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An Interdisciplinary Monograph Series

Chemical Mutagens

ENVIRONMENTAL EFFECTS ON BIOLOGICAL SYSTEMS

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Foreword

There is growing concern over the possibility that future generations may suffer from genetic damage by mutation-inducing chemical substances to which the population at large is unwittingly exposed. The hazards of radiation mutagenesis have, of course, been long recognized, and both scientific awareness and public health concern have been extensive. A belief persists that potentially irreversible damage could occur without warning due to the some 10,000 or more natural and synthetic chemical agents in the environment as well as to the chemicals continually being synthesized by man, which assuredly will find widespread use in the future. The induction of mutations in man by environmental agents is of sufficient concern to warrant major efforts directed to improving existing techniques and to develop new ones for detecting mutagenic agents.

Concurrently, a battery of procedures using a spectrum of microbial and more complex submammalian species is being used to identify compounds with mutagenic properties. Compounds chosen for study have been primarily selected by virtue of chemical similarities to established mutagens, carcinogens, or other toxicants. While these have served well as broad guidelines, each has important limitations. Contributing to the acuity of the problem has been the recent observation that the traditional concepts that mutagenic agents are also carcinogenic or toxic is not necessarily always correct. The most vexing frustration is the difficulty inherent in attempting to relate experimental findings of mutagenicity to man.

The demonstration of a mutagenic effect in a microbial system fails to provide important information on whether the responsible environmental agents can reach the genetic material in the nucleus of responsible organs and whether the metabolic capabilities of man would permit the expression of a mutational event. The provocative studies on cell culture and submammalian test systems which have demonstrated mutagenicity, however, make it especially urgent that comparable studies be undertaken on intact mammals and that simultaneously the feasibility of epidemiologic studies be assessed to seek out human data which might indicate the existence of an environmental chemical mutagen.

Numerous advisory groups are addressing themselves to the problems of environmental chemical mutagenesis, and despite differences in sponsorship, organizational titles, and discipline emphasis the problems they face and the questions they ask are similar. First, what guidelines should be established in developing a systematic approach to selecting compounds for testing? Second, how can one best test for mutagenicity; what are adequate tests or test systems? This is a particularly difficult problem since test systems which are relatively simple are those with least relevance to man. The closer one approaches man in the assay of chemical mutagens, the more complex the problems. Third, recognizing the limitations of simple test systems, can chromosome breakage be correlated with mutagenesis and is it feasible to monitor the human population using this gross observation so that mutations due to environmental causes can be detected sufficiently early to prevent catastrophic consequences—a genetic disaster? Recognizing that this genetic monitoring would demand an unachievable effort of monumental proportions in scope and time, there appears to be at present universal agreement that at the very least a registry of environmental mutagens can be established and maintained with ready availability of its information.

The use of different species of progressively increasing phylogenetic complexity for bioassay systems, though sound from a theoretical and feasible from a protocol viewpoint, has contributed little to our understanding of the extent of the mutagenic hazard to man associated with environmental chemicals. This is so for deficiencies already noted plus the additional factor to which this book addresses itself, and that is the need to systematically assay for chemical mutagens even with our less than ideal systems. A crucial problem facing those with public health responsibility is the difficulty in assessing the magnitude of the influence of agents whose potential effects occur rarely and with extreme variability. As an example there is the argument that inasmuch as man has been drinking coffee for 200 years and we have been unable to identify any differences between coffee drinkers and noncoffee drinkers in terms of chromosomal damage, let alone phenotypic variations, there is no hazard associated with drinking coffee. While this may be so, we can only speculate that it is so, particularly since recent evidence has destroyed the shibboleth that "What doesn't show, doesn't hurt." Prior to discussing such problems as the mutagenic effect of coffee drinking, it is imperative that we ask: "How hard has the look been both experimentally and epidemiologically?" Delayed effects due to minimal insults ultimately appear responsible for the diverse epidemiological characteristics of arteriosclerotic cardiovascular disease, degenerative pulmonary disease, and neoplasia. For the future it is crucial to determine how our techniques using the many models available can be united into a constellation of approaches yielding maximum capability for judgment as to whether an environmental mutagen is indeed a hazard to man. To further

confound the problem, one must always bear in mind the mutagenic potential resulting from natural phenomena, including differences in the metabolic characteristics of man himself.

