tal science, which it must become if man is ever to exert real control on his atmosphere. Fortunately, the first step has been made with cumulus clouds. These are of vital importance to man in themselves, a key part of the larger-scale atmospheric machinery, and a prototype of the widespread geophysical phenomenon of thermal convection.

### References and Notes


1. V. J. Schaefer, *Science* **104**, 457 (1946).
2. B. Vonnegut, *J. Appl. Phys.* **18**, 593 (1947).
2a. I. Langmuir, *Final Report Project Cirrus* (General Electric Company Research Laboratory, Schenectady, 1948), p. 120.
3. T. Bergeron, *Mem. Union Géod. Géophys. Intern. Lisbon* (1933).
4. J. S. Malkus, *Weather* **8**, 291 (1953).
5. ———, *J. Meteorol.* **11**, 220 (1954); ———. and G. Witt, *The Atmosphere and the Sea in Motion* (Oxford Univ. Press, New York, 1959), pp. 425–439.
6. J. S. Malkus, *Science* **141**, 767 (1963).
7. ———, survey paper presented at the 3rd Technological Conference on Hurricanes and Tropical Meteorology, Mexico City, 11 June 1963; for a summary of the conference, see *Science* **141**, 935 (1963).
8. ———, *Cumulus Dynamics* (Pergamon, New York, 1960), pp. 65–84.
9. R. H. Simpson, *Sci. Am.* **190**, 32 (1954); ———, A. W. Johnson, R. C. Gentry, *Weatherwise* **9**, 459 (1955).
10. R. H. Simpson and J. S. Malkus, *Science* **142**, 498 (1963).
11. R. H. Simpson, M. A. Ahrens, R. D. Decker, *Natl. Hurricane Res. Proj. Rept.* **60** (1963).
11a. E. B. Kraus and P. Squires, *Nature* **159**, 489 (1947).
12. J. Levine, *J. Meteorol.* **16**, 653 (1959).
13. J. S. Malkus and R. T. Williams, *Meteorol. Monographs* **5** (1963), p. 59.
14. H. Stommel, *J. Meteorol.* **4**, 91 (1947).
15. B. R. Morton, G. I. Taylor, J. S. Turner, *Proc. Roy. Soc. London* **A234**, 1 (1956).
16. J. S. Malkus and R. H. Simpson, *J. Appl. Meteorol.*, in press.
17. D. A. Andrews, thesis, University of California, Los Angeles (1964).
18. J. S. Malkus and H. Riehl, *Tellus* **12**, 1 (1960); H. Riehl and J. S. Malkus, *Geophysica, Helsinki* **6**(3-4), 503 (1958).


19. Our colleague, Captain Max Eaton (U.S. Navy) made this study possible by his efficient direction of the vital part of the operation contributed by the U.S. Navy. We are deeply grateful to the crews of the Navy's VW-4 Squadron and the U.S. Weather Bureau's Research Flight Facility, who went far beyond the call of duty in carrying out the experiment. The analysis of results was mainly carried out under the National Science Foundation's grant No. GP-1158 to the University of California, Los Angeles. This article is University of California (Los Angeles) Department of Meteorology paper No. 106.


**CURRENT PROBLEMS IN RESEARCH**

# Enzymatic Alteration of Nucleic Acid Structure

Enzymes put finishing touches characteristic of each species on RNA and DNA by insertion of methyl groups.


P. R. Srinivasan and Ernest Borek


Nucleic acids, both RNA and DNA, contain several minor components in addition to the four main bases of their primary structure. In the DNA of bacteria the minor component is 6-methyladenine (*1*) and in that of plants and animals it is 5-methylcytosine (*2*). The presence of methyl derivatives of the bases has also been reported in ribosomal RNA, but the compounds have not as yet been characterized. In transfer RNA, on the other hand, no fewer than ten methylated bases have been demonstrated (*3*). In addition, transfer RNA contains an unusual riboside, pseudouridine, a compound in which the ribose is attached to carbon atom 5 of the base (*4*) (Fig. 1).

The presence of methylated bases in nucleic acids has, until recently, presented several paradoxes. In the first place, no monomeric methylated precursors were ever found within any tissue examined. Moreover, it was difficult to visualize how transfer RNA, which contains ten different methylated bases and pseudouridine, could be derived by complementary alignment from DNA, which contains but one methylated base. Indeed, the Watson-Crick hypothesis for the replication of DNA itself, or for its transcription into transfer RNA, offers no mechanism for the determination of the sequence of methylated bases in a nucleotide chain.

All of these paradoxes were resolved by the discovery, in our laboratory, of enzymes, which activate transmethylation, at the polymer level of the previously formed transfer RNA. This work revealed the mechanism of insertion of the methylated bases into both RNA and DNA. The bases are methylated *after* the formation of the polymeric nucleic acids.

