(*2*). Therefore 5-methylcytosine in DNA can be labeled by tritium. The methyl group of thymine for DNA stems from formate, and therefore is not labeled by [methyl-^{3}H]SAM. It can be differentially labeled with [^{14}C]formate. When Scarano found that a portion of the thymine in the DNA of sea urchin embryo which had been incubated with [methyl-^{3}H]methionine bore the tritium label, he concluded that this "minor thymine" came from the deamination of [methyl-^{3}H]methylcytosine.

Indeed, Scarano demonstrated the existence of an enzyme, DNA cytosine deaminase, but this enzyme does not have the ubiquitous occurrence in tissues that would be expected if it had an important regulatory function. The best source of the enzyme is donkey spleen. On the basis of these findings Scarano *et al.* proposed the "synchron model of differentiation" (*3*). The model described by Holliday and Pugh is an extension of Scarano's model.

However, there is another pathway possible for the entry of tritium label into the "minor" thymine of DNA. There is thymine in tRNA as well, but this thymine is synthesized at the polymer level by the addition of an intact methyl group from SAM (*4*). Should there be a salvage pathway for the thymine resulting from the turnover of tRNA, tritium-labeled thymine might find its way into DNA.

Excess thymine in tissues is catabolized to β-aminoisobutyric acid (βAIB), which is excreted in the urine. By taking advantage of the different pathway of synthesis of the thymines in tRNA and DNA, we have been able to show by differential labeling that βAIB has a dual origin; it stems from the degradation of thymine of DNA as well as thymine of RNA (*5*). Weber has shown that in rapidly growing tumor tissue the catabolic degradation of thymine to βAIB is greatly diminished (*6*). We have confirmed his in vitro observation by in vivo experiments. The excretion of βAIB—stemming from both DNA and RNA—by rats with rapidly growing Novikoff hepatoma diminishes. In turn, in those rats injected with [methyl-^{3}H]methionine, the tritium in thymine of DNA in the tumor tissue is significant, approximately 5 percent of the total thymine (*7*). This may stem from the deamination of 5-methylcytosine in DNA or from a salvage of tritium-labeled thymine from tRNA. The concomitant diminution of the excretion of βAIB suggests, but does not prove, the existence of such a salvage pathway.

Holliday and Pugh invoke a number of reversible modification mechanisms of DNA: two different DNA deaminases, two different DNA reaminases, DNA demethylases, and DNA methylases. Of these only the last one is unequivocally in the realm of reality. A number of groups of investigators, including my own group, have searched for DNA demethylases without success.

Nor are there demethylases for tRNA. This is the reason for the excretion of methylated purines and pyrimidines in the urine which result from the turnover of tRNA (*8*).

Model building of differentiating systems by developmental biologists can be stimulating; but we must bear in mind that even though of necessity the interaction of macromolecules must be invoked, biochemistry is still the ultimate arbiter of the validity of models.

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References

1. R. Holliday and J. E. Pugh, *Science* **187**, 226 (1975).
2. B. Sheid, P. R. Srinivasan, E. Borek, *Biochemistry* **7**, 280 (1968).
3. E. Scarano, M. Iaccarino, P. Grippo, E. Pansi, *Proc. Natl. Acad. Sci. U.S.A.* **57**, 1394 (1967).
4. L. R. Mandel and E. Borek, *Biochemistry* **2**, 555 (1963).
5. H. R. Nielson, K. E. Sjolin, K. Nyholm, E. Borek, *Acta Pathol. Microbiol. Scand.* **80**, 687 (1972).
6. G. Weber, S. F. Quiner, J. A. Ferdinandus, *Adv. Enzyme Regul.* **9**, 63 (1971).
7. H. R. Nielsen, K. E. Sjolin, K. Nyholm, B. S. Baliga, R. Wong, E. Borek, *Cancer Res.* **34**, 1381 (1974).
8. L. W. Mandel, P. R. Srinivasan, E. Borek, *Nature (Lond.)* **209**, 586 (1966).

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While we can agree with Borek's last sentence, we must point out that it would greatly impede biological research if every theory or hypothesis was discounted because of the lack of direct biochemical evidence. Again and again biochemical predictions have been made, which were only confirmed by direct evidence much later on. To give just three examples: the prediction of amino acid adaptors containing nucleotides predated the characterization of tRNA; numerous discussions of genetic repair mechanisms preceded the identification of repair enzymes, and the operon model of genetic regulation was proposed 5 years before the isolation of a protein repressor. Of course, numerous predictions have not been confirmed by subsequent biochemical observations, and it remains to be seen whether the interaction between DNA base sequences and specific modification enzymes we and others have proposed (*1*) actually does occur in eukaryotic organisms.

We do not think it particularly significant that deaminated adenine has not been detected in DNA. Since only a proportion of the DNA is transcribed and the transcription unit is very large in eukaryotes, a single modified base in a controlling sequence would constitute an extremely small proportion of the overall base composition, perhaps less than 0.01 percent. As Borek makes clear, the status of "minor thymine" derived from cytosine is controversial. With regard to specific modification enzymes, we pointed out that these might be extremely hard to detect, as the substrate would be a defined base sequence present in only one or a few copies in the total genome. Moreover, contrary to Borek's comment about the ubiquity of such enzymes, we would expect them to be confined to particular cell types or tissues, and in many cases for rather short periods of time during development. Finally, our article does explain that enzymes which demethylate bases are not essential for our general hypothesis, since methyl groups can also be lost by genetic replication. If such enzymes do exist, they may be found only in the germ line, or even just in meiocytes. For all these reasons, we strongly disagree with Borek's statement that some of the assumptions we make can be readily refuted by existing biochemical data.

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References

1. E. Scarano, *Adv. Cytopharmacol.* **1**, 13 (1971); R. Holliday and J. E. Pugh, *Science* **187**, 226 (1975); A. D. Riggs, *Cytogenet. Cell. Genet.* **14**, 9 (1975); M. Rossi, G. Augusti-Tocco, A. Monroy, *Q. Rev. Biophys.* **8**, 43 (1975); R. Sager and R. Kitchin, *Science* **189**, 426 (1975); D. C. Hawthorne, personal communication.

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Catalysis of NO + CO

In the report on the Japanese-American seminar on Prospects in Organotransition-Metal Chemistry (*1*) we are reported to have observed the catalytic conversion of CO and NO to CO_2 and N_2.

What we actually observe is the catalysis of the reaction

$$2NO + CO \rightarrow N_2O + CO_2$$

by solutions of dinitrosyl bis(phosphine) complexes of group VIII metals in *N,N*-dimethylformamide. We are attempting to determine a mechanism for the catalytic cycle, and to correlate trends in catalytic activity with trends in the structures of such complexes.

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References

1. Y. Ishii and M. Tsutsui, *Science* **188**, 1224 (1975).

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