In organizing the text, the authors have wisely divided the book into two parts. The first is concerned with a lucid discussion of the modern concepts of the gene at the molecular and biochemical levels. This simple, though emphatically not superficial section, neatly demonstrates important acquisitions of knowledge during the past decade while at the same time noting the many important deficiencies in our knowledge. This part relates importantly to the second part in that it assures that judgments and conclusions have taken into account the existing balance between knowledge and ignorance. Further, the part dealing with individual chemical mutagens provides within one text an opportunity for comparative evaluation of environmental mutagens in addition to assessing their intrinsic hazard. The authors have wisely chosen to review environmental chemical mutagens in a multifactorial manner integrating chemical structure, biological availability and biochemical behavior, environmental presence, and economic use thereby enhancing its single source value. This assures its utility to the student, the investigator, and those charged with public health responsibility. The extensive bibliography precludes the necessity for an encyclopedic text while at the same time assuring comprehensiveness. The intent of the authors and the very nature of the text assure its becoming less all inclusive with time. Serving as an information source for past research and a critical review of current efforts, it should provide inspiration and guidance for the future. It will most assuredly increase intelligent awareness of existing problems and expand interests and efforts in monitoring this growing hazardous area and contribute to its own obsolescence.

The authors have successfully completed a major effort. It provides in a comprehensive manner an authoritative and responsible assessment of chemical mutagens in man's environment, a worthy benchmark for future studies.

Paul Kotin
Director
NIEHS

Preface

This work has of necessity touched upon a number of different and often unrelated subjects: e.g., industrial application, genetics, cytogenetics, molecular biology, biochemistry, and organic and analytical chemistry. The depth to which any particular area could be explored was understandably limited, but we have tried to provide the reader with a key to the literature so that additional information could be obtained as required.

Though the central theme and overriding purpose of this book was to bring together relevant facts about mutagenic chemicals (both synthetic and naturally occurring) which are a part of man's environment today, we also believed it important to explain the theories, ideas, and facts concerning the mechanism by which all types of mutations arise, and we have described, in some detail, how such mutations have been detected and studied.

We have stressed the importance of knowing as many of the salient facts as possible, such as the stability of the substance under various conditions, their degradation products, if any, the frequency and physical form in which they are encountered by man, whether they are systemically absorbed and by what route, how they are distributed among the cells, tissues, and organs of the body, and how quickly they are metabolized, which alternate pathways are used, what their effects on the genetic material (DNA) may be, and whether these effects can be repaired.

Although we have considered chemical mutagens as discrete entities, it is recognized that man is exposed to a broad galaxy of environmental agents, and hence considerations relative to possible synergistic, potentiating, comutagenic, and/or antagonistic interactions of mutagenic and nonmutagenic chemicals are of vital importance.

All of the above factors must be envoked before valid assessments regarding the genetic hazards presented by these environmental chemicals can be made. Since the organism we are most concerned with is man, the difficulty is enhanced. Though results of studies on other mammals often are considered extrapolatable to man, much more evidence must be brought forth to determine if this extrapolation is done correctly.

To solve some of the basic problems, it is imperative that we improve our understanding of the underlying molecular mechanisms by which these effects are mediated. It is hoped that this work will serve such a purpose.

L. Fishbein
W. G. Flamm
H. L. Falk

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CHAPTER 1

Introduction

Some appreciation of heredity is older than written history, although the science concerned with this subject is in its infancy—dating back no further than to Mendel's discoveries in the latter part of the nineteenth century (1). The discoveries, however, went unnoticed for 34 years until independently rediscovered by de Vries, Correns, and Tschermark.

The failure of Mendel's observations to have an impact upon the biologists of his time is attributed to his having been years ahead of his contemporaries; however, by 1900 more biology was known, and the role of the cell nucleus and the chromosomes was beginning to be understood (2). Their part in the process of inheritance was beginning to be realized, and thus the climate was right when Mendel's work was brought to the attention of biologists.

Mendel succeeded in unlocking one of nature's most important secrets because he chose to consider only a few of the physical characters of the offsprings he bred, and this allowed him the opportunity to examine all the individuals of each generation and to look for quantitative differences. Hence he was able to establish that hereditary traits segregate among the progeny in a mathematically predictable way, and thus he opened the door to the study of genetics.

