communication, 1963). Thus we begin to have a better understanding of the Cotton effects of nucleic acids.

In a preliminary communication (Yang and Samejima, 1963b) we observed that dCMP will dominate the 290 mμ peak in a mixture of deoxyribonucleotides. Therefore, a close relationship may exist between the (cytosine + guanine) content and the 290 mμ peak for DNA when its secondary structure is destroyed; that is, the higher the (C + G) content, the larger the magnitude of the peak. Of course, the ORD of the mononucleotides could vary not only in magnitude but even in sign when they are incorporated into a polynucleotide chain. This hypothesis could be tested by studying the Cotton effects of DNA of various species. Furthermore, a comparative study of the Cotton effects of the DNA's in their native state might provide some information concerning the stacking interactions of the base pairs in the different species.

ORD of RNA.—Figure 4 suggests that RNA also possesses a certain degree of secondary structure and that its Cotton effects are significantly changed in alkaline solution or at high temperature. The red shift of the 280 mμ peak and 252 mμ trough (curves 2 and 3) might indicate the disruption of intramolecular hydrogen bonding between the stacking chromophores (bases). On the other hand, the weak 222 mμ peak actually increases its magnitude in alkaline solution or at high temperature. Why the peaks shift in opposite directions is not clear. Of interest is the similarity of the ORD curve for DNA (Fig. 2, curve 4) and RNA (Fig. 4, curve 3) in alkaline solution. DNA and RNA also appear to have very similar strong peaks at 200 mμ and 195 mμ, respectively, with nearly identical magnitude (Figs. 2 and 4). Since we are still unable to measure this Cotton effect at high temperature because of experimental difficulties, it is not certain whether this peak is closely related to the secondary structure or not. Note also that the measurements below 200 mμ approach the instrument limit, and therefore should be viewed with some reservation until we have more concrete evidence by studying the Cotton effects of various nucleic acids.

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Species Variation of the RNA Methylases*

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The complex of enzymes which methylate t-RNA at the polynucleotide level were found to be species specific. Therefore a preparation of t-RNA (transfer ribonucleic acid) from a given source, while fully methylated with respect to its homologous enzymes, will receive methyl groups *in vitro* from a heterologous enzyme source. Some closely related organisms exhibit a similarity in their pattern of methylation of foreign t-RNA's. A study of amino acid–specific t-RNA's with heterologous enzymes revealed that the different t-RNA's do not behave uniformly as substrates for methylation. Therefore the species variation may be restricted only to some of the t-RNA's.

It has recently been demonstrated (Borek *et al.*, 1962; Fleissner and Borek, 1963) and confirmed in several laboratories (Svensson *et al.*, 1963; Starr, 1963; Gold *et al.*, 1963; Comb, 1963) that the methylated bases of t-RNA are acquired by the methylation of the preformed polynucleotide chain by a complex of enzymes. The demonstration of the sequential nature of t-RNA synthesis was made possible by the discovery of a mutant, *Escherichia coli* $K_{12}W6$, in which, as a result of two independent mutations, the primary synthesis and the methylation of the product are uncoupled (Borek *et al.*, 1955). During methionine starvation this organism produces a species of t-RNA devoid of methylated derivatives of the component bases (Mandel and Borek, 1963). The availability of a naturally produced nonmethylated t-RNA as a substrate made possible the demonstration of the existence of a t-RNA-methylating enzyme. The enzyme system proved to be a complex whose individual members are endowed with highly restricted specificities (Fleissner and Borek, 1963; Gold *et al.*, 1963).

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The nonmethylated t-RNA, when isolated from the prestarved methionine auxotroph, can accept methyl groups not only from homologous enzymes but from enzymes extracted from every other species of organism tested.

In studies of the interactions of heterologous enzymes and methyl-deficient t-RNA control experiments were performed using fully methylated t-RNA from *E. coli* K_{12} in logarithmic growth phase. Instead of the anticipated negligible interactions, extensive transfer of supernumerary methyl groups into the otherwise fully methylated t-RNA by several heterologous enzymes was observed. Such overmethylation of normal t-RNA by heterologous enzymes is not restricted to RNA from *E. coli*, but is a rather generalized phenomenon: successful interactions of heterologous RNA and enzyme are far more frequent than negative findings. The distribution of methyl groups in t-RNA is apparently a species characteristic attribute; therefore an RNA, while fully methylated with respect to its own enzyme, can offer new sites for methylation to heterologous enzymes (Srinivasan and Borek, 1963). The species specificity of RNA methylases has been observed in other laboratories as well (Svensson *et al.*, 1963; Gold *et al.*, 1963).