These conclusions were made possible by an unexpected observation made 10 years ago (*5*) on an anomalous attribute of the auxotroph *Escherichia coli* $K_{12}W6$. This organism is unique among amino-acid-requiring microorganisms in that RNA synthesis continues during starvation of its essential amino acid, methionine, whereas in every other auxotroph RNA synthesis ceases in the absence of an essential amino acid.

Recently Stent and Brenner (*6*) have made an important contribution to the genetics of *E. coli* $K_{12}W6$ by demonstrating that the relaxed control over RNA synthesis in this organism is due to a genetic aberration which can be transferred during conjugation. The appropriate amino-acid-requiring recombinants accumulate RNA in the absence of any requisite amino acid.

Examination of the RNA which accumulates during methionine starvation revealed no moleculear species different from those found in normal microorganisms. However, we observed a profound alteration in the structure of the newly formed transfer RNA. It lacked the methylated bases, including thymine. This finding pointed to the possibility that methionine is the source of methyl groups for all of the methylated bases in RNA. This could be anticipated in part, for methionine, or rather its activated derivative S-adenosylmethionine, is the methylating agent in most biological reactions. However, that the thymine of RNA should stem from this source was completely unexpected. It had been unequivocally established earlier, by Kornberg and his associates, that the thymine in DNA is a product of a different reaction: a condensation of a


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uracil derivative with activated formic acid and subsequent reduction of the latter moiety to a methyl group. Experiments with $C^{14}$-labeled methionine revealed that indeed there is a dichotomy in the pathway of synthesis of thymine for DNA and RNA (Table 1). Moreover, all of the methylated bases of RNA had identical specific radioactivity, indicating that they all stem from the same methyl pool (*7*).

The synthesis, during methionine starvation, of transfer RNA that lacked the methylated bases and the origin, under normal growth conditions, of all these bases, including thymine, from the same methyl pool pointed to the possibility that the methylated bases are acquired by transmethylation, at the polynucleotide level, of the previously-formed transfer RNA.

According to this hypothesis the accumulation of methyl-deficient transfer RNA in *Escherichia coli* $K_{12}W6$ is the result of two genetic lesions: the relaxed control of RNA synthesis which permits accumulation of RNA in the absence of the full complement of amino acids and the chance coincidence of the inability to synthesize methionine for the methylation of the nascent transfer RNA.

At first, in vivo evidence was sought for methylation at the level of the previously formed polymer. The number of methyl groups introduced per nucleotide bond formed in normal organisms and in RNA-enriched organisms recovering from previous methionine starvation was determined by simultaneous labeling with $P^{32}O_4$ and $C^{14}$ methyl methionine. The results of such double-labeling experiments (Fig. 2) indicated a higher incorporation of methyl groups, relative to the number of nucleotide bonds formed, in the recovering organisms with the excess RNA (*8*).

## The RNA Methylases

The results of the in vivo experiments augured well for a search, in a cell-free system, for an enzymatic activity which would effect methylation of the RNA macromolecule. The natural choice for a substrate was the methyl-deficient soluble RNA accumulated during methionine starvation. If such methyl-deficient transfer RNA is incubated with a soluble extract of *Escherichia coli* cells and with adenosylmethionine, methyl groups are added to the various bases in the transfer RNA to the level of the normal endowment in cells grown on a complete medium. Several lines of evidence demonstrated unequivocally that the methylation by the enzymes occurs at the level of the macromolecule. These observations have been confirmed in many laboratories (*9–12*).

Table 1. Source of the methyl group for ribosylthymine.

| Compound* | Specific radioactivity (count/min μmole) |
|---|---|
| 1) DNA thymine | 200 |
| 2) RNA thymine riboside | 37,000 |
| 3) Thymine riboside from compound 2 diluted 30-fold with non-radioactive synthetic thymine riboside | 1,300 |
| 4) Thymine degraded from the diluted thymine riboside (compound 3) | 1,300 |

* *Escherichia coli* was grown on methyl-$C^{14}$-methionine; thymine and thymine riboside were isolated from DNA and RNA, respectively.

The enzyme with the potential to methylate transfer RNA (we have named it "RNA methylase") has several different substrates: uracil and cytosine (in both of which C–$CH_3$ linkages are achieved), adenine (in which both nitrogen and carbon atoms can be the receptors of the methyl groups), and guanine (in which three different nitrogen atoms are known to be methylated). It was anticipated that no single enzyme could have such prodigious versatility, and the existence of several enzymes was conjectured.

Fractionation of the enzyme extracts confirmed the prediction of multiplicity of the enzymes (*13*). According to the latest report from the workers most intensively engaged in purification of the enzymes—Gold and Hurwitz—there are no less than six enzymes involved in the introduction of methyl groups into various positions in transfer RNA (*14*).

The RNA methylases proved to be

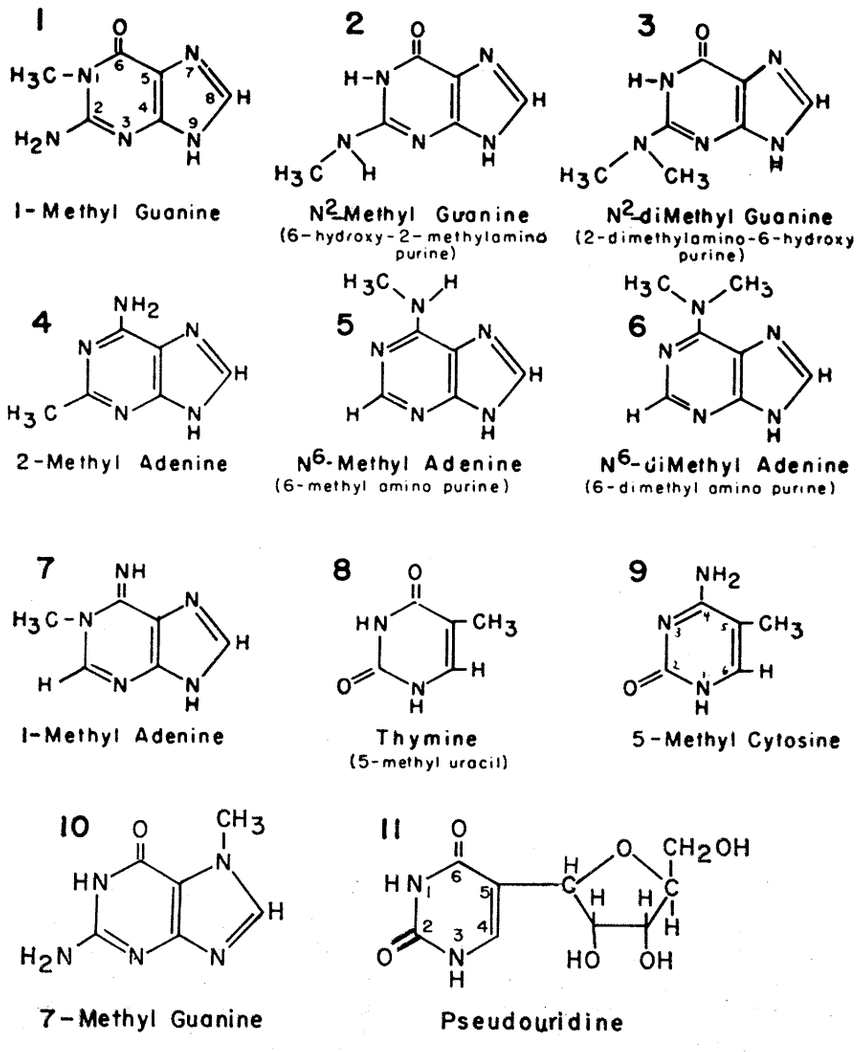


Fig 1. The minor components of RNA and DNA.

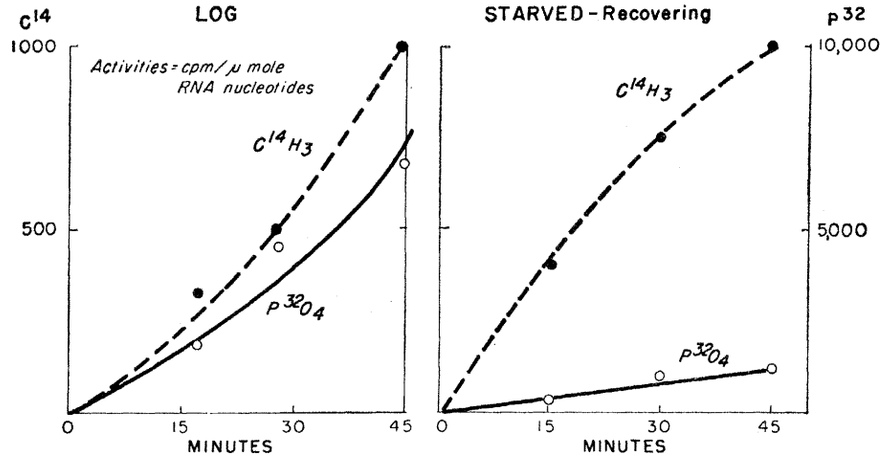


Fig. 2. Incorporation of $P^{32}$ and $C^{14}H_3$ methionine into RNA of *Escherichia coli* $K_{12}W6$ during the logarithmic growth phase and in the recovery phase after starvation of the organism (see *8*).

ubiquitously distributed. Every tissue examined contains these enzymes. However, the distribution of the enzymes specific for the various bases is not the same in all species. The result of such uneven RNA methylase potencies is reflected in variations in the distribution of the methylated bases in the transfer RNA's of different species of organisms (Table 2).

In studies of the distribution of the enzymes, fully methylated transfer RNA of *Escherichia coli* was used as the substrate for determining the minimum, control, level of the reaction with the methyl-deficient transfer RNA. It was discovered almost simultaneously in three different laboratories that enzymes from heterologous sources can still add methyl groups in vitro to fully methylated transfer RNA of *E. coli* (*11, 15, 16*).

Such interactions between otherwise fully methylated transfer RNA's and heterologous enzymes were found to be widespread. The pattern of interactions, however, is complex. Enzymes from closely related organisms often do exhibit similarities in their pattern of methylation of heterologous RNA's. However, there are some infractions of the anticipated rules of homology (*17*) (Table 3).

More detailed study may reveal the meaning of homologies based on patterns of methylation, but it is already apparent that both the substrate and the enzyme are endowed with a directive specificity which is a species characteristic. In turn, the transfer RNA's of each species must have a structural characteristic conferred on them by the number and position of the methyl groups.

Table 2. Distribution and molar proportions (moles per 100 moles of uracil) of methylated bases in ribonucleic acids (*3*) [*, not examined; —, none detected (less than 0.05 percent); +, present but not estimated].

| Source of RNA | Thymine | 2-Methyl-adenine | 6-Methyl-amino-purine | 6-Dimethyl-amino-purine | 1-Methyl-guanine | 2-Dimethyl-amino, 6-hydroxy-purine |
|---|---|---|---|---|---|---|
| *Escherichia coli* B/r | 0.9 | 0.5 | 0.4 | 0.3 | * | * |
| *Escherichia coli* 15T- | 1.0 | .3 | * | * | * | * |
| *Aerobacter aerogenes* | 1.2 | .3 | .3 | .1 | 0.1 | — |
| *Staphylococcus aureus* | 0.9 | .05 | .3 | — | * | * |
| Yeast | .6 | .1 | .2 | .05 | .02 | * |
| Rabbit liver | — | — | .3 | — | + | * |
| Rat liver microsomes | — | — | .5 | .1 | .09 | 0.09 |
| Wheat embryo | 3.7 | .1 | 3.7 | .06 | .6 | .5 |
| *Beta vulgaris* leaves | 0.23 | * | 0.2 | * | .2 | .1 |
| *Nicotiana glutinosa* leaves | * | * | * | * | .2 | .06 |
| Turnip yellow mosaic virus | — | — | — | — | — | — |
| Tobacco mosaic virus | — | — | — | — | — | * |

## The DNA Methylases

The discovery of the species specificity of the RNA methylases pointed to the probability that the DNA-methylating enzymes too are species specific. It should be recalled that the distribution of methyl groups in DNA is not only species but even kingdom specific: bacterial DNA contains 6-methyladenine, and the DNA's of plants and animals contain 5-methylcytosine. Indeed, Kornberg had speculated that the 5-methylcytosine of DNA may be produced by the methylation of the polymer. His search for DNA-methylating enzymes was unsuccessful because of the lack of a suitable recipient methyl-deficient substrate.

The methylation of DNA by heterologous enzymes was successful in our hands (*18*) and was reported from Hurwitz's laboratory (*16*). According to reports from that laboratory, two separate enzyme activities exist, one of which methylates cytosine, the other adenine.

The species specificity of the DNA methylases indicates that the distribution of the methyl groups in DNA is likely to be a species characteristic. That the substrate DNA itself has a directive influence on the number of methyl groups it accepts is suggested by experiments performed in our laboratory by Ann Ryan. It is known that ultraviolet irradiation produces in vivo structural alterations in DNA. It was found that in bacterial populations which were irradiated with ultraviolet at a dosage which was sufficiently high to inhibit DNA synthesis completely, the incorporation of methyl groups continued at a significant rate (*19*). Therefore, in cells in which the DNA already had its normal endowment of methyl groups, the homologous enzymes searched out new sites for methylation as a result of the radiation-produced structural alteration of the DNA.

It is very significant that both the RNA and the DNA methylases proved to be species specific. In the past, biochemical unity has been overemphasized in studies of reactions of enzymes on simple substrates. But as we approach the study of structural enzymes we should expect to find increasing biochemical diversity.

In this connection it is interesting to speculate on the origin of pseudouridine in transfer RNA. The Watson-

Crick hypothesis offers no mechanism for the determination of the position of this minor component by the seminal information within the DNA template. There have been suggestions in the literature that pseudouridine is the result of an intramolecular rearrangement as a result of which the ribose of uridine migrates from the nitrogen atom to carbon atom 5. The discovery of enzymes of methylation which alter the structure of RNA and DNA at the macromolecular level makes it appear highly probable that enzymes which achieve the ordered synthesis of pseudouridine at the macromolecular level also exist. The species specificity of pseudouridylating enzymes—which can be confidently predicted—could be employed in a search for their presence.

