Molecular Biology of Mutagens and Carcinogens

B. Singer

and

D. Grunberger

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B. Singer
University of California at Berkeley
Berkeley, California

and

D. Grunberger
College of Physicians and Surgeons of Columbia University
New York, New York

PLENUM PRESS • NEW YORK AND LONDON

Library of Congress Cataloging in Publication Data

Singer, B. (Beatrice), date–
Molecular biology of mutagens and carcinogens.

Bibliography: p.
Includes index.
1. Carcinogens. 2. Chemical mutagenesis. 3. Molecular biology. I. Grunberger, Dezider 1922– . II. Title.
RC268.6.S56 1983 616.99'4071 83-17683
ISBN 0-306-41430-9

Preface

This book originated in numerous Gordon Research Conferences and many other meetings of scientists working in chemistry, biophysics, biochemistry, and biology related to mutagenesis and carcinogenesis. It seemed the appropriate time to sit back and summarize the results of several decades of research in laboratories in different countries.

We are very grateful to the Rockefeller Foundation for inviting us to formulate and begin writing the book at the Center for International Studies in Bellagio, Italy, where we were Resident Scholars.

We are fortunate to have had the assistance of so many colleagues around the world who cheerfully sent original data, figures, and preprints and listened patiently to us as we worked out the various conflicting ideas in this fast-moving field. The names of these scientists are found within the tables, figures, and references.

There is one person whose contributions we especially wish to acknowledge. Professor Heinz Fraenkel-Conrat was present at the inception of this book and throughout the writing encouraged and criticized in approximately equal proportion. Finally, his editing and amalgamation of our two styles gave us great comfort.

B.S.
D.G.

Contents

I

Introduction

Cancer, as an ultimate result of exposure to chemicals, has been described for at least 200 years and it has probably existed as long as man. When the span of human life was short due to epidemic disease, war, and famine, cancer was not a major concern. However, as most causes of early death have been conquered, cancer emerges as one of the most common causes of death in late life. Evaluation of the extent to which exposure to, or ingestion of, chemicals causes cancer is not the purpose of this book. Rather, we will examine what is known of the chemical and biochemical reactions of different classes of carcinogenic chemicals, or their metabolites, with cellular components. This study will focus primarily on nucleic acids, and most specifically on the mechanism of the initiation stage of carcinogenesis. Initiation in this context is the term for the covalent binding of a reactive group to a nucleotide in an informational macromolecule. For this reason, reaction with other cellular components such as proteins, lipids, and polysaccharides is not included.

However, following this initial binding, there are many factors that influence whether the nucleic acid modification will lead to cancer. Among these factors are repair, promotion, inhibition, and genetic susceptibility. Thus, cancer is termed a multifactorial disease. In spite of the multifactorial and multistage character of cancer, this book will be restricted to the initial events in the process of carcinogenesis and mutagenesis. Enzymatic repair of adducts in eukaryotic cells soon after initiation is one other area where the biochemistry is now advancing, and it is the subject of a chapter.

While the aim of understanding cancer is to prevent it in man, much of the data to be discussed use simpler models, such as nucleosides, synthetic polynucleotides, or natural nucleic acids. Information gained in this way is then used to design experiments in the more complex systems represented by bacteria and mammalian cells in culture, and by whole animals.

It is generally accepted that ultimate carcinogens, whether metabolites or directly acting, are mutagens. That is, using one or more of a variety of test systems, the chemical can be shown to cause a heritable change in the genetic material. However, many effective mutagens, such as sodium nitrite, have not at this time been found to be carcinogenic. This may be due to the fact that mutation can be achieved by direct modification of a biologically

active nucleic acid *in vitro*, while carcinogenesis is the end result of a long series of biological processes.

Mutagenesis, whether directly caused by chemicals or as an intrinsic property of nucleic acids, is an inescapable event. Certainly in the context of Darwinian evolution, survival of the fittest implies that mutation can confer a genetic advantage, as well as disadvantage. However, the mutations we observe are usually detected as errors. Genetic diseases that can be shown to result from mutation are by no means uncommon and it has been estimated that as many as 2% of live births include dominant, recessive, X-linked, or chromosomal abnormalities. It has been established biochemically that a single amino acid exchange in hemoglobin is responsible for sickle cell anemia. There are also a considerable number of diseases resulting from the absence or near absence of an enzyme or a transport or receptor protein that are classified as inborn errors of metabolism. These include defects in DNA repair such as xeroderma pigmentosum, ataxia telangiectasia, and Falconi's anemia.