It has been suggested by Kornberg and his associates (Kornberg *et al.*, 1959) that the 5-methylcytosine in germinating wheat DNA may be acquired by enzymatic methylation of the preformed polymer. The absence of a nonmethylated substrate DNA for a homologous enzyme is a handicap for the search for a DNA methylase. However, the discovery of species variation of RNA methylase made the species variation of DNA methylases even more likely since the distribution of methylated bases in DNA is even kingdom specific: animals and plants contain 5-methylcytosine, bacteria contain 6-methyladenine. The species specificity of DNA methylases has been demonstrated in Dr. Hurwitz's laboratory and in our own (Gold *et al.*, 1963; P. R. Srinivasan and E. Borek, in preparation). We report here detailed studies on the species specificity of the RNA methylases.

Materials and Methods

The bacterial strains used in this investigation were all ultimately derived from stocks of the American Type Culture Collection. The organisms were grown on nutrient broth except for *E. coli* K_{12}, which was grown on defined media. All the organisms were harvested in logarithmic growth phase. For the preparation of methyl-deficient t-RNA see Mandel and Borek (1963). The enzyme extract from yeast was prepared from commercially available baker's yeast. *E. coli* B and yeast t-RNA's were purchased from General Biochemicals, Inc., Chagrin Falls, Ohio. *S*-Adenosyl-L-methionine-methyl-^{14}C with a specific activity of 26.11 mc/mM was obtained as an aqueous acid solution from Tracerlab, Inc., Waltham, Mass. It was neutralized with 1 M Tris to *p*H 5.0 and diluted to yield an activity of 10 μc/ml.

Preparation of Enzyme Extracts and t-RNA.—The preparation of liver and spinach extracts has been described in detail in an earlier publication (Srinivasan and Borek, 1963). To obtain the bacterial and yeast-enzyme extracts the cells were disrupted in a Waring Blendor with glass beads; the cell debris and unbroken cells were eliminated by centrifugation at 20,000 *g* for 20 minutes, and a clear extract was obtained by centrifugation at 105,000 *g* in the Spinco preparative ultracentrifuge for 1 hour. This procedure is similar to that described by Chamberlin and Berg (1962) and is described in detail by Fleissner and Borek (1963). The extracts were stored at 2° with a few drops of toluene and under these conditions they remained active for at least a week.

The t-RNA preparations from each strain were prepared by phenol extractions of the supernatant which results from centrifugation at 105,000 *g* for 2 hours. The RNA was precipitated by alcohol, dialyzed, and lyophilized.

The reaction mixtures contained 100 μmoles of Tris buffer, *p*H 8.2, 10 μmoles each of $MgCl_2$ and reduced glutathione, 0.05 ml of *S*-adenosylmethionine-methyl-^{14}C, 2 mg of t-RNA, and 1 ml of enzyme extract in a total volume of 2.0 ml. The incubations were carried out at 37° for 45 minutes. The precipitation of t-RNA from the reaction mixture and the subsequent washing procedure to remove contamination of adenosylmethionine have been described earlier (Srinivasan and Borek, 1963). With each extract a control experiment was performed to assess the incorporation in the absence of any added t-RNA. Such background values were subtracted from the results presented in the various tables. All the radioactivity measurements were carried out in a Nuclear Chicago gas-flow counter and sufficient counts were recorded to give a probable error of ±5%.

Results and Discussion

In Table I the results of interactions of enzymes from liver, spinach, and yeast with t-RNA from a variety of sources are presented. In any attempt at interpretation it must be borne in mind that these data are the results of a highly complex system of interactions. The substrates are the different amino acid–specific t-RNA's, which number over twenty, and several different RNA methylases have been identified to date (in *E. coli*).

Table I
Interaction of Heterologous t-RNA's and RNA Methylases[a]

Source of t-RNA	Source of Enzyme Extract		
	Liver	Spinach	Yeast
Liver	0.01	0	0.13
Spinach	0.03	0.01	0.07
Yeast	0.08	0.04	0.07
E. coli K_{12} (methyl deficient)	0.21	0.12	1.23
E. coli K_{12} log	0.13	0.08	0.58
E. coli B	0.15	0.09	0.72
B. megatherium	0.12	0.08	0.50
B. cereus	0.17	0.06	0.24
Ps. aerugenosa	0.13	0.06	0.46
Rh. spheroides	0.11	0.09	0.55
S. typhimurium	0.21	0.08	0.76

[a] Activity is expressed as mμmoles $^{14}CH_3$ incorporated into 2 mg t-RNA with $^{14}CH_3$ *S*-adenosylmethionine as the methyl donor.

The following generalizations may be drawn. The methylation of the isolated t-RNA by the appropriate homologous enzyme system is, as a general rule, negligible except by the enzymes from yeast which consistently produce a small but significant incorporation. The reason for this is obscure at the present time. It is possible that in the RNA extracted from yeast a small fraction is in a transient state between the primary synthesis and methylation. The existence of such t-RNA, transiently denuded of methyl groups,

TABLE II
INTERACTION OF BACTERIAL t-RNA'S AND RNA METHYLASES

Source of Enzyme Extract	Source of t-RNA[a]								
	E. coli K_{12} (*starved*)	*E. coli* K_{12} (log)	*E. coli* B	*S. typhimurium*	*B. cereus*	*B. megatherium*	*Ps. aeruginosa*	*Ps. fluorescens*	*Rh. spheroides*
E. coli K_{12}	2.6	0.01	0	0	0.08	0.10	0	0.25	0.03
E. coli B	2.4	<0.01	0	0.08	0.09	0.07	0.02	0.25	0.07
S. typhimurium	2.8	0	0	0	0.01	0.14	0	0.15	0
B. cereus	1.2	0.29	0.56	0.27	<0.01	<0.01	0.19	0.27	0.22
B. megatherium	1.4	0.50	0.70	0.50	0	0	0.16	0.35	0.02
Ps. aerugenosa	1.35	0.01	0.02	0.01	0.12	0.42	<0.01	0.29	0.01
Ps. fluorescens	2.0	0.50	0.64	0.28	0.16	0.37	0.09	0.04	0.05
Rh. spheroides	0.39	<0.01	<0.01	<0.01	<0.01	<0.01	0	0.01	0

[a] Activity expressed as in Table I.

has been observed in the nuclei of the water mold (Comb, 1963) and of peas (Birnstiel *et al.*, 1963). The accessibility of yeast RNA for further methylation may also be due to conditions during the extraction of the RNA which propitiate the activity of some demethylating enzymes.

The methyl-deficient t-RNA which is extracted from *E. coli* K_{12}W6 after methionine starvation appears to be a universal substrate for the enzymes from eleven different sources (Tables I and II). This is to be expected in as much as this product offers two sets of sites for methylation by the heterologous enzymes: the sites left vacant by the indigenous enzymes during methionine starvation and the sites where the heterologous enzyme introduces the supernumerary methyl groups in the normal t-RNA of *E. coli* K_{12}W6.

In Table II the results of interactions of enzymes from eight different strains of microorganisms with nine different t-RNA's are presented. The pattern of interactions is complex. It is interesting to note that the organism *Rhodopseudomonas spheroides*, whose RNA is the poorest substrate for enzymes from other organisms, also yields the least versatile enzymes. This organism is unique in that its enzymes methylate only the methyl-deficient t-RNA of *E. coli* K_{12}W6. On the other end of the scale is *Pseudomonas fluoroscens*, whose enzymes methylate every RNA except that of *Rhodopseudomonas spheroides*. In turn, the RNA of *Pseudomonas fluorescens* is methylated by all enzymes except by those of *Rhodopseudomonas spheroides*.

The negligible methylation *in vitro* of t-RNA's by their homologous enzymes pointed to the possibility that enzymes from closely related organisms may exhibit some similarity in their pattern of methylation of heterologous t-RNA's. The enzymes of *E. coli* B and of *E. coli* K_{12} seem to fulfill such a prediction. The enzymes of *Bacillus cereus* and of *B. megatherium* are similarly paired. However, the enzymes of the two strains derived from the genus *Pseudomonas* defy such generalization. More data are needed to determine the frequency of such homologies in closely related organisms and to assess the meaning of exceptions.

So far no correlations have emerged between the guanine-cytosine content of the bacterial DNA's and the cross methylations of RNA's by enzyme extracts. However, this is not unexpected in as much as results drawn from quantitative hybridization experiments indicate that only a very small region of the DNA serves as the primer for t-RNA synthesis (Giacomoni and Spiegelman, 1962; Goodman and Rich, 1962).

It is possible that some subtle homologies will emerge from the analysis of the reciprocal effects of methylating enzymes and substrate RNA's at the level of the methylated bases in amino acid–specific transfer RNA's. That the methylating enzymes have high specificity toward amino acid–specific transfer RNA's is apparent from Table III. Different fractions of yeast t-RNA's separated by countercurrent distribution, which were kindly provided for us by Dr. Holley, were exposed to the methylating enzymes from three different sources. The six fractions of t-RNA range from no. 1, which is enriched with alanine t-RNA, to no. 15, which is the enriched tyrosine t-RNA (Doctor *et al.*, 1961). The three enzyme sources were liver, *E. coli* K_{12}, and *Pseudomonas fluorescens*. It is significant that the enzymes from the last source, which were proved to have the greatest versatility toward heterologous substrates, are highly selective toward the different fractions of yeast t-RNA. The tyrosine t-RNA, which is known to have a large number of methylated bases (eight; Holley *et al.*, 1963), is the best substrate for further methylation. The first three fractions of the t-RNA's are essentially inert as substrates for these enzymes. On the other hand, the enzymes from liver methylated the same six fractions in a practically reverse order of effectiveness. The pattern of methylations by these two sets of enzymes seem to indicate that the species variation of RNA methylases which is observed with the total t-RNA's may be restricted to only some of the specific t-RNA's. In other words, yeast RNA fractions 1, 5, and 8 are saturated with respect to the homologous enzymes from yeast or to the heterologous enzymes of *Pseudomonas fluorescens*, but the tyrosine t-RNA is saturated with respect to the homologous enzyme only. On the other hand, the enzymes from liver search out new sites for methylation in all the fractions of yeast t-RNA. Therefore, in none of these fractions is there a coincidence of the methylating pattern by the enzymes from yeast and from liver. Svensson *et al.* (1963) have attempted a correlation between the distribution of t-RNA's in a countercurrent system and their capacity to accept methyl groups. It is interesting to note that tyrosine t-RNA of *E. coli* 30SOA5 was a poor substrate for the enzymes of yeast. The converse, that tyrosine t-RNA of yeast is also a poor substrate for enzymes from *E. coli*, is evident from Table III.

The necessity of species variation for t-RNA is totally obscure at the present time, as is the function of the methyl groups themselves in the t-RNA. Once the function of the latter is elucidated the need for species specificity may become apparent.

An interesting suggestion has been made for the need for species specificity of DNA by M. Meselson (personal communication). The introduction of methyl groups into DNA undoubtedly alters the conformation of the

TABLE III

INTERACTION OF HETEROLOGOUS ENZYMES WITH YEAST t-RNA FRACTIONS FROM COUNTERCURRENT DISTRIBUTION[a]

Substrate	Source of Enzyme		
	Ps. fluorescens	Liver	*E. coli* K_{12}
E. coli methyl deficient t-RNA	12,200	1800	10,200
Yeast t-RNA	1,440	650	1,120
Fractionated yeast t-RNA			
(a) Fraction 1	0	530	Not done
(b) Fraction 5	0	650	0
(c) Fraction 8	40	530	60
(d) Fraction 12	100	340	120
(e) Fraction 14	170	360	40
(f) Fraction 15	1,940	290	90

[a] The reaction mixture employed contained 1 mg of the indicated t-RNA, 100 μmoles of Tris buffer, *p*H 8.2, 10 μmoles of reduced glutathione, 10 μmoles of $MgCl_2$, 0.02 ml of *S*-adenosylmethionine-methyl-^{14}C, and 0.5 ml of enzyme extract in a total volume of 1.0 ml. The activity is expressed as counts per minute.

DNA, conferring upon it a species individuality. Such structural individuality might render difficult the integration of foreign DNA (from some infecting parasite) into the DNA of the host, and thus the species-specific methylation would serve as a guardian of DNA.

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ADDED IN PROOF

The tyrosyl t-RNA (fraction 15, Table III) which had been exposed to the methylating enzymes of *Ps. fluorescens* has now been examined for the pattern of methylation. Almost all of the radioactivity, and therefore methyl groups, was found in methyladenylic acid. It is noteworthy that a species of RNA which already contains eight methylated bases should be the recipient of another methyl group, in contrast to the valyl and alanyl t-RNA's which contain but a few methylated bases initially and receive no more even from the most efficient heterologous enzymes.

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Interactions of Purine with Proteins and Amino Acids*

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The effect of purine on the transition temperature (T_m) of bovine serum albumin, ribonuclease, and lysozyme has been studied by optical rotation at 589 mμ. It is found that purine decreases the T_m of these proteins in proportion to the activity of purine in solution. In appropriate concentration of purine (higher required for bovine serum albumin) the thermal denaturation of lysozyme and bovine serum albumin is instantly reversible, a phenomenon not observable in the absence of purine. In comparison to urea on activity basis, purine is 6- to 10-fold more effective in lowering the T_m of the proteins. Purine (0.3 molal) increases the solubility of tyrosine (300%), of tryptophane (250%), of phenylalanine (40%), and of histidine (15%), but does not increase the solubility of glycine and alanine. Secondary structures of proteins appear to be much less sensitive to the influence of purine than do those of nucleic acids. Purine may serve as a selective denaturant for nucleic acids in protein solution. A distinction between the mechanism of purine denaturation and urea denaturation has also been made.

Optical rotation measurements in the visible region have been successfully employed in the study of the interaction of nucleic acids with various ultraviolet-absorbing compounds of biological importance (Ts'o *et al.*, 1962a). This technique is very sensitive to changes in conformation of biopolymers, and optical activity versus temperature profiles of the biopolymers

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