Chemical Dosimetry in Somatic Cells and Its Utility to Mutagenesis

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In assessing the potential health hazard a given mutagen might present to man, a number of extrapolations must be made from the test systems which are currently available. For the L5178Y mouse lymphoma cell assay system that we use, these extrapolations include: somatic versus germinal cells; single cells versus whole animals; transformed versus “normal” cells; and mouse versus human cells. All of these factors represent potentials for ameliorating or enhancing the mutagenic effects of a given agent, especially by altering the extent of interaction between mutagen and DNA. By quantitating this interaction for the various test systems, the confidence with which mutagenic data can be meaningfully extrapolated to man is greatly improved.

Problems of dosage interpretation of mutagenic agents arise as a consequence of innate differences in the many different mutagen assay systems. Dr. Rall has outlined some of these problems earlier (1). It is important for us to realize that the interactions of a chemical agent with DNA in a test tube may not mimic the reaction that occurs in a mammalian cell. While the interaction itself may be interesting and educational, it may or may not relate to the actual mutagenic initiation event in an intact animal. Certain classes of environmental agents are known to interact either physically or chemically with DNA. Any foreign chemical which has an affinity for and a tendency to react with specific components of genetic material may possess the potential for causing a mutagenic event to occur.

Alkylating agents make up the largest group of chemicals which are known to act as mutagens. Included in this group are nitrogen, sulfur, and oxygen mustards; nitrosamines and nitrosamides; epoxides, lactones, aziridines, aldehydes, alkane sulfonic esters, dialkyl sulfates, and many related derivatives. Other chemicals which interact with DNA are a variety of antibiotics and mycotoxins, nitrous acid and nitrates, bisulfites, peroxides, acridines, heavy metal salts, and polynuclear aromatic hydrocarbons. In addition, some chemical compounds are metabolically converted to new compounds which can then react with DNA.

The half-life of a reactive compound further governs whether an agent can enter the nucleus in sufficient quantities to interact with the genetic apparatus. For instance, if the compound in question has a halflife of less than 1 min, the chance of the compound getting into a germ cell nucleus before reacting with extracellular or cytoplasmic nucleophils is relatively small.

Because of the variety of reaction options presented to a chemical agent entering a cell, it becomes extremely difficult to predict how an untested agent will react in any given mutagenic assay system. Since no single assay system now available can detect the whole spectrum of mutagenic effects, it

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December 1973
is important to study the interaction of an agent with cells and cell components in several assay systems. Increasing credence is being placed on mutation studies in somatic mammalian cells. This makes it necessary to compare and contrast the binding of chemical agents to cellular components in germ cells and somatic cells from the same species. The theoretical and practical attractiveness of some mammalian somatic cell culture assay systems has already been discussed in these proceedings.

The relationship between the number of mutagenic events induced and the number of insults visited upon specific cellular macromolecules by chemical compounds is a central one in the study of chemical mutagenesis. This relationship can be referred to as a dosimetric one. By performing well-designed dosimetry experiments, an investigator can determine how much of an administered chemical is actually reacting with DNA, RNA, or proteins (e.g., general proteins or specific ones such as histones or DNA polymerase). This short discussion will be restricted to the interactions of alkylating agents with DNA, although one should appreciate that alterations induced into the enzymes of DNA metabolism and replication can significantly alter the fidelity of replication. It has been reported recently (2) that mutations introduced into the gene for phage T4 DNA polymerase can cause the enzyme to act as a mutator (decreased fidelity), an antimutator (increased fidelity), or the mutation rate may be unaltered. While these observations concern mutations in the enzyme instead of nongenetic lesions (e.g., alkylation of an amino acid residue in or near an active-site), it is theoretically possible to understand how an alkylation of DNA polymerase in mammalian cells could result in an increased rate of mutation.

Nearly all of the dosimetric studies performed until now have been done in the name of chemical carcinogenesis; however, most of the experimental conditions apply themselves as well to chemical mutagenesis. Such studies require that the DNA be isolated free of all other cellular components, then degraded and chromatographed. These operations become necessary in order to ascertain to what extent certain chemicals have a tendency to react with specific components of DNA.

DNA has generally been degraded by some form of acid hydrolysis. However, it has been appreciated for at least a decade that some alkylated bases are dealkylated by the conditions necessary for hydrolysis to occur [deoxy-(0-6)-methylguanosine yields guanine on hydrolysis with perchloric acid] (3). In the presence of alkali, 1-methyladenosine is converted to N-6-methyladenosine (4); 2-amino-6-methoxy purine is dealkylated at pH 1, 100° C (5). These are simply examples to illustrate that no single-step chemical method of degrading DNA is likely to yield all of the products of alkylation.

An encouraging development in the hydrolysis of DNA has been the introduction of controlled enzymatic hydrolysis methods. Treatment of DNA with DNase yields an oligonucleotide mixture; addition of snake venom phosphodiesterase or lambda exonuclease (6) yields 5'-mononucleotides, further incubation with bacterial alkaline phosphatase yields nucleosides from the mononucleotide-5'-phosphate mixture (7). A complicating factor in the use of enzymatic hydrolysis is the evidence that some chemically altered DNA may be partially resistant to enzyme degradation (8).

The \( R_f \) values and spectral properties of a large number of DNA hydrolysis products and base derivatives have been determined for various solvent systems (7,9). The basic methods now available for the separation of nucleotides are paper chromatography (9–11) and column chromatography (9,12) [e.g., Dowex-1 (13,14), DEAE-cellulose, 7M urea (15,16), DEAE-Sephadex, 7M urea (17)]. Nucleosides can be separated by chromatography on columns of Dowex-50 (ammonium form) (18–20), kieselguhr (aqueous and organic solvents) (7), Sephadex LH–20 (organic solvents) (21), and Sephadex G–10 (water or buffers) (18). Sephadex G–10 (22,23) and Dowex-50 (H'-form eluted with 2N HCl) (18,24) are the
methods of choice for separating bases. None of these chromatographic techniques will provide absolute separation of all the altered bases that might occur in alkylated DNA. However, a prudent combination of the more effective methods should allow detection of most of the altered bases as they exist in the cell.

The chromatographed products are usually detected and identified by their ultraviolet absorption properties in the 210–350 nm range. Radioactively labeled mutagens provide greater sensitivity for the detection and identification of small quantities of DNA products. Of course, this technique requires that the labeled moiety of the mutagen becomes chemically bound to a component of the NA. Sugimura et al. (25) used doubly-labeled \(^{3}H\)-methyl-\(^{14}C\)-guanidine (MNNG) in \textit{in vitro} binding experiments to macromolecules. The \(^{3}H\)-methyl was preferably bound to DNA and poly A (low for RNA and poly U) and the \(^{14}C\)-guanidine was preferably bound to proteins (histone > cytochrome C > RNase > globulin). Lijinsky et al. (26) employed mass spectroscopy to determine that dimethylnitrosamine (labeled with deuterium in the methyl group) injected into rats produced 7-methylguanine in liver DNA in which the methyl group was an intact CD\(_{3}\). This provided proof that diazomethane is not the methylating species derived from dimethyl

nitrosamine as had been suggested.

Investigators have used combinations of these various methods in an effort to produce, isolate, and identify those altered bases which may be directly responsible for the initiation of a carcinogenic event. The answers can be applied as well to questions related to anomalous base pairing leading to a mutagenic event.

The major products of DNA alkylation are generally 7-alkylguanine, 3-alkyladenine and 7-alkyladenine; a number of other minor alkylated base products occur, the respective levels varying with the alkylating agent and the exposure conditions.

Lawley and Shah (18) examined the alkylation products of methyl nitrosourea (MNU) and dimethyl sulfate (DMS) in RNA \textit{in vitro}. They found that 7-methylguanine is the major product of reaction with both compounds but the distribution of minor methylation products differs significantly. The principle minor product of DNA alkylation by MNU and MNNG is 0–6-methylguanine; however, it is not detected upon alkylation with DMS. The principal minor products from methyl methanesulfonate (MMS) and DMS alkylation are 1-methyladenine and 3-methylcytosine (27). Even though the 0–6 atom of guanine is involved in hydrogen bonding, the relative degree of 0–6 alkylation in DNA is about twice that in RNA. Loveless (28) reacted MNU with deoxyguanosine and determined that the O–6 methyl derivative accounted for about 10% of the total alkylation products; the ethyl nitrosourea (ENU) reaction product yielded three to four times as much O–6-ethylguanine. When MMS, ethyl methanesulfonate (EMS), and DMS were reacted with deoxyguanylic acid, only EMS yielded the O–6 alkyl product. Loveless (29) had earlier determined that whereas both MMS and EMS produce 7-alkylguanine derivatives in the DNA of T-even phages, only EMS is mutagenic. However, both MNU and ENU were found to be mutagenic in T2 phage (30). These data led Loveless to hypothesize that since the 1-position of guanine no longer bears a proton after O–6-alkylation, such a reaction fosters anomalous base pairing.

Several investigators have since found O–6-alkylguanine after treating cells with alkyl-nitrosamides, alkyl-nitrosamidines and EMS. Lawley and Thatcher (5) found the N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) treatment to yield about 7% O–6-methyl guanine both \textit{in vitro} and in mouse cells, whereas DMS did not yield that product. MNNG is known to induce mutations in \textit{Neurospora}, 81% of which exhibit nonpolarized complementation patterns, indicating the probable presence of a complete polypeptide chain; among MMS-induced mutants the figure is only about 31% (31). MMS also induced chromosome abnormalities, whereas MNNG did not. These data show that MNNG

December 1973
acts as a specific inducer of point mutations, indicating that MNNG and other alkylnitro-
samides and alkylnitrosamidines, as well as EMS, may have different reactive specificities
than MMS and DMS.

The $S_1$ chemical reaction mechanism in-
volves the production of a reactive carbon-
ium ion, whereas the $S_2$ reaction mechanism
involves the formation of a transition com-
plex between the nucleophilic and the elec-
trophilic species. $S_1$ type reagents attack
chemical groups independently of their nucleophilicity; $S_2$ reagents are increasingly
reactive toward more nucleophilic groups.
The sites in DNA generally attacked by MMS and DMS are groups of known high
nucleophilicity. Since the $S_1$ reaction is
more indiscriminate in its reactive target,
it may well be that alkylation of the O-6
of guanine is indicative of an agent which
reacts by the $S_1$ mechanism (5). The alky-
ation of the O-6 of guanine in DNA could
therefore be related to the high mutagenic
efficiency of $S_1$ type alkylating agents. The
presence of 3-methylguanine residues in
DNA treated with MMS and DMS provides
a possible answer for the mutagenicity of $S_2$
alkylating agents, since 3-methylguanine is
a potentially mispairing base (32).

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Environmental Health Perspectives

198
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