ENVIRONMENTAL PATHOLOGY

Edited by

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Preface

It has long been recognized that the occurrence of most diseases results from the interaction of an exogenous etiologic agent with a susceptible individual. Perhaps it was the recognition of this fact that led Rudolf Virchow (1821–1902), the scientist who first related clinical disease to cellular pathology, to devote so much of his time to the development of public health laws in Germany. It was his view that "the ultimate task of medicine is the constitution of society on a physiological basis." Even at the time of Virchow's death, seven of the ten leading causes of death in the United States of America were infectious (biotic) agents (bacterial, viral, parasitic, or fungal). After the battle against infectious diseases was won by public health measures, improved nutrition and living standards, and antibiotics in medicine, the leading causes of fatal diseases in industrial countries changed dramatically. Chronic diseases came to the fore, especially cancer and cardiovascular disease, and questions were raised about environmental and occupational exposures to chemical and physical agents. Population growth and the development of industrial structures sufficient to support the population increased exposures to unusual concentrations and new forms of chemicals as well as radiation.

The goal of this book is to bring together for the first time a comprehensive synthesis of information about the increasingly important non-biotic etiologic agents of human disease. The book is intended to serve as a text and reference for pathologists, toxicologists, veterinarians, occupational physicians, and environmental health scientists seeking further understanding of the role of environmental chemical and physical agents in the pathogenesis of human disease. Much of the relevant information has developed within these sciences.

Because of the diverse origins of the book's subject matter, the contributing authors include toxicologists, pathologists, veterinarians, clinical physicians, and environmental health scientists. All have emphasized human disease and have sought to make their disciplines understandable to others in related fields. The authors' occupations include industrial, governmental, research, and administrative positions, which attests to the breadth of this new field.

Although this is a "pathology" text, it deals as much with subcellular and molecular effects of chemical and radiation exposures as with the more conventional histopathology. Etiology and pathogenesis are the focus rather than differential diagnosis. The book is divided into two parts: (1) four

chapters that present pathologic processes affecting the body as a whole and (2) ten chapters based on organ systems. It is hoped that this organization will enable the reader to quickly find sought-after information.

Even on brief perusal of the book, it will be apparent that the state of knowledge about environmental pathology varies considerably from one organ system to another. The disease processes caused by environmental chemicals in the respiratory, urinary, and nervous systems have been much more extensively studied than those of the immune and gastrointestinal systems. I was unable to justify chapters on the endocrine and musculoskeletal systems and pancreas because of similar limitations.

Almost a decade ago I organized and began teaching a course in environmental pathology at the University of Washington. It was taken by medical, dental, and public health students, as well as graduate and postgraduate students in the environmental sciences. Several of the authors have participated in the course. The approach the book takes is, in part, an outgrowth of our experience teaching the course. I am most grateful for the many useful suggestions made by the students. Their positive response to the course served as a stimulus to develop the book.

Finally, I wish to thank all of those who contributed to this book as chapter authors and in other ways. My wife, Nancy, assisted greatly in the preparation of the prospectus and draft outlines of the overall plan, format, and index. Mr. Ralph L. Body spent much of his spare time doing library searches to confirm references and develop background information. Mrs. Maureen K. Levell worked on the organization, manuscript development, and galley and page proofs. Many others contributed in different ways. To all who helped I express thanks.

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Contents

I

General Reactions to Injury by Environmental Agents

1

The Molecular Basis of Environmental Mutagenesis

John R. Silber and Lawrence A. Loeb

Concern has been mounting during the last decade about the possible deleterious effects of the introduction of an increasing number of exobiotic substances into the environment. It has been estimated that approximately 50,000 chemical compounds are presently used for industrial and domestic purposes, and that this number is increasing by 1000 new compounds yearly (Ames, 1979). The growing ubiquity of these compounds can be appreciated when it is realized that the introduction of many of them into the environment, either as manufactured goods or polluting waste products, is measured in the millions of pounds.

The toxicologic properties of most of these compounds as well as many natural substances are only now beginning to be evaluated. It has been found that many of these compounds as well as ionizing and ultraviolet radiation from man-made and natural sources can chemically alter DNA. The alterations produced, called DNA damage, can lead to two deleterious consequences. First, the damage can grossly interfere with the essential functioning of DNA to such an extent as to cause cell death. Second, in some instances the damage is an intermediate in the production of mutations. This is an immediate concern, because mutations have been implicated in the pathogenesis of many inherited and somatic human disease states. The danger to human health from mutagenic environmental agents is best perceived in their possible etiologic role in the causation of cancer. It is currently argued that the majority of human cancers are due to environmental factors. The finding that many mutagens are also carcinogens has increased the trepidation about the increasing presence of toxicologic agents in the environment. More importantly, mutations in germ cells cause deformations at birth and increase the incidence of inherited disease. These consequences of mutagenesis are more costly to society than diseases such as cancer that primarily afflict older individuals.

This chapter will introduce mechanisms by which environmental agents can produce mutations. Special emphasis will be placed on the repair of DNA damage, because much mutagenesis by exogenous agents in experimental systems has been found to be the result of deficient or faulty DNA repair. Possible mechanisms of mutagenesis due to alterations in the fidelity of DNA replication by exogenous agents also will be detailed.

The Primacy of DNA

In order to define clearly what a mutation is, it is first necessary to elucidate briefly

some of the structural and functional properties of DNA molecules.

DNA is an informational molecule. It contains all of the instructions necessary to define the total behavioral repertoire of a cell. Considering its important role, DNA is a surprisingly simple molecule composed of two strands wound about each other forming a double helix. Each strand of the helix is made up of only four deoxyribonucleotides covalently bound together by phosphodiester linkages. The nucleotides differ by containing either a purine base, adenine or guanine, or a pyrimidine base, thymine or cytosine. The two strands are held together by weak hydrogen bonding between complementary base pairs. As shown in Figure 1-1, the complementarity of the bases is highly specific, with adenine hydrogen bonding with thymine, and guanine bonding with cytosine. Complementarity enables a DNA molecule to act as a template to direct its faithful replication by DNA polymerases and the synthesis of RNA transcripts by RNA polymerases.

The information in a DNA molecule is contained in the linear sequence of its nucleotides on each strand. For this information to be biologically expressed, the sequence of nucleotides of a gene must be converted into the sequence of amino acids of a protein. The translation of the one sequence into the other requires a complementary RNA transcript of the gene to direct the protein-synthesizing machinery of the cell and a genetic code that allows only four different nucleotides to specify for the 20 amino acids from which proteins are constructed. The genetic code is composed of sets of three nucleotides, call triplet codons, each of which corresponds to an amino acid. Of the 64 possible codons, 61 specify an amino acid, which necessitates some amino acids being represented by more than one codon. The three remaining codons code for no amino acids and act as stop signals to denote the termination of an amino acid chain.

The primacy of DNA in the scheme of life should be apparent from this short discussion. In its nucleotide sequences resides the information to build the characteristic amino acid chains of the proteins whose enzymatic and structural properties determine all cellular behavior. The template properties afforded to DNA by base pair complementarity allow this information to be accurately expressed as well as duplicated for future generations. The dominant role that DNA plays in the expression and perpetuation of life, however, designates it as the critical target for the action of mutagenic environmental agents.

Mutations

A mutation is a change in the nucleotide sequence of a gene. The change can come about by one of two processes, which are outlined in Table 1-1.

Line A of Table 1-1 lists the nucleotide sequence of a hypothetical gene coding for a protein composed of six amino acids. The sequence has been grouped as triplet codons with the abbreviations for the corresponding amino acids listed beneath them. A simple way to create a mutation in this sequence is to substitute guanine for cytosine in the third triplet; the codon for the amino acid leucine has been converted into that for phenylalanine. The simple act of replacing one nucleotide in the sequence with another has resulted in an alteration of the amino acid sequence of the protein. Another consequence of a single nucleotide substitution is shown in line C. When the adenine at the second position of the third codon is replaced with thymine, a premature stop signal is introduced into the gene,

Table 1–1. Possible Modes of Nucleotide Sequence Mutation

(A)	TAC	AGA	AAC	TGT	GTG	TGT	ATC
	MET	SER	LEU	THR	HIS	THR	STOP
(B)	TAC	AGA	AAG	TGT	GTG	TGT	ATC
	MET	SER	PHE	THR	HIS	THR	STOP
(C)	TAC	AGA	ATC	TGT	GTG	TGT	ATC
	MET	SER	STOP	THR	HIS	THR	STOP
(D)	TAC	AGA	AAC	GTG	TGT	GTA	TC
	MET	SER	LEU	HIS	THR	HIS	—

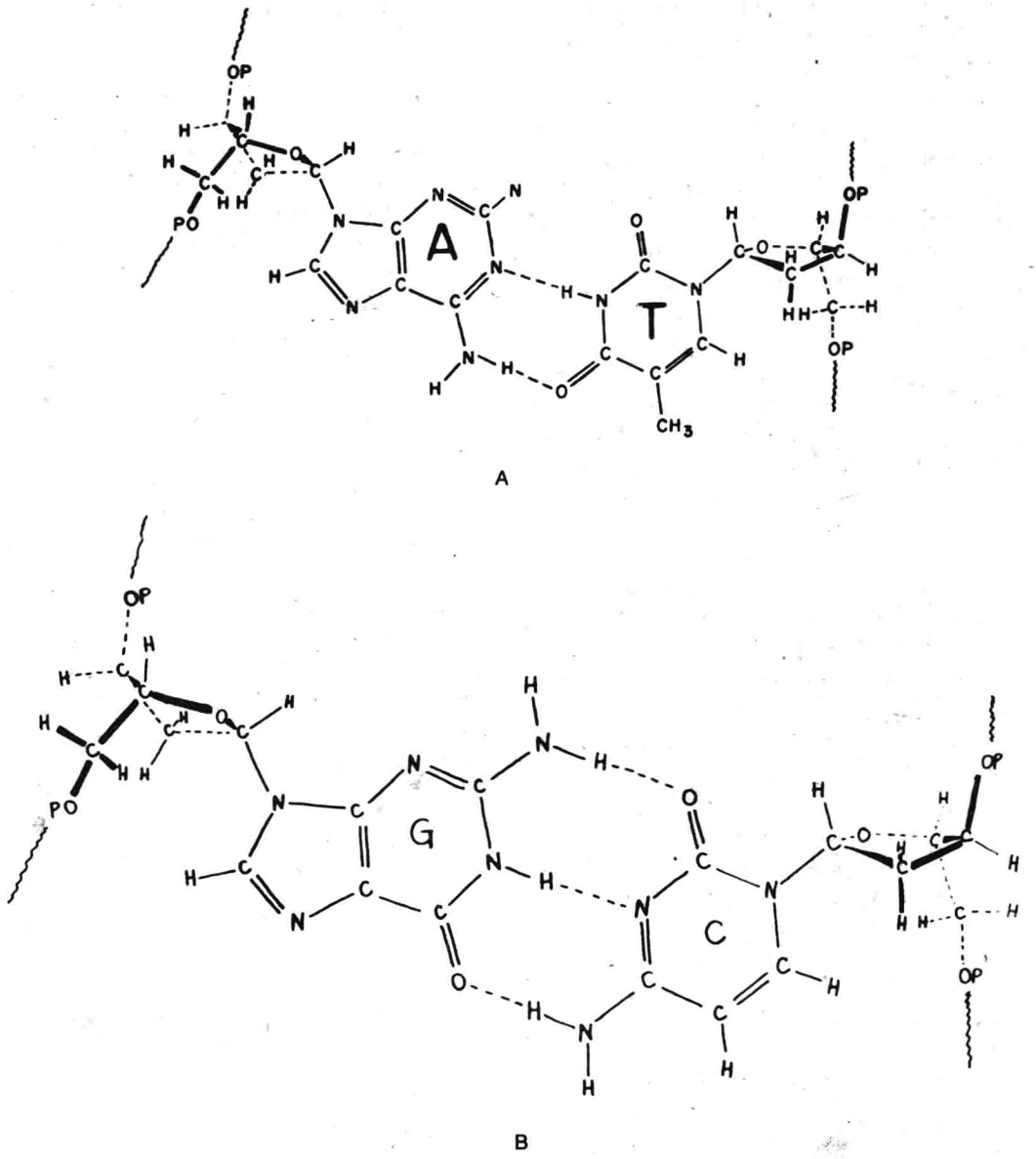

Figure 1–1. Complementary base-pairing (A) between adenine and thymine, and (B) between guanine and cytosine.

which now codes for a trucated protein of only two amino acids.

The other way to produce a mutation is to alter the frame in which the nucleotides of a gene are read as triplet codons. Frameshift mutations are created by adding or deleting one or more nucleotides in the sequence of the gene. The result of deleting the thymine in the first position of the fourth codon from the nucleotide sequence coding for the hypothetical protein of Table 1-1 is shown in line D. With one less nucleotide, the reading frame of the gene after the third codon will be shifted to the right to re-establish a sequence of codons. The result of this is to change the sequence of the final three amino acids of the protein, and to destroy the termination codon.

Although a mutation is always produced when the nucleotide sequence of a gene is changed, all changes are not biologically expressed. Mutations may be silent. This is most easily understood for single-base substitutions. The substitution of one amino acid for another may have no effect on a protein if the change has no effect on its functional properties. The same may be true for substitutions that produce premature termination signals if the abbreviated amino acid chain can still form a biologically active protein. It is also possible that a base substitution will convert one triplet into another that codes for the same amino acid. Frameshift mutations can be unexpressed if the addition or deletion of a limited number of nucleotides at one position can be compensated by a similar event elsewhere in the nucleotide sequence, which will reestablish an amino acid sequence that is still biologically active.

There are no absolute rules that limit the changes that can occur to a sequence of nucleotides. A purine nucleotide may be substituted for another purine, or may be substituted for by a pyrimidine nucleotide. Frameshift mutations may be the result of the addition or deletion of a single nucleotide or many. In some instances, a significant fraction of a chromosome may be relocated from one position to another. The addition to or the loss from the genome of an entire chromosome is also possible. Regardless of how a change is effected, it is conserved and is normally propagated from one generation to the next.

Considering the permanency of mutations, it is of interest to know how frequently they occur. During normal DNA replication, spontaneous single-nucleotide changes are estimated to occur at a frequency of less than one for every 10^9 nucleotides replicated (Loeb et al., 1979). Alteration of the nucleotide sequence of DNA, therefore, is an exceedingly rare event, and under normal circumstances, the sequence is highly conserved. However, there are environmental agents that interact with DNA and increase the frequency of mutagenesis by orders of magnitude. These agents are mutagens and are the topic of the next section.

Mutagens and DNA Damage

The number of mutagenic agents from both natural and man-made sources in the environment is enormous. Table 1-2 lists a variety of mutagens that might be encountered in everyday life. From this list, it is apparent that even seemingly innocuous endeavors can bring one in contact with a great diversity of mutagenic substances.

Mutagens vary greatly in their form and mutagenic potential. They are classified somewhat arbitrarily as either physical or chemical in nature. Examples of physical mutagens include heat, ultraviolet light, and ionizing radiation such as X rays and γ rays. Chemical mutagens include substances as simple as inorganic salts of metals to complex polycylic aromatic compounds. Regardless of their heterogeneity, most mutagens (but not all) share a common property of being able to interact chemically with DNA. The production of new chemical structures in a DNA molecule, DNA dam-

Table 1–2. Common Environmental Mutagens

Mutagen	Source
Ultraviolet light	Sunlight
Ionizing radiation	Cosmic rays, medical X rays
Aflatoxin B_1	Fungi-contaminated peanuts and grains
Flavonoids (quercetin kaempferol)	Fruits and vegetables
Chloroform	Chlorinated water
Nitrosamines	Beer and whiskey
Pyrolysis products of tryptophan	Broiled meat
Nickel	Metal alloys, mines
Saccharin	Sweeteners
Hydrazine	Cigarettes and wood smoke
Benz[*a*]pyrene	Cigarettes and wood smoke
Vinyl chloride	Plastics
Benzidine	Textile dyes; manufacture of leather and paper
2-Naphthylamine	Textile dyes; manufacture of leather and paper
Imuran	Anticancer drugs
Nitrogen mustards	Anticancer drugs
Cyclophosphamide	Anticancer drugs

age, can have profound effects on its biologic functions.

The variety of DNA damage produced by mutagenic compounds is extremely large. This reflects the large number of mutagens that react with DNA and the diversity of products that result from these reactions. Experimental evidence suggests, however, that alterations of the purine and pyrimidine bases of DNA are responsible for most of the biologic consequences of DNA damage. Five classes of altered base damage, along with mutagens that produce them, are discussed below (Setlow and Setlow, 1972; Cerruti, 1975; Miller, 1978; Lindahl, 1979; W. Harm, 1980).

1. *Small adducts.* Some chemical mutagens such as methylmethane sulfonate, dimethylnitrosamine, and ethylnitrosourea can transfer a short aliphatic chain to a base. Figure 1-2A shows two possible adducts that are formed when dimethylnitrosamine reacts with guanine. A single methyl group from the mutagen is covalently bound to either the N-7 or 0-6 position of guanine. The addition of a small group to a base may cause it to be unable to hydrogen-bond properly with its complementary base. Usually, however, a small adduct will create only a minor distortion in the double-helical structure of DNA (Cerruti, 1975). Physical mutagens are also capable of producing small adducts in bases. One example is shown in Figure 1-2B, in which the double bond of thymine has been saturated by water. This type of damage can be induced by ultraviolet light and γ rays.

2. *Large adducts.* Other chemical mutagens including benz[a]pyrene, aflatoxin B_1, and 3-methylcholantherene can add large chemical structures to bases. This is shown in Figure 1-3A and B, in which aflatoxin B_1 and benz[a]pyrene have formed adducts at two different positions on guanine. The addition of such a large chemical moiety cannot be accomodated by the DNA helix. Such lesions destroy the base-pairing specificity of the altered base and disrupt hydrogen bonding of adjacent base-paired nucleotides (Cerruti, 1975).

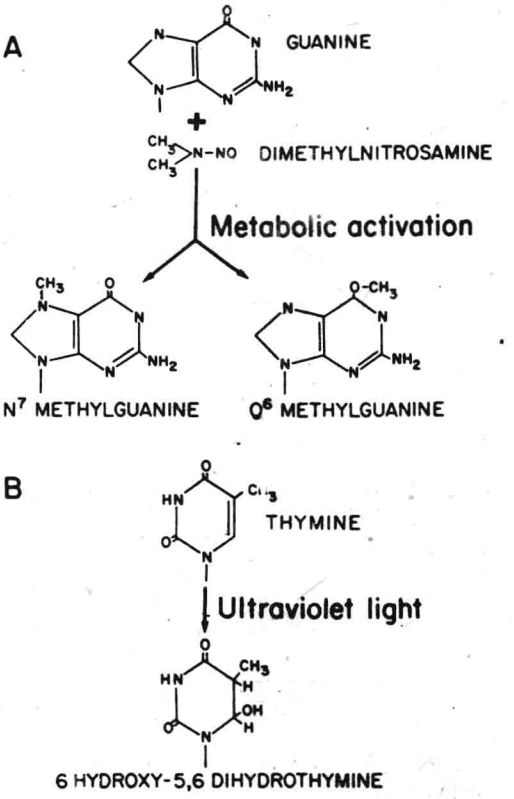

Figure 1–2. Small adducts produced by chemical and physical mutagens. (A) Methyl group adducts at the N-7 or O-6 positions of guanine caused by reaction with metabolically activated dimethylnitrosamine. (B) Saturation of the double bond of thymine with water, induced by ultraviolet light.

Notice in Figure 1-3 that the original chemical structure of benz[a]pyrene has been modified prior to its binding to guanine. Many chemical mutagens will not react with the DNA unless they are first metabolically activated into a more electrophilic or electron-seeking compound (Miller, 1978). The activated compound will readily react with the many electron-rich nucleophilic sites on a DNA molecule. Metabolic activation is performed by cellular enzymes, which normally detoxify the cell of endogenous and exogenous substances. There are a variety of activating enzymes generically referred to as mixed-function oxidases, which are found in a great many cell types. The activation of chemical mutagens is a consequence of these

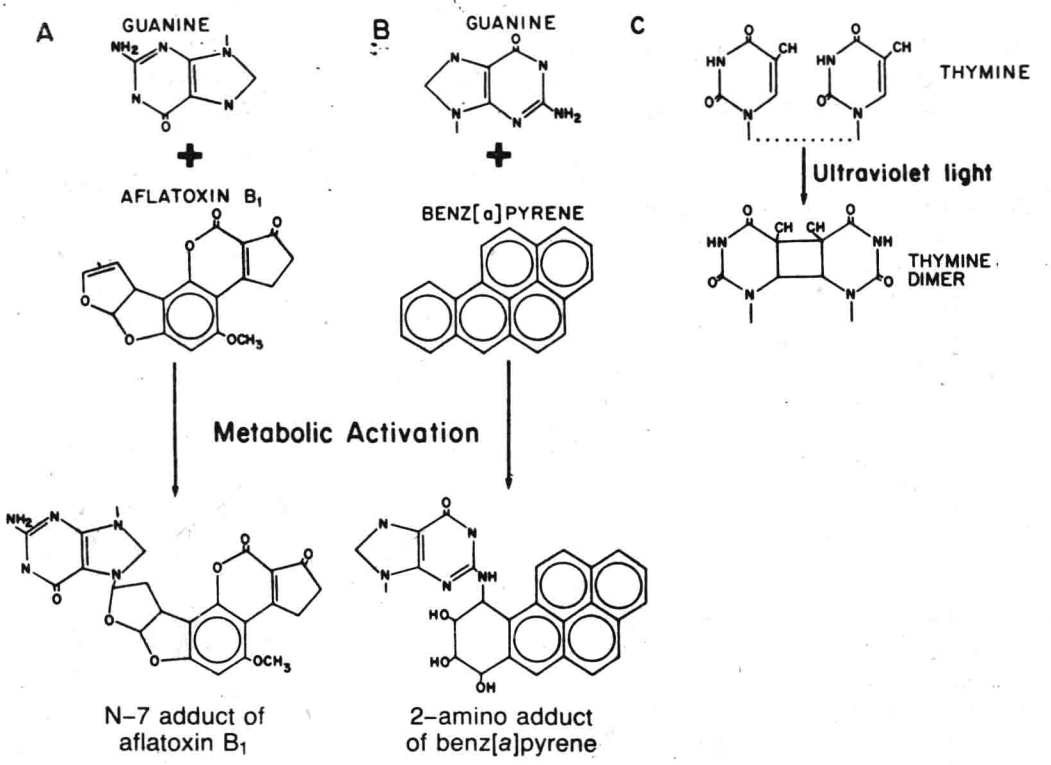

Figure 1–3. Large adducts produced by chemical and physical mutagens. (A) Adduct of metabolically activated aflatoxin B_1 at N-7 position of guanine. (B) Adduct of metabolically activated benz[*a*]pyrene at the 2-amino position of guanine. (C) Ultraviolet light–induced thymine–thymine dimer.

enzymes performing the normal detoxification of the cell.

Physical mutagens also are capable of producing large adducts. The classic example in this case shown in Figure 1-3C is the ultraviolet light–induced pyrimidine dimer (Setlow and Setlow, 1972). In this example, adjacent pyrimidine bases on the same strand of DNA undergo a reaction that covalently binds each to the other. The pyrimidine dimer is a model example of a large adduct and is the most studied lesion of this class of damage.

3. *Cross-links.* Certain chemical mutagens, such as mitomycin C, nitrogen mustard, and light-activated psoralins can link one chemical moiety to two bases on opposing strands of a DNA molecule. Cross-links covalently bind the two strands together and create large distortions in the structure of the double helix, although the exact structure of the strand-to-strand DNA cross-links is not known (W. Harm, 1980).