As was previously discussed for carcinogens, only certain well-studied mutagenic chemicals will be included. We will not consider intercalating agents, which are generally frameshift mutagens, ultraviolet or other radiation damage, nor the effects of base analogs.

Research in chemical modification of macromolecules and carcinogenesis has expanded enormously in the last decade. It therefore would be virtually impossible to cite all the relevant papers. Fortunately there are many excellent review articles and book chapters that summarize specific areas and contain references to the original literature. We will generally cite, at the end of each chapter, reviews and occasionally a particularly recent or fundamental paper. References to specific data are given in tables and figures. We hope that our colleagues and workers in this field will understand that this type of book cannot give due credit to all of these scientists who have contributed so much. Without their results, this book could not have been written.

II

Chemicals as Environmental Mutagens and Carcinogens

There has been a constant and dramatic increase in the number of chemicals synthesized and encountered in the environment. However, in spite of new chemicals accruing at the rate of 6000 per week, the number of chemicals in "common use" is estimated to be about 65,000 (of the greater than 4,000,000 known) and the number of chemicals for which there is evidence of carcinogenicity is remarkably small.

There is no estimate of the number of naturally occurring compounds in foods, drinks, seeds, nuts, etc. Some of the most potent carcinogens known, such as aflatoxin, safrole, estragole, and cycasin, are natural products. Aflatoxin has been classified as a human carcinogen in epidemiological studies in Africa where large amounts of peanuts contaminated with this mycotoxin are consumed. In other countries the level of exposure to aflatoxin and other natural carcinogens appears to be quite low, but not zero.

While concern has been expressed regarding the potential hazards of synthetic and natural chemicals in the environment, it is not generally realized that some of the best studied and most effective mutagens are synthesized in humans as the result of normal metabolic processes. These include nitrosamines and other *N*-nitroso compounds, bisulfite, and hydroxylamines, some in very considerable amounts. While the presence of these endogenous mutagens may well contribute to the incidence of cancer, they are unavoidable. Their normal occurrence *in vivo* makes the presence in the environment of low levels of such compounds of lesser importance than those that may be avoided if harmful.

The International Agency for Research on Cancer (IARC), which has as its function the continuous review of cancer-causing agents, has several categories of certainty in classifying chemicals as carcinogens. It must be remembered that this classification is based largely on epidemiology. Thus, the chemicals listed in Tables II-1 and II-2 are primarily industrial, or are widely used and many people may become exposed to them. Epidemiology does not deal with individuals but rather arrives at statistical data based on large groups

Table II-1. Chemicals or Industrial Processes Carcinogenic for Humans

Chemicals	Industries or industrial processes
Industrial chemicals	Manufacturing industries
Aromatic amines	15. Auramine
1. 4-Aminobiphenyl	16. Isopropyl alcohol
2. Benzidine	17. Boots, shoes, and other leather goods
3. 2-Naphthylamine	18. Furniture and cabinetry
Others	Other industrial processes
4. Benzene	19. Hematite mining (? radon)
5. Bis(chloromethyl)ether	20. Nickel refining
6. Vinyl chloride	21. Exposure to soots, tars, oils
Inorganic substances	
7. Arsenic and As compounds	
8. Asbestos	
9. Chromium and Cr compounds	
Drugs	
Alkylating agents	
10. Chlornaphazine	
11. Melphelan	
12. Mustard gas	
Hormones	
13. Diethylstilbestrol	
14. Conjugated estrogens	

Table II-2. Chemicals or Groups of Chemicals Probably Carcinogenic for Humans

High degree of human evidence	Lower degree of human evidence
Drugs	Drugs
1. Chorambucil	1. Iron dextran
2. Cyclophosphamide	2. Oxymetholone
3. Thio-TEPA	3. Phenacetin
Inorganic compounds	Industrial chemicals
4. Cadmium and Cd compounds	4. Acrylonitrile
5. Nickel and Ni compounds	5. Auramine
Food contaminant	6. Carbon tetrachloride
6. Aflatoxins	7. Dimethyl carbamoyl chloride
	Alkylating agents
	8. Dimethylsulfate
	9. Ethylene oxide
	Others
	10. Aminotriazole
	11. Polychlorinated biphenyls

of potentially carcinogen-exposed populations as compared to appropriate control groups.

The value of epidemiology has been shown by the striking correlation between the amount and duration of cigarette smoking and incidence of cancer of the lung and other squamous cell tissues. Predictably, the increase in such cancers in women, who became heavy smokers later than men, started decades after a similar increase in men. At this time, the slopes of the cancer rates of the two sexes are similar.

Figures II-1 and II-2 show some epidemiological data for a variety of cancers, and it is comforting that, notwithstanding our living in this "sea of chemicals," all but the cancers attributed to cigarettes are decreasing, or not increasing at a significant rate.

There are many interesting illustrations of the application of epidemiology to the identification of carcinogens and two are cited here. In an old study, the high incidence of stomach cancer in Japan was compared with that of Japanese in Hawaii (before statehood) and in the United States. It became evident that this incidence, which is the highest in the world, was lower for the Japanese who had moved to Hawaii and even lower for those who had moved to the United States. American-born Japanese have an even lower stomach cancer rate. Since the same racial group was being compared, the

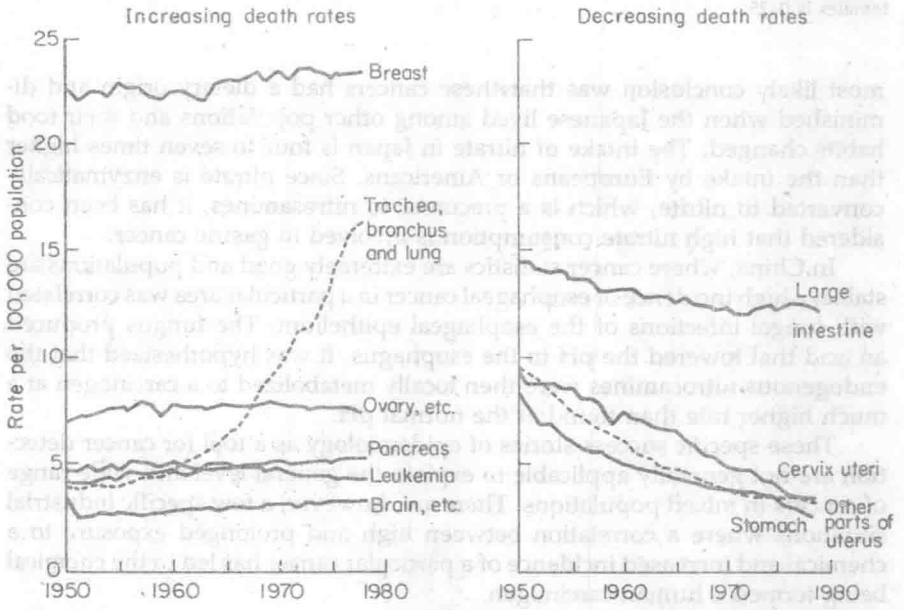

Figure II-1. Age-adjusted death rates for white females for leading sites of malignant neoplasms: United States, 1950–1980.

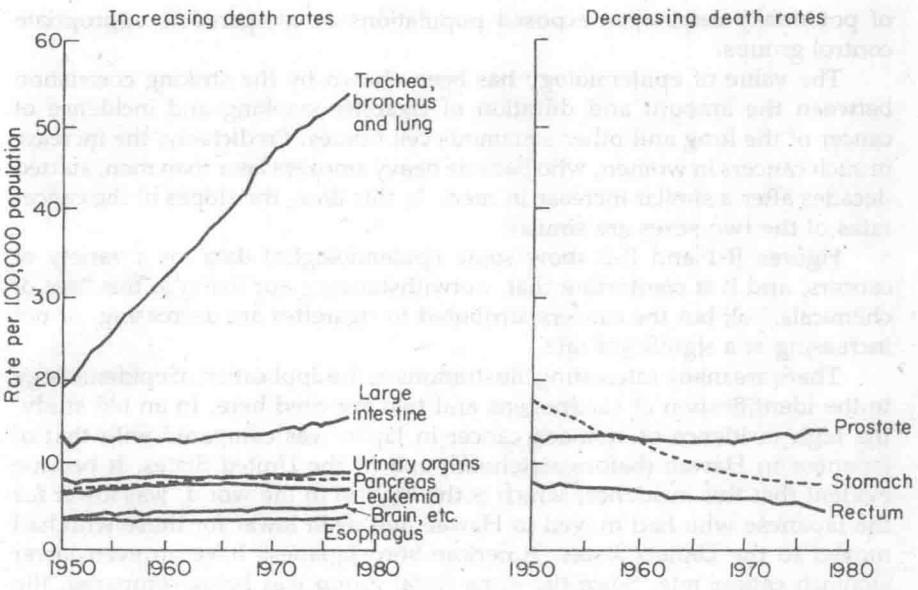

Figure II-2. Age-adjusted death rates for white males for leading sites of malignant neoplasms: United States, 1950–1980. Note that the scale for death rates for males is 0–60 while that for females is 0–25.

most likely conclusion was that these cancers had a dietary origin and diminished when the Japanese lived among other populations and their food habits changed. The intake of nitrate in Japan is four to seven times higher than the intake by Europeans or Americans. Since nitrate is enzymatically converted to nitrite, which is a precursor to nitrosamines, it has been considered that high nitrate consumption is involved in gastric cancer.

In China, where cancer statistics are extremely good and populations are stable, a high incidence of esophageal cancer in a particular area was correlated with fungal infections of the esophageal epithelium. The fungus produced an acid that lowered the pH in the esophagus. It was hypothesized that the endogenous nitrosamines were then locally metabolized to a carcinogen at a much higher rate than found at the normal pH.

These specific success stories of epidemiology as a tool for cancer detection are not generally applicable to explain the general level and wide range of cancers in mixed populations. There are, however, a few specific industrial situations where a correlation between high and prolonged exposure to a chemical and increased incidence of a particular cancer has led to the chemical being termed a human carcinogen.

The impossibility of using humans as test subjects for carcinogens has led to the development of animal testing of suspected cancer-causing chem-

icals. Since the number of animals in any one group (level of chemical, sex, age, controls) is necessarily limited for economic and other reasons, high doses, which may be four to six orders of magnitude greater than the potential human exposure levels, are needed to be able to detect tumors. Such tests have led to a considerable number of chemicals becoming identified as possible carcinogens. It is, however, not certain that extrapolation from very high doses used in animal studies to very low amounts ingested by humans, possibly over long time periods, is justified and valid as a basis for concluding that a chemical is a human carcinogen.

Examples for this are the concerns raised about the use of cyclamates and saccharin, which in an experiment with 80 rats fed together at very high doses for 78 weeks led to bladder tumors. Two government commissions examined all the data in this and every other related study. At the end it was concluded that the carcinogenicity of cyclamate had not been established. Nor does saccharin convincingly act as a carcinogen.

The important question that such issues raise involves the so-called "threshold theory." Hypothetical results that might be obtained with an animal carcinogenesis test are illustrated in Figure II-3. The dotted line indicates

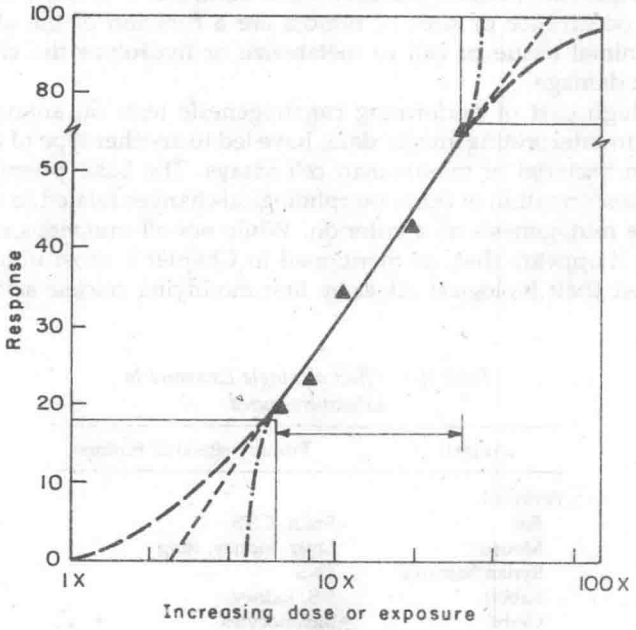

Figure II-3. Data from a typical animal experiment designed to test the carcinogenicity of a chemical. The boxes indicate extrapolation to very low or high doses using different mathematical models. Linear extrapolation (- - - -), sigmoid extrapolation (– –), and no extrapolation (– · –).

a simple mathematical extrapolation which would lead to the conclusion that since the intercept is below zero, a chemical such as saccharin is not a carcinogen at normal levels of exposure. The threshold would be a dose level that was proven to be harmless. On the other hand, one can hypothesize that no safe or threshold level exists and therefore arbitrarily extrapolate to zero by drawing a sigmoid curve or by choosing a slightly different slope toward zero, which is always possible since the points are rarely on a straight line.

