
21. Yang, S., Reinitz, E. and Geffer, M.L. (1973) *Arch. Biochem.* 157, 55-62.
22. Bjork, G.R. and Isakson, L.A. (1970) *J. Mol. Biol.* 51, 83-100.
23. Wainfan, E., Tscherne, J.S., Maschio, F.A. and Balis, M.E. (1977) *Cancer Res.* 37, 865-869.
24. Moore, B.G. and Smith, R.C. (1969) *Canad. J. Biochem.* 47, 561-565.
25. Liao, M.C., Hunt, J.B., Smith D.W. and Hurlbert, R.B. (1973) *Cancer Res.* 33, 321-331.
26. Wainfan, E. and Landsberg, B. (1971) *Fed. Eur. Biochem. Soc. Letters*, 19 144-148.
27. Brada, Z., Bulba, S. and Cohen, J. (1975) *Cancer Res.* 35, 2674-2683.
28. Peterkofsky, A. (1965) in "Transmethylation and Methionine Biosynthesis" Shapiro, S.K. and Schlenk, F., eds. Univ. of Chicago Press, p.137.
29. Cox, R. and Smith, R.C. (1969) *Arch. Biochem. Biophys.* 129, 615-619.
30. Lambardini, J.B., Coulter, A.W. and Talalay, P. (1970), *Molec. Pharmacol.* 6, 481-499.