## Function of Methyl Groups

In addition to the transfer RNA and DNA, the occurrence of methylated components in ribosomal RNA have been reported by two different investigators, Boman (*11*) and Starr (*9*). Recently we have demonstrated the existence of enzymes which methylate ribosomal RNA at the polynucleotide level (*20*). Therefore, to date at least ten different enzymes have been identified whose function is the methylation of nucleic acids.

But once the methyl groups are inserted, what is their function? That they are important there can be no doubt; for example, if *Escherichia coli* $K_{12}W6$ is deprived of methionine for 3 hours and the amino acid is then restored, within 15 minutes (long before protein synthesis recommences) the accumulated methyl-deprived transfer RNA becomes fully methylated (*21*).

The introduction of methyl groups must produce profound alterations in the structure of nucleic acids. Such changes can stem from two different kinds of perturbations: steric and electronic. The methyl group is a bulky, hydrophobic structure which must induce alterations in the conformation of nucleic acids; moreover, the introduction of a methyl group must affect the electron density distribution within the recipient purines or pyrimidines. There is evidence that methylation has both of these effects. Shugar and W. Szer (*22, 23*) have investigated the physical properties of polyuridylic and polyribothymidylic acids. (The latter might be considered a highly methylated derivative of the former.) The phase transition or melting point of polyuridylic acid is 8.5°C, indicating that this polymer exists in solution as a random coil; polyribothymidylic acid, on the other hand, has a "melting point" of 36°C, indicating that the introduction of the methyl groups into the 5-position of uracil brought about a highly ordered structure. Moreover, they have also shown that polyribothymidylic acid is more extensively hydrogen-bonded to polyadenylic acid than is polyuridylic acid. The introduction of methyl groups apparently either augments the strength of hydrogen bonding or alters the conformation so that there are more sites for such bonding.

The profound electronic perturbation which can be brought about by methylation is best illustrated by the properties of 7-methylguanosine or 7-methyldeoxyguanosine. The introduction of a methyl group into the 7-

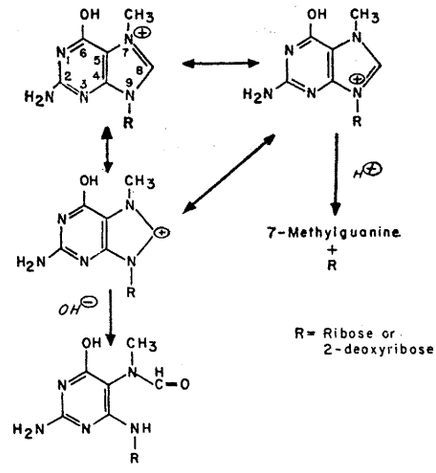


Fig. 3. Resonance structures of 7-methylguanine nucleosides and their behavior under mildly acidic and basic conditions.

position renders the whole structure sufficiently unstable to cause the cleavage of the glycosidic bond (*24*) (Fig. 3).

The ultimate biological function of the methylation of nucleic acids remains obscure. In the case of DNA we must visualize that germinal substance as carrying the information to produce an enzyme that will alter its own secondary structure, by the insertion of methyl groups in designated positions. One function of such methylations might be that of achieving an individuality of structure which could serve to protect the DNA. This most precious hoard of a living cell is made up of permutations of the same four bases in every organism. Therefore the physical characteristics conferred on the DNA's of different organisms by their primary sequence are nearly identical in every case. This might render the integration of a foreign DNA of some infecting organism too easy. A species-specific alteration in the physical conformation of the DNA would guard against such a calamity. It will be interesting to study the patterns of methylation in DNA's of putatively different microorganisms among which transformation can be successfully achieved. Another possible protective function of special, characteristic, methylation of DNA might be that of rendering the methylated DNA resistant to homologous deoxyribonucleases. Some intraspecies-specificity of deoxyribonucleases has been reported by Catlin of Marquette University (*25*).

If the function of methylation is indeed the protection of DNA, then we

Table 3. Interaction of RNA methylases and transfer RNA's from various sources. The transfer RNA's were exposed to the different enzyme extracts in the presence of S-adenosylmethionine-methyl-$C^{14}$, under appropriate conditions (*17*).

| Source of transfer RNA | Source of enzyme extract | | | |
|---|---|---|---|---|
| | Liver (count/min) | Spinach (count/min) | Yeast (count/min) | *Pseudomonas fluorescens* (count/min) |
| Liver | 210 | 0 | 2,600 | 8,700 |
| Spinach | 520 | 260 | 1,300 | 740 |
| Yeast | 1,530 | 690 | 1,340 | 10,600 |
| *Escherichia coli* $K_{12}$, methyl-deficient | 4,270 | 2,450 | 24,600 | 36,600 |
| *Escherichia coli* $K_{12}$, logarithmic growth phase | 2,540 | 1,580 | 11,500 | 10,000 |
| *Escherichia coli* B | 2,890 | 1,820 | 14,500 | 12,700 |
| *Bacillus megaterium* | 2,460 | 1,640 | 9,900 | 7,400 |
| *Bacillus cereus* | 3,340 | 1,240 | 4,700 | 3,300 |
| *Pseudomonas aeruginosa* | 2,640 | 1,240 | 9,200 | 1,700 |
| *Rhodopseudomonas spheroides* | 2,140 | 1,710 | 10,900 | 1,000 |
| *Salmonella typhimurium* | 4,140 | 1,680 | 15,100 | 5,600 |

must visualize the evolution of a new species of organism as requiring a sequence of mutations among which must be the acquisition of a methylating potency characteristic of the new species.

The function of the methyl groups in transfer RNA is also obscure. Emerging data from several laboratories indicate that the methylated bases are not uniformly distributed in the transfer RNA's specific for different amino acids (*26*). The possibility that the number and variety of methylated bases form a code whose nature is not yet recognized cannot be excluded. Such a code might have as one of its functions the recognition by the activating enzyme of the appropriate transfer RNA for the attachment of the amino acid. Starr (*27*) of Northwestern University, Boman (*11*) of Uppsala, and Littauer (*12*) of the Weizmann Institute reported that the methyl-deficient transfer RNA which accumulates in *Escherichia coli* $K_{12}W6$ deprived of methionine can accept amino acids in vitro. However, it should be pointed out that all of these preliminary experiments were performed with a mixture of methylated and nonmethylated transfer RNA's in which the abundance of the latter may have been as low as 40 percent. (Before the organisms can be subjected to starvation they must be grown in a complete medium, and during this period normal, methylated transfer RNA is formed.) Moreover, it is possible that the methyl groups form a code of exclusion. In other words, the methyl groups in an amino-acid-specific transfer RNA could serve to bar all activating enzymes save the appropriate one. Should this be the mechanism, then the nonmethylated transfer RNA's could be expected to accept amino acids promiscuously.

This problem will become resolvable once a specific transfer RNA devoid of methyl groups is available. Attempts to isolate such a product are being made in several laboratories, including our own.

We have recently completed a study, in collaboration with Elsie Wainfan, which indicates that messenger RNA does not contain any methylated bases (*28*). This finding provides added support for the view that the methylated bases cannot be involved in coding for the amino acids themselves. But such a view was implicit earlier in the finding that the RNA viruses (which are essentially messenger RNA wrapped in a protein coat) lack methylated bases.

Finally, the methylated bases in DNA and RNA may have functions which are as yet unrecognized. That the enzymes which form the methylated bases (and therefore the distribution of the bases) are characteristic of the species points to the possibility of some function unique for the species. The biological function that has maximum species individuality is differentiation, which, at present, is visualized as being controlled, in part, by the cueing in or out of existing capacities for enzyme synthesis. One is tempted to conjecture on a possible role for the methylated bases in such control mechanisms.

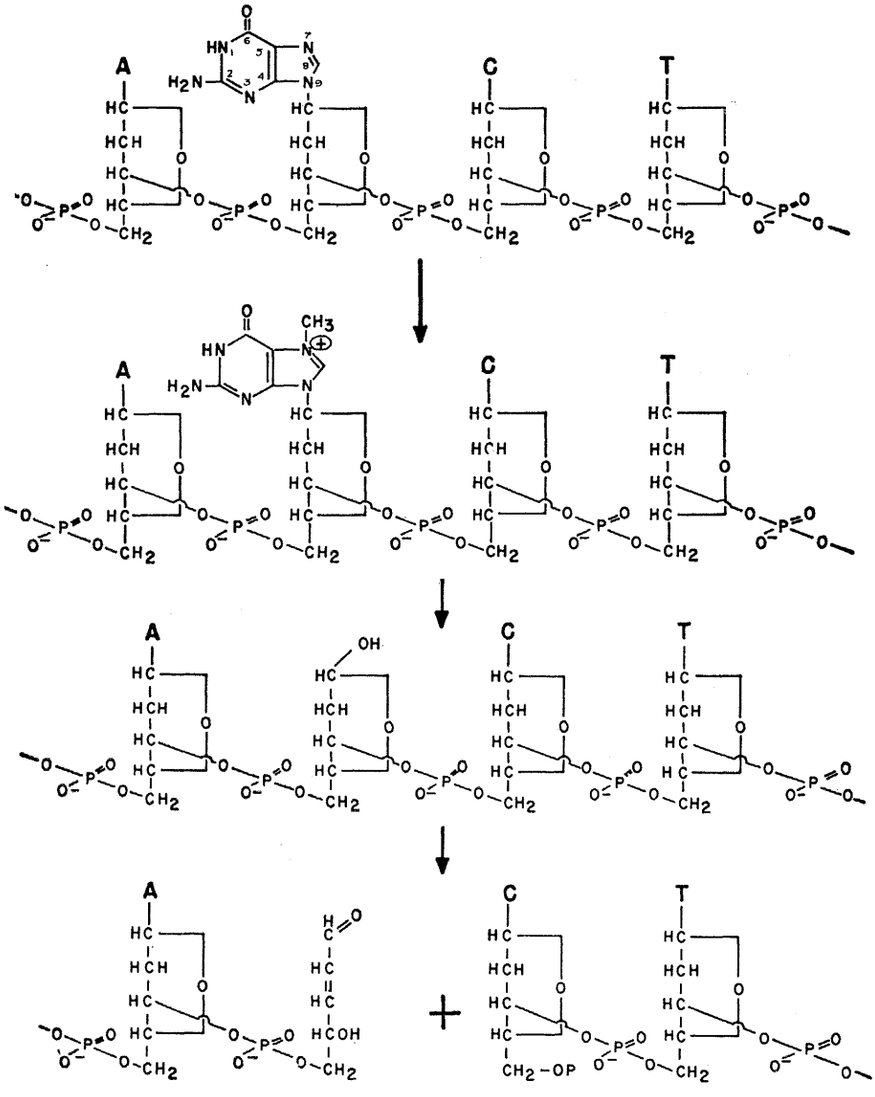


Fig. 4. Possible alterations in the structure of DNA attributable to methylation of the 7-position of guanine.

## Methylating Enzymes as Possible Natural Oncogenic Agents

As soon as the species specificity of enzymes that activate the methylation of nucleic acids was discovered it occurred to us that some methylating enzyme complex may be a naturally occurring carcinogen (*15*). Such a hypothesis is based on the following information. It was shown recently by Magee and Farber that alkylating carcinogens alkylate transfer RNA much more than they do DNA (*29*). Moreover these authors reported that the pattern of such alkylation is aberrant; sites in RNA which are normally free of methyl groups are alkylated—for example 7-methylguanine, a normally rare methylated base, occurs in abun-

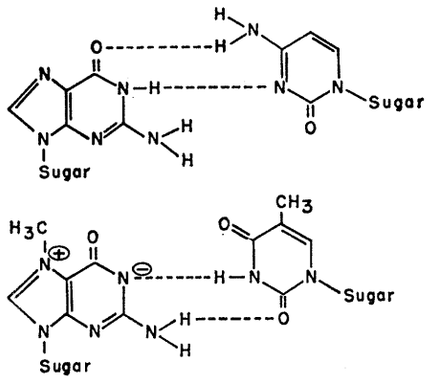

Fig. 5. (Top) Normal base pairing between guanine and cytosine; (bottom) possible aberrant base pairing between 7-methylguanine and thymine.

dance in the transfer RNA of animals which had been exposed to carcinogenic methylating agents. If, as seems likely, these imposed alkylations by chemical carcinogens have a causal relation to the tumors they produce, then such chemical alkylations may have counterparts, as oncogenic agents, in aberrant or excessive methylations of RNA or DNA by naturally occurring methylating enzymes.

The introduction of a novel enzyme-synthesizing capacity by an invading viral parasite has been frequently demonstrated in bacterial virus-host systems since the first demonstration by Seymour Cohen (*30*). In like manner an infecting oncogenic virus could introduce a capacity for the synthesis of a methylating enzyme foreign to the host. If the gene responsible for the capability were incorporated into the genome of the host, replication of the gene and consequent continuing synthesis of the oncogenic enzyme would render growth of the tumor independent of the initial infection.

Alternatively, the oncogenic virus could introduce an enzyme capable of methylating DNA in the 7-position of guanine. The conversion of nitrogen-7 into a quaternary state might render the DNA unstable enough to induce a scission of the glycosidic bond. The DNA with a lacuna of a guanine molecule might follow any one of the following paths in the process of uncoiling and replication.

1) The injury might be repaired by the reinsertion of a guanine molecule. (At present no mechanism for such restoration is known.)

2) In place of guanine, another base may be incorporated, an error in coding thus being introduced.

3) The lacunal DNA chain may be cleaved as a result of a process of beta elimination of a phosphate ester bond (Fig. 4).

The significantly increased excretion of 7-methylguanine and 7-methyl-8-hydroxyguanine in the urine of leukemia patients seems to favor the foregoing speculations.

In addition to initiating processes resulting in scissions, the insertion of a methyl group into the 7-position of deoxyguanosine would produce a marked enhancement of ionization of the proton from the nitrogen in the 1-position at *p*H 7.0. Such an alteration could induce 7-methylguanine to pair erroneously with thymine instead of with cytosine, producing a mutation which would be perpetuated during subsequent replications (see Fig. 5). Lawley and Brookes have postulated such a mechanism to explain the biological effects of chemical alkylating agents, several of which produce 7-alkylguanine in the DNA of organisms exposed to them (*24, 31*).

In addition to anomalies in the DNA, methylating enzymes foreign to the host cell could yield aberrant methylation of the transfer RNA as well, with anomalies both in the concentration and in the distribution of the methyl groups.