There are several additional difficulties in classifying chemicals as carcinogens on the basis of animal experiments. Different results are obtained when different sexes, age groups, and modes of introducing the chemical are compared. Particularly dramatic differences are noted when comparing different species. An example is shown in Table II-3. A single dose of 20 mg/kg ethylnitrosourea administered to 10-day-old BD IX rats will ultimately lead to > 95% of the animals developing specifically brain tumors. In contrast, the adult rat does not develop any malignancy with a single dose but only upon long-term repeated administration, and in this case tumors are noted at many sites. Other animals also developed tumors on sites that are related to species or sex. The toad *Xenopus laevus*, after several years of observation, is resistant to the analogous carcinogen, methylnitrosourea, even though it can be shown that the expected chemical reactions have occurred. It is likely that the variations in occurrence or sites of tumors are a function of the ability of the specific animal tissue or cell to metabolize or hydrolyze the chemical and repair the damage.

The high cost of performing carcinogenesis tests on animals, and the problems in interpreting animal data, have led to another type of test, namely short-term bacterial or mammalian cell assays. The basic premise is not to test for transformation or other morphological changes related to malignancy, but to use mutagenesis as a criterion. While not all mutagens need be carcinogens, it appears that, as mentioned in Chapter I, most ultimate carcinogens exert their biological effect by first modifying nucleic acids, and this

Table II-3. Effect of Single Exposure to Ethylnitrosourea

Animal	Primary site(s) of tumors
Perinatal	
Rat	Brain, CNS
Mouse	Liver, kidney, lung
Syrian hamster	PNS
Rabbit	NS, kidney
Gerbil	Melanocytes
Adult	
Rat	Brain, hemopoietic system
Mouse F	Lymph nodes, lung
M	Liver, kidney, lung

initial event or damage, when expressed, would be termed mutation. Before discussing specific tests and their use, it is necessary to understand how we classify a chemical as a mutagen.

Historically, a mutation was observed as a result of producing a phenotypic change. Testing was primarily done by measuring forward mutations of *in vitro*-mutagenized phage, transforming DNA, seeds, TMV RNA, or *Drosophila*. No metabolic or enzymatic activation of chemicals occurred and there was no toxicity to consider, but a danger did exist that the nucleic acid might be degraded by the conditions of chemical treatment and lose its biological activity, if any. With these tests, mutagenesis could theoretically be related to specific chemical modification of nucleic acid, even though the detection of the mutational events required replication *in vivo*. Using *in vitro*-treated nucleic acids, the observed mutagenicity and resultant amino acid exchanges generally correlated with either a base change (i.e., nitrous acid deamination) or a tautomeric shift (i.e., hydroxylamine or methoxyamine replacement of the amino group of cytidine). This simple explanation of mutation as a function of a single base change, a point mutation, cannot be extended to *in vivo* systems where the reagent must penetrate the cell wall and may need to be activated, and where the modification of the nucleic acid may be repaired by several pathways that themselves may be mutagenic (i.e., induced error-prone repair). Under these conditions it is not surprising that, just as in animal testing, the detection and quantitation of mutation is greatly affected by the test system. The use of back mutation, i.e., restoration of a specific gene function lost through a mutational event, has been found to be particularly useful. In this way very large numbers of bacteria can be "mutagenized," but only those with the mutation at a locus that is nonlethal under specific conditions will be replicated. Reversants that regain viability under the nonpermissive conditions can then be easily detected, isolated, or counted.

Regardless of whether forward or back mutations are being studied, bacteria certainly do not metabolize carcinogens in exactly the same way as animal cells and they may therefore fail to detect the mutagenicity of a proximate carcinogen. It was therefore a great advantage in mutagen testing when a rat liver extract was included in the *Salmonella typhimurium* system developed by Ames and co-workers. In this way it became possible to test for the necessity for metabolic activation of a potential carcinogen by a comparison of mutation with and without the rat liver enzymes. When the mutation frequency was greatly increased, or if mutation occurred only in the presence of the liver extract, it was apparent that one or more metabolites of the potential carcinogen were responsible for mutation. Other animal species and organs have also been used as the source of activating enzymes and the mutagenicity of some carcinogens is quite different under such varying conditions. Similarly, the various tester strains have given different results as a consequence of their particular genetic characteristics.

In the best studied short-term tests employing *Salmonella* strains or the Chinese hamster cell lines CHO and V79, mutation assays are performed with