Initially, the concept of a gene envisaged a stable structure since hereditary characters are passed from one generation to the next, i.e., they breed true. If, however, genes were absolutely immutable, how would organic evolution be mediated? Obviously, the stability of a gene is less than absolute; occasionally, though infrequently, a change will arise, but, like the predecessor, the new product is also highly stable. The new gene is a mutant, and the process is known as mutation, the latter mediating evolution (3). Because the development of a mutant is ordinarily a rare and unpredictable event, attempts to study the process are greatly handicapped. One could look at the result, but an understanding of mutation depends upon the development of methods which can produce mutants on demand. H. J. Muller, whose contributions

to genetics are well known, discovered that mutants could be induced artificially by x-rays, and thus he opened a new approach which has revealed many important genetic facts (4).

The nature of the gene and of mutation can be understood better when chemicals are used for the induction of mutants, since it is through knowledge of the properties of the chemicals that inferences can be drawn regarding the structure of the other reactant (the gene) and (since we now know the genes to be composed of nucleic acid) of the characteristics of the intragenic site that has undergone mutation.

The search for chemical mutagens covered a period of 20 years (1920–1940) before one such substance was found. The discovery was not entirely accidental. Robson and Carr, who had been studying the effects of mustard gas, noted that the inflammation and interference with cell division caused by a chemical were similar to that caused by x-rays. Believing that the substance might be mutagenic, they enlisted the help of Charlotte Auerbach (5) who confirmed their suspicions, but the work was classified and it was not until after the war that Dr. Auerbach's paper appeared.

Soon many chemical mutagens were known, but the interest in them was attributable to their usefulness as tools for unveiling genetic facts. Only recently has much attention been paid to the possibility that some of these agents might pose a threat to man. In fact, it has been argued that mutagenic agents are more helpful than harmful and without them evolution of species would have been arrested at a very primitive stage.

As long as the natural environment was entirely responsible for the existence and production of physical and chemical mutagens, this argument has justification. However, within the last two or three decades man has introduced into his environment a variety of chemical substances which are known to be mutagenic in certain test organisms.

It is the purpose of this book to focus on such agents. For convenience the book is divided into two parts: the first is instructional and begins with the modern concepts of the gene in molecular terms, then deals with the different types of mutations and how they form as well as the biological systems used for their detection. The metabolic events which are concerned with the repair of gene damage are outlined in Chapter 4 and the relevance of these metabolic events to mutation frequency are discussed. The second part of this book deals with the individual chemical mutagens of environmental significance, with their manufacture and occurrence, their method of detection, their degradation and metabolism (when known), and with the types of mutation they induce in the various test systems which have been utilized.

We cannot assess as yet the magnitude of their genetic impact on man, but we will show that the techniques for doing so are still in the developmental stage. There is no doubt that important new chemical mutagens have been

added to man's environment, although, perhaps, the mutagenic agents of greatest importance are still contributed by nature or even produced by our own normal metabolism.

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CHAPTER 2

Nature of Genetic Material

I. Genes and DNA

To understand why certain chemicals have a potential for producing changes within the hereditary material of a living organism while others do not, we must concern ourselves with the chemical foundation of heredity. It is necessary but insufficient to know that heredity is determined and mediated by individual units called genes which are organized, at stages in the cell cycle, into microscopically visible structures called chromosomes which contain (in higher organisms) thousands of such units; that each gene possesses the necessary information for specifying the formation of a particular enzyme or other protein; and that the spectrum of these enzymes and other proteins are primarily responsible for the differences among organisms and species.

Evidence of an indirect and direct nature has accumulated so that it is now universally accepted that deoxyribonucleic acid (DNA) is the material of which genes are composed (with the exception of certain ribonucleic acid viruses which do not contain DNA). Chemical mutagens have been instrumental in providing some of this evidence, for as C. Auerbach stated over 20 years ago, "If we assume a mutation is a chemical process, then knowledge of the reagents capable of initiating this process should throw light not only on the reaction itself but also on the nature of the gene, the other partner in the reaction" (1). And so it has been, although direct and indirect evidence has been derived from many other sources.

It is beyond the scope of this review to consider all the evidence on which the concept is based [see reviