[CANCER RESEARCH 35, 2830-2855, October 1975]

Ethionine-induced Changes in Rat Liver Transfer RNA Methylation[1]

Elsie Wainfan, Mary L. Moller, Frank A. Maschio, and M. Earl Balis

The Lindsley F. Kimball Research Institute of The New York Blood Center [E. W., F. A. M], and Memorial Sloan-Kettering Cancer Center [M. L. M., M. E. B.], New York, New York 10021

SUMMARY

We have confirmed the finding by Rajalakshmi that transfer RNA (tRNA) from livers of ethionine-treated rats can act as a substrate for homologous tRNA-methylating enzymes *in vitro*. This methyl-deficient tRNA from liver can be methylated *in vitro* by enzymes from normal or ethionine-treated rats. The *in vivo* inhibition of tRNA methylation that follows ethionine treatment can be at least partially relieved *in vitro*.

The liver extracts from ethionine-treated animals contained a low-molecular-weight inhibitor of tRNA methylation. Dialysis of enzyme preparations from ethionine-treated, but not control, rats resulted in large increases in tRNA methylase activity, with either *Escherichia coli* or homologous tRNA's as substrate. Furthermore, the tRNA methylase activity of control rat liver enzyme extracts was greatly depressed by dialysate from liver homogenates of ethionine-treated rats.

After 5 days of ethionine administration the liver tRNA methylase activities were significantly higher than those of control preparations despite the continued presence of the dialyzable inhibitor(s). The liver tRNA's from these animals were poorer methyl acceptors than those from 3-day-treated rats, although still better than tRNA's from untreated rats.

These observations have been interpreted to indicate that ethionine causes the accumulation in the liver of inhibitors of tRNA methylation. Early in the course of ethionine administration, methyl-deficient tRNA can be isolated. When the period of ethionine treatment is extended, the organism attempts to maintain homeostasis by production of increased amounts of tRNA-methylating enzymes. The increased quantities of these enzymes are able to overcome, at least partially, the effects of the inhibitors and to decrease the extent to which methyl-deficient tRNA is produced.

INTRODUCTION

A recent report by Rajalakshmi (13) indicated that methyl-deficient tRNA can be isolated from the livers of rats that had been given injections of large doses of ethionine. This finding appeared to be contradictory to the earlier observations by Hancock (4), Hancock and Forrester (5), and Moore and Smith (11) that administration of ethionine to animals results in elevation of the levels of liver tRNA-methylating enzymes. Increased levels of tRNA-methylating enzyme activity have also been found after administration of other carcinogens (16, 19). The present study describes experiments designed to determine the reasons for these seemingly anomalous reports from several different laboratories. Our results indicate that the various effects of ethionine administration that can be observed *in vivo* and *in vitro* are not inconsistent but that the responses are time dependent and may represent the interplay of factors involved in regulation of tRNA methylation *in vivo*.

MATERIALS AND METHODS

Animals. Female CFN rats of the Wistar strain, obtained from Carworth Farms, New City, N. Y., were used for all experiments. The rats were maintained on a diet of commercial laboratory rat chow and given food and water *ad libitum*. These animals were given sodium pentobarbital (50 mg/100 ml) in their drinking water for 3 days prior to the drug treatment and maintained on this regimen until sacrificed. Control animals also received pentobarbital in the drinking water. [Addition of pentobarbital to the drinking water has been reported to minimize RNase activity in liver (10).] Experimental animals were given i.p. injections for either 3 or 5 consecutive days with a daily dose of DL-ethionine (250 mg/kg body weight) and adenine (120 mg/kg body weight) suspended in carboxymethylcellulose and were sacrificed by decapitation on the following morning. Some animals were given injections of adenine only, while control rats did not receive any injections.

Enzyme Extracts. Liver was quickly excised after the animal was sacrificed and then disrupted by mincing, followed by homogenization at 0° for about 2 min in a Potter-Elvehjem homogenizer in 0.25 M sucrose:0.005 M $MgCl_2$. The suspension was centrifuged at 10,000 × *g* for 20 min, and the supernatant was centrifuged at 100,000 × *g* for 1 hr. The supernatant from this centrifugation contained most of the tRNA methylase activity of the cell homogenates. Volumes were adjusted so that about 6 g of liver yielded 10 ml of the 100,000 × *g* supernatant.

Where dialysis was used the enzyme extract was dialyzed at 4° against large volumes of 0.01 M Tris:0.005 M $MgCl_2$:0.005 M mercaptoethanol, pH 8, for 2 hr.

[1] The work was supported in part by USPHS Grant HL-09011 and National Cancer Institute Grant CA-08748.

Received May 1, 1975,; accepted June 20, 1975.

Preparation of tRNA. tRNA from *E. coli* was prepared by a modification of the procedure of Fleissner and Borek (1) described earlier (17).

For preparation of tRNA from liver, the tissue was first minced with scissors; then homogenized at 4° for 30 sec in 0.01 M Tris (pH 7.0), 0.15 M NaCl:0.006 M EDTA (pH 7.0), and 0.005 M mercaptoethanol; and then stirred with an equal volume of buffer-saturated phenol for 30 min at 4° followed by 30 min at room temperature. Isolation of the RNA from the phenol extract was accomplished by substantially the same procedures as those used for *E. coli* (17).

Enzyme Assays. Each incubation tube contained about 75 to 100 μg tRNA as substrate, 0.1 μCi [*methyl*-^{14}C]-*S*-adenosylmethionine (specific activity, 55 μCi/mmole; ICN Corp., Irvine, Calif.) as methyl donor and enzyme extract as indicated, in a volume of about 0.5 ml, all in a final buffer concentration of 0.01 M Tris:0.005 M $MgCl_2$:0.005 M mercaptoethanol, pH 8.0. Some samples also contained 0.2 M ammonium acetate. Incubation was carried out at 35° for 40 to 60 min; 0.2 ml of 1.5 M NH_4OH, pH 7.5, was added to each tube; and the incubation was continued for 10 min longer. Samples were chilled and 2 mg carrier RNA, followed by 4 ml of 0.5 M NaCl in 70% ethanol, were added. Precipitates were collected by centrifugation in the cold, washed with ether:alcohol (2:1), and extracted with 2 M NaCl. RNA was reprecipitated from the extracts by addition of 50% trichloroacetic acid solution. The precipitates were washed in the cold with 10% trichloroacetic acid solution, alcohol, and ether. The RNA, dissolved in 1.6 ml of 2 M NH_4OH, was assayed for radioactivity in a scintillation counter.

Extraction with NaCl, as first described by Kaye and Leboy (7), is necessary to obtain low and reproducible enzyme blank values.

Base Specificity of tRNA Methylation. The incubation mixture for the isolation of [*methyl*-^{14}C]-tRNA was the same as described above, except that 0.2 to 0.4 mg of tRNA was used and all other quantities were accordingly increased. Enzyme quantities were at saturation level. After 1 hr of incubation at 35° the samples were treated as described above, but no carrier RNA was added.

The isolated RNA was hydrolyzed to nucleotides with 0.4 N NaOH at 37° for 18 hr. The RNA hydrolysate was deionized and subjected to paper electrophoresis in ammonium acetate buffer (pH 2.75), followed by chromatography in 2-propanol:H_2O (70:30) with NH_3 in the vapor phase, using the methods described by Ingram and Pierce (6). Radioactivity in the methylated base nucleotides was assayed by placing cut strips of the chromatograms into vials and counting in a scintillation counter.

Preparation of Dialysate Containing Inhibitor. Appropriately treated animals were sacrificed by decapitation and bled. Livers were removed and rinsed several times with ice-cold CO_2-saturated distilled water, pH 4.5 to 5. Six livers were minced and then homogenized in a Potter-Elvehjem homogenizer with 50 ml of the ice-cold water. The homogenate was centrifuged at about 15,000 × *g* at 5° for 15 min. The supernatant was removed and dialyzed *versus* 175 ml of the same ice-cold water for 4 hr. The dialysate was lyophilized and resuspended in 9 ml cold water. Thus, 0.05 ml of dialysate represents about 6% of the total obtained from 1 rat liver. Dialysates were stored frozen.