Bergquist and Matthews (*32*) have already shown that some tumor tissues contain the highest amounts of methylated bases in transfer RNA ever reported. However, studies to find whether changes occur in the patterns and levels of methylating enzymes of tumor tissues will provide a more searching test of the hypothesis of oncogenesis which we have proposed. Such studies are under way in our laboratory.

**References and Notes**


1. D. B. Dunn and J. D. Smith, *Biochem. J.* **68**, 627 (1958).
2. R. Wyatt, *ibid.* **48**, 584 (1951); E. Chargaff, in *The Nucleic Acids*, E. Chargaff and J. N. Davidson, Eds. (Academic Press, New York, 155), vol. 1, p. 307.
3. J. W. Littlefield and D. B. Dunn, *Biochem. J.* **70**, 642 (1958); J. D. Smith and D. B. Dunn, *ibid.* **72**, 294 (1959); D. B. Dunn, *Biochim. Biophys. Acta* **34**, 286 (1959); ———, *ibid.* **38**, 176 (1960); ———, *ibid.* **46**, 198 (1961).
4. W. E. Cohn, *Biochim. Biophys. Acta* **32**, 569 (1959); F. F. Davis and F. W. Allen, *J. Biol. Chem.* **227**, 907 (1957).
5. E. Borek, A. Ryan, J. Rockenbach, *J. Bacteriol.* **69**, 460 (1955).
6. G. S. Stent and S. Brenner, *Proc. Natl. Acad. Sci. U.S.* **47**, 2005 (1961).
7. E. Borek, *Cold Spring Harbor Symp. Quant. Biol.* **28**, 139 (1963); L. R. Mandel and E. Borek, *Biochemistry* **2**, 555 (1963).
8. E. Fleissner and E. Borek, *Biochemistry* **2**, 1093 (1963).
9. J. L. Starr, *Biochem. Biophys. Res. Commun.* **10**, 175 (1963).
10. M. Gold, J. Hurwitz, M. Anders, *ibid.* **11**, 107 (1963); D. G. Comb, *Federation Proc.* **22**, 354 (1963).
11. I. Svensson, H. G. Boman, K. G. Eriksson, K. Kjellin, *J. Mol. Biol.* **7**, 254 (1963).
12. U. Z. Littauer, K. Muench, P. Berg, W. Gilbert, P. F. Spahr, *Cold Spring Harbor Symp. Quant. Biol.* **28**, 2 (1963).
13. E. Fleissner and E. Borek, *Federation Proc.* **22**, 229 (1963); M. Gold and J. Hurwitz, *ibid.*, p. 230.
14. M. Gold and J. Hurwitz, *ibid.* **23**, 374 (1964).
15. P. R. Srinivasan and E. Borek, *Proc. Natl. Acad. Sci. U.S.* **49**, 529 (1963).
16. M. Gold, J. Hurwitz, M. Anders, *ibid.* **50**, 164 (1963).
17. P. R. Srinivasan and E. Borek, *Biochemistry* **3**, 616 (1964).
18. ———, in preparation.
19. A. Ryan and E. Borek, *Federation Proc.* **23**, 374 (1964).
20. P. R. Srinivasan, S. Nofal, C. Sussman, *Biochem. Biophys. Res. Commun.* **16**, 82 (1964).
21. E. Fleissner, thesis, Columbia University (1963).
22. W. Szer, paper presented at a meeting of the American Chemical Society, Denver (1964).
23. D. Shugar and W. Szer, *J. Mol. Biol.* **5**, 580 (1962).
24. P. D. Lawley and P. Brookes, *Biochem. J.* **89**, 127 (1963).
25. B. W. Catlin, *J. Bacteriol.* **79**, 587 (1960).
26. R. W. Holley, J. Apgar, G. A. Everett, J. T. Madison, S. H. Merrill, A. Zamir, *Cold Spring Harbor Symp. Quant. Biol.* **28**, 117 (1963); V. Ingram and J. A. Sjoquist, *ibid.* **28**, 133 (1963).
27. J. L. Starr, *Biochem. Biophys, Res. Commun.* **10**, 181 (1963).
28. E. Wainfan, P. R. Srinivasan, E. Borek, paper presented at a meeting of the American Chemical Society, Denver (1964).
29. P. N. Magee and E. Farber, *Biochem. J.* **83**, 114 (1962).
30. J. G. Flaks and S. S. Cohen, *J. Biol. Chem.* **234**, 1501 (1959); J. G. Flaks, J. Lichtenstein, S. S. Cohen, *ibid.* p. 1507.
31. P. D. Lawley and P. Brookes, *Nature* **192**, 1081 (1961).
32. P. L. Bergquist and R. E. P. Matthews, *Biochem. J.* **85**, 305 (1962).


33. The work discussed in this article was supported by grants from the U.S. Public Health Service, the National Science Foundation, and the Atomic Energy Commission.