4. *Nucleotide analogs.* These lesions are produced when base analogs such as 5-bromouracil and 2-aminopurine are incorporated in the place of thymine and adenine, respectively, during DNA replication. Such analogs may also arise via the deamination by heat or chemical treatment of cytosine or adenine to produce nucleotides containing uracil or hypoxanthine (Lindahl, 1979). Many analogs produce noncomplementary base-paired nucleotides in DNA that result in only minor distortions of the double helix.

5. *Missing bases.* Depurination, the loss of the purine bases adenine and guanine from a DNA strand, probably is the most frequently occurring type of DNA base damage. It is the result of the breaking of the glycosylic linkage between the purine base

and the deoxyribose moiety of the nucleotide. This damage is very stable, having a half-life of 200 hours. Heat is a causative agent of depurination. It is estimated that at physiologic temperatures, as many as 10,000 purines may be lost from the genome of a mammalian cell in one generation (Lindahl, 1979). Furthermore, the addition of alkyl and large chemical groups can increase the rate of depurination 1000 to 10,000 times. It is possible that depurination is the common fate of many diverse types of adducted bases. Depyrimidization also occurs but at a rate 100- to 1000-fold less than that of depurination.

From the standpoint of biologic effect, it is useful to reclassify DNA base damage as consisting of either blocking or nonblocking lesions (Cerruti, 1975). Blocking lesions cause large distortions in the DNA molecule, such as those produced by the benz[a]pyrene adduct of guanine or by ultraviolet light–induced dimers. These lesions interfere with the template function of DNA by blocking the progress of DNA polymerases during replication and RNA polymerases during transcription (Moore et al., 1981). It has been proposed that DNA polymerases are blocked by this type of lesion as a result of the damaged base not being able to properly base-pair with any other nucleotide (Radman, 1974, 1975; Radman et al., 1978). During synthesis, the DNA polymerase randomly incorporates a nucleotide opposite the noncoding damaged nucleotide in the template strand. Before the polymerase continues its synthesis, a proofreading function excises the newly incorporated nucleotide (Burtlag and Kornberg, 1972). The role of the proofreading function is to guarantee that during DNA synthesis each newly incorporated nucleotide is properly hydrogen-bonded with its template complement before replication can continue. Because any nucleotide incorporated opposite the noncoding damaged base will not be correctly base-paired, the proofreading function removes it. The polymerase then incorporates another nucleotide at the same site, only to have it also excised by proofreading. Evidence for such a mechanism has come from studies with bacteria.

Whereas the blockage of RNA polymerase at the damaged site in the gene may be absolute, the DNA polymerase will only hesitate temporarily at the site of the blocking lesion before skipping over it to reinitiate synthesis farther along on a template strand (Hanawalt et al., 1979). This produces a single-strand gap as large as several thousand nucleotides in the newly synthesized DNA molecule. The gap represents lost genetic information. The DNA strand opposite the gap is also susceptible to being broken and degraded by nucleases. This will cause the loss of the genetic information in this DNA strand, disrupt the physical continuity of the DNA molecule, and possibly inhibit the coordinate expression of genes on the two separated pieces of DNA. The fragmentation of the DNA molecule is assured if the gapped strand is used as a template for subsequent DNA replication. The presence of a single-strand gap, therefore, can be of very grave consequence to a cell.

Nonblocking lesions do not prohibit synthesis by DNA and RNA polymerases (Saffhill, 1974). These lesions are usually the result of the incorporation or formation of base analogs in a DNA molecule or are the result of the addition of small groups to a base. Some small adducts are innocuous and produce no biologic consequences. Other adducts as well as nucleotide analogs change the base-pairing specificity of the damaged base. This can have two consequences. The first is unfaithful gene expression during transcription. The presence of a miscoding lesion in the DNA sequence of a gene will change the sequence of its messenger RNA transcript (Singer and Kröger, 1979). The transcript will direct the synthesis of an altered protein that could have deleterious effects on normal cellular behavior. The second consequence comes into being when the DNA strand containing the miscoding lesion is replicated. The newly synthesized DNA strand will contain a nucleotide that is complementary to the miscoding lesion rather than to the original, undamaged base. Thus, an altered nucleotide sequence is cre-