Table 1

Methyl group acceptance by tRNA from various sources during in vitro methylation with control rat liver enzymes

Each incubation tube contained, in a volume of 0.4 to 0.5 ml, tRNA, as indicated in the table; 0.04 to 0.1 μCi [*methyl*-^{14}C]-*S*-adenosylmethionine (specific activity, 55 μCi/mmole); 0.2 M ammonium acetate, where indicated; and enzyme extract from control rat liver, in a final buffer concentration of 0.01 M Tris: 0.005 M $MgCl_2$: 0.005 M mercaptoethanol, pH 8. After 40 to 50 min of incubation at 35°, the reaction was terminated by the addition of 0.2 ml of 1.5 M hydroxylamine, pH 7.5, and incubation was continued for 10 min longer. Samples were then chilled and RNA was precipitated by the addition of 4 ml 0.5 M NaCl in 70% ethanol plus 2 mg carrier RNA. Samples were washed and assayed for radioactivity as indicated above in "Materials and Methods."

Experiment	Source of tRNA	tRNA (μg)	0.2 M ammonium acetate	$^{14}CH_3$ incorporated
1	*E. coli*	100	–	1884
	NRL	102	–	67
2	*E. coli*	100	+	2518
	NRL	100	+	41
3	*E. coli*	100	+	4126
	E. coli	100	–	1895
	PRL[a]	110	+	91
4	*E. coli*	100	+	1757
	APL	100	+	65
5	*E. coli*	75	–	2613
	E-3	78	–	1047
6	E-3[b]	100	+	1700
	E-3[b]	100	–	570
7	E-3	100	+	1001
	E-5	100	+	649
	E-5	100	+	540

[a] PRL, tRNA from rats given no injections, but receiving pentobarbital in the drinking water; APL, tRNA from rats that were given daily injections of adenine for 3 consecutive days and received pentobarbital in the drinking water; E-5, tRNA from livers of rats given daily injections of ethionine + adenine for 5 consecutive days; these rats received pentobarbital in the drinking water.

[b] 40 to 60% saturated ammonium sulfate precipitate and dialyzed enzyme was used.

RESULTS

Samples of tRNA were prepared from livers of control rats, of rats given injections of ethionine plus adenine for either 3 consecutive days or for 5 days, from rats injected with adenine only for 3 days, and from *E. coli*. Each of these tRNA preparations was tested for its ability to act as methyl group acceptor in the *in vitro* reaction catalyzed by the tRNA-methylating enzymes extracted from livers of control rats (Table 1). The heterologous tRNA from *E. coli* was an excellent substrate for this *in vitro* methylation reaction. Under the same conditions, NRL,[2] the homologous substrate, was unable to accept a significant level of

[2] The abbreviations used are: NRL, tRNA of livers of untreated rats, no injections; no pentobarbital in the drinking water; E-3 RNA, tRNA from livers of rats given daily injections for 3 consecutive days of ethionine plus adenine; these rats received pentobarbital in the drinking water.

radioactive methyl groups. These results serve as controls which show that these rat liver tRNA-methylating enzymes are active when presented with a suitable heterologous substrate but that all available sites for methyl groups on the NRL RNA have already been filled by these enzymes *in vivo* (9, 15). In contrast, E-3 RNA was seen to be a good *in vitro* substrate for methylation by control rat liver enzymes. When ethionine plus adenine injections were continued for several additional days the RNA from these animals, although still able to act as a substrate *in vitro*, had a lower capacity for methyl acceptance from homologous enzymes than did E-3 RNA. These results are consistent with measurements made on several separate preparations from groups of untreated and ethionine-treated animals. The tRNA from animals given injections of adenine alone or from animals that received no injections but were given pentobarbital in the drinking water, like that from control rats, did not accept methyl groups *in vitro* in the reaction catalyzed by the rat liver enzymes. This confirms the finding of Rajalakshmi (13) that large doses of ethionine for several days result in accumulation of incompletely methylated tRNA in the liver.

Covalent attachment of the methyl groups to bases in the tRNA during *in vitro* methylation by homologous enzyme extracts was shown by analysis of the methylated base content of the E-3 RNA. About 9.5% of the total radioactivity from $^{14}CH_3$ was in methyladenine plus methylcytosine, 88% was in methylguanines, and about 2.5% was in methyluracil (Table 2). When enzymes from the same source precipitated by 40 to 60% saturated $(NH_4)_2SO_4$ were used after dialysis, the relative proportion of methylguanines to methyladenine + methylcytosine was slightly decreased, probably due to some fractionation of enzyme activities during this treatment. The methylated base ratios were not altered by addition of ammonium acetate.

Enzymes from the 3- and 5-day ethionine-treated animals were also able to catalyze methylation of homologous E-3 RNA *in vitro* (Table 2; Chart 1). As shown in Table 2, the distribution of radioactive methyl groups among the various bases after methylation of E-3 RNA with the enzymes from the 3-day ethionine-treated rats was quite similar to that obtained with control liver enzymes. The ability of the

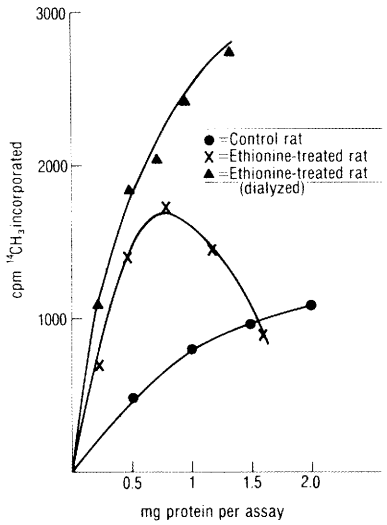

Chart 1. Methylation of tRNA from ethionine-treated rats by rat liver enzymes. Each incubation tube contained, in a volume of 0.55 ml, 87 μg E-3 tRNA, 0.1 μCi [*methyl*-^{14}C]-*S*-adenosylmethionine (specific activity, 55 μCi/mmole), and varying amounts of enzyme extract, in a final buffer concentration of 0.23 M ammonium acetate: 0.01 M Tris: 0.005 M $MgCl_2$: 0.005 M mercaptoethanol, pH 8.0. After 50 min of incubation at 35°, the reaction was terminated by the addition to each tube of 0.2 ml 1.5 M hydroxylamine at pH 7.5. After 10 min of additional incubation, the tubes were chilled and then RNA was precipitated by the addition of 2 mg of carrier RNA plus 4 ml of 0.5 N NaCl in 70% ethanol. Samples were washed and assayed for radioactivity as indicated in "Materials and Methods." ●, enzyme from control rat liver, undialyzed; ×, enzyme from 5-day ethionine-treated rat liver, undialyzed; ▲, enzyme from 5-day ethionine-treated rat liver, dialyzed.

Table 2

The methylated base composition of tRNA after in vitro methylation

Samples were incubated under the same conditions as described for Table 1, except that larger quantities of RNA and enzyme were used, and carrier RNA for precipitation was omitted. The RNA was hydrolyzed in 0.4 N NaOH at 37° for 16 hr. Nucleotides were separated by paper electrophoresis at pH 2.75 followed by chromatography in isopropanol:H_2O (7:3) with NH_3 in the vapor phase (6). Each value represents the average of values obtained from 2 separate assays.

Source of enzyme	0.2 M ammonium acetate	RNA	^{14}C-Methylated nucleotides as % of total radioactivity			Ratio MeG: MeC + MeA
			MeA[a] + MeC	MeG	MeU	
Control rat	–	E-3	9.4	87.8	2.8	9.3
3-day ethionine rat	–	E-3	11.3	84.0	4.8	7.4
Control rat [40–60% saturated $(NH_4)_2SO_4$ enzyme]	–	E-3	13.5	74.0	12.5	5.6
	+	E-3	17.0	77.0	6.0	4.5
Control rat	+	*E. coli*	21	78.5	0.5	3.8
Control rat (+ ethionine-3 dialysate)	+	*E. coli*	18.5	78.5	3.0	4.3

[a] MeA, methyladenylic acid; MeC, methylcytidylic acid; MeG, methylguanylic acid; MeU, methyluridylic acid.

enzymes from the ethionine-treated rats to catalyze methylation of the RNA from the same source indicates that the incomplete methylation of liver tRNA in these animals *in vivo* was not due to the absence of competent enzymes. Furthermore, the ability of these enzymes to catalyze methylation of E-3 RNA *in vitro* indicates that the inhibition of methyl transfer to tRNA that occurred *in vivo* was at least partially relieved *in vitro*.

Measurement of the effect of varying enzyme concentration on the rate of tRNA methylation by enzyme extracts from liver of ethionine-treated rats showed a pattern that is typical for preparations that contain inhibitors (Charts 1 and 2). When the amount of enzyme extract added to an incubation tube containing about 100 μg of *E. coli* tRNA or E-3 RNA exceeded the equivalent of about 0.8 to 0.9 mg protein, more extract caused a decrease in methyl group transfer to tRNA. In contrast, similar measurements with extracts of control rat liver showed that a plateau value for methylation of this amount of RNA was reached with about 1.5 mg protein per tube.

Further experiments indicated that a low-molecular-weight inhibitor was present in enzyme preparations made from tissues of ethionine-treated animals. Table 3 shows the effects of dialysis on the tRNA methylase activity of control and ethionine-treated preparations when *E. coli* tRNA was used as substrate. The activity of preparations from 3- or 5-day ethionine-treated animals was increased significantly after dialysis. Control preparations did not show increased activity after the same treatment. The data presented in Chart 1 show that the ability of preparations from 5-day ethionine-treated rats to methylate E-3 RNA was similarly increased after dialysis. The activity of both dialyzed and undialyzed preparations was increased about 3-fold by addition of ammonium acetate to a final concentration of 0.2 M.

Further evidence for a dialyzable inhibitor was obtained when the addition of aliquots of the concentrated dialysate from ethionine-treated rat liver caused significant inhibition of the tRNA methylase activity of normal liver enzyme preparations (Table 4). This inhibition did not appear to be selectively directed against any one of the base-specific methylases, as shown by lack of change in relative proportions of the bases which were methylated compared with that seen in controls without added dialysate (Table 2).

The levels of activity of the enzymes from 3-day ethionine-treated animals were quite variable. In most samples, no increase in specific activity over that of the controls was seen, although in a few cases elevated levels were observed when measurements were made at low protein concentration. On the other hand, undialyzed enzymes from 5-day-treated animals had consistently higher specific activity than did controls. Dialysis increased the activity to greater levels. Thus, after 5 days of ethionine injection, the livers of these animals were found to have higher than normal tRNA methylase activity when either homologous (Chart 1) or heterologous (Chart 2) tRNA was used as substrate.

DISCUSSION

Evidence from a number of laboratories indicates that the tRNA-methylating enzyme activity of tumor tissues differs from that of normal tissues in both amount and specificity (9). It is not clear, however, whether these changes are concomitant events in tumor development or whether they play some causative role in carcinogenesis. It was therefore

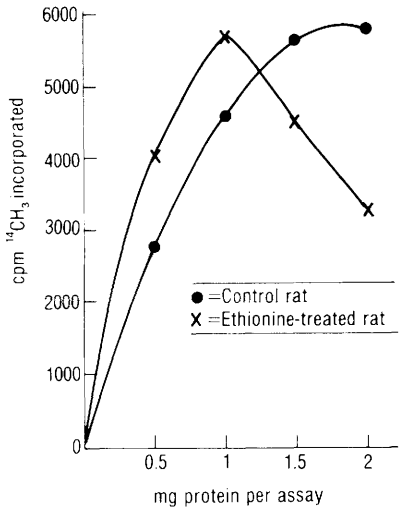

Chart 2. Methylation of *E. coli* tRNA by rat liver enzymes. The conditions were the same as described for Chart 1 except that 100 μg of *E. coli* B tRNA were used as substrate. ●, enzyme from control rat liver; ×, enzyme from 5-day ethionine-treated rat liver.

Table 3

Effect of dialysis on activity of tRNA-methylating enzymes in liver preparations from control and ethionine-treated rats

Experimental conditions were the same as described for Table 1.

Source of enzyme	Enzyme extract/ tube (mg protein)	RNA	0.2 M ammonium acetate	$^{14}CH_3$ transferred	
				Undialyzed (cpm)	Dialyzed (cpm)
Control rat	0.85	*E. coli*	-	4430	4070
	1.7		+	6700	6260
5-day ethionine-treated rat	0.9	*E. coli*	-	5710	6685
	1.8		+	6000	9418
3-day ethionine-treated rat	2	*E. coli*	-	500	680
	5		-	720	1150

Table 4

Inhibition of liver tRNA-methylating enzymes by dialysate from ethionine-treated rat liver

Experimental conditions were similar to those described for Table 1. From 180 to 200 μg of *E. coli* B tRNA were used. Methylation was carried out in the absence of ammonium acetate.

Sample	Additions	Incorporated (cpm)	% of control
1	None	5,030	
1E	+0.09 ml E-3 liver dialysate	3,071	61
1N	+0.09 ml normal liver dialysate	5,159	101
2	None	12,600	
2E	+0.09 ml E-3 liver dialysate	6,525	52

of considerable interest when Hancock (4), Hancock and Forrester (5), Stewart and Pegg (16), and Wilkinson and Pillinger (19) found that preparations from the tissues of animals treated with ethionine, or several other carcinogens, showed increased tRNA-methylating enzyme activity. In these assays, heterologous tRNA was the methyl acceptor.

The recent findings, that incompletely methylated tRNA was present in the livers of rats treated with large doses of ethionine for a short time, implied that tRNA methylation was inhibited *in vivo* after ethionine administration. This observation was consistent with our finding that incubation of *E. coli* B with ethionine, under appropriate conditions, also caused production of methyl-deficient tRNA (18). It appeared, therefore, that ethionine could cause interference with tRNA methylation *in vivo* in widely divergent types of living systems and that tumor development was not a necessary prior condition for this to occur.

The finding of incompletely methylated tRNA in the livers of animals given injections of ethionine would at first appear to be contradictory with the earlier reports of elevated tRNA-methylating enzyme activity. However, the results that we have presented here seem to indicate that these effects are not mutually inconsistent but that the changes in the tRNA and in the enzyme levels are time dependent.

The data indicate that relatively low-molecular-weight material that inhibits tRNA methylation accumulates shortly after ethionine is given. The inhibitory factor(s) prevents complete methylation of the tRNA *in vivo*. Subsequently, in the presence of the inhibitor(s), tRNA methylase activity increases. Under these conditions, the *in vivo* inhibition is partially overcome and tRNA, which is more nearly normally methylated, is produced. It seems likely that *S*-adenosylethionine which has been reported to be formed in livers of rats receiving ethionine (12, 14) is one of the components of the low-molecular-weight inhibitor fraction generated after ethionine is given. *S*-Adenosylethionine was shown by Moore and Smith (11) to be an inhibitor of tRNA methylation *in vitro*. Several groups (2, 3, 8) have reported finding inhibitors of tRNA methylation in liver. It is not possible at this time to exclude the identity of any of those substances with the dialyzable inhibitor(s) that we have observed. However, the ability of ammonium acetate to stimulate dialyzed as well as undialyzed preparations and the apparent absence of the inhibitory factor(s) from control preparations suggest that the glycine methylase described by Kerr (8) is not the responsible agent.

A possible sequence of events following administration of ethionine would be: (*a*) production of inhibitors; (*b*) reduction in methylation of tRNA; (*c*) derepression of the locus for tRNA methylase synthesis; and (*d*) nearly normal methylation due to excess enzyme. The development of increased levels of tRNA-methylating enzyme activity in liver after the temporary *in vivo* inhibition of this activity suggests that control of the synthesis of these enzymes in liver may, at least in part, be exerted by the presence or absence of substrate, *i.e.*, under methylated tRNA.

ACKNOWLEDGMENTS

The authors wish to acknowledge the excellent technical assistance in this work by George F. Brown.

REFERENCES

1. Fleissner, E., and Borek, E. Methyl-Poor sRNA. *In:* G. L. Cantoni, and D. R. Davies, (eds.), Procedures in Nucleic Acid Research, pp. 461–465. New York: Harper and Row, 1966.
2. Gross, H. J., and Wildenauer, D. *In vitro* Inhibition of tRNA and Protein Methylation by Nicotinamide and Isonicotinic Acid Hydrazide: Activation of a *S*-adenosylmethionine-splitting Enzyme in Rat Liver. Biochem. Biophys. Res. Commun., *48:* 58–64, 1972.
3. Halpern, R. M., Chaney, S. Q., Halpern, B. C., and Smith, R. A. Nicotinamide: A Natural Inhibitor of tRNA Methylase. Biochem. Biophys. Res. Commun., *42:* 602–607, 1971.
4. Hancock, R. L. Ethionine Induced sRNA Neomethylase Activity. Biochem. Biophys. Res. Commun., *31:* 77–81, 1968.
5. Hancock, R. L., and Forrester, P. I. Increase of Soluble RNA Methylase Activities by Chemical Carcinogens. Cancer Res., *33:* 1747–1753, 1973.
6. Ingram, V. M., and Pierce, J. G. Chemical Studies on Amino Acid Acceptor Ribonucleic Acids. I. Some Properties of Yeast Amino Acid Acceptor Ribonucleic Acid and Mapping of the Oligonucleotides. Biochemistry, *1:* 58–594, 1962.
7. Kaye, A. M., and Leboy, P. S. Methylation of RNA by Mouse Organs and Tumors: Ionic Simulation *in vitro*. Biochim. Biophys. Acta, *157:* 289–302, 1968.
8. Kerr, S. J. Natural Inhibitors of the Transfer Ribonucleic Acid Methylases. Biochemistry, *9:* 690–695, 1970.
9. Kerr, S. J., and Borek, E. The tRNA Methyltransferases. Advan. Enzymol., *36:* 1–27, 1972.
10. Louis-Ferdinand, R. T., and Fuller, G. C. Suppression of Hepatic Ribonuclease during Phenobarbital Stimulation of Drug Metabolism. Biochem. Biophys. Res. Commun., *38:* 811–816, 1970.
11. Moore, B. G., and Smith, R. C. *S*-adenosylethionine as an Inhibitor of tRNA Methylation. Can. J. Biochem., *47:* 561–565, 1969.
12. Orthwerth, B. J., and Novelli, G. D. Studies on the Incorporation of L-Ethionine-ethyl-1-^{14}C into the Transfer RNA of Rat Liver. Cancer Res., *29:* 380–390, 1969.
13. Rajalakshmi, S. A Liver tRNA for the Assay of Rat Liver Methylase *in vitro*. Proc. Am. Assoc. Cncer Res., *14:* 39, 1973.
14. Smith, R. C., and Salmon, W. D. Formation of *S*-Adenosylethionine by Ethionine-Treated Rats. Arch. Biochem. Biophys., *111:* 191–196, 1965.
15. Srinivasan, P. R., and Borek, E. Species Variation of the RNA Methylases. Biochemistry, *3:* 616–619, 1964.
16. Stewart, B. W., and Pegg, A. E. Changes in tRNA Methylase Activity

of Rat Kidney following Administration of the Carcinogen Dimethylnitrosamine. Biochim. Biophys. Acta, *281:* 416–424, 1972.

17. Wainfan, E., and Chu, J. A Simple Specific Assay for Uracil tRNA Methylases of Enteric Bacteria: Use of Ribothymidine-Deficient tRNA. Anal. Biochem., *55:* 388–393, 1973.
18. Wainfan, E., and Maschio, F. A. Inhibition of Methylated Nucleoside Synthesis *in vivo:* Accumulation of Incompletely Methylated Transfer RNA in Ethionine-Treated Cells of *Escherichia coli B*. Ann. N. Y. Acad. Sci., *255:* 567–575, 1975.
19. Wilkinson, R., and Pillinger, D. J. Effect of the Carcinogen *N*-nitrosomethylurea on the tRNA Methylase Activity of Cells Cultured *in vitro*. Intern. J. Cancer, *8:* 401–409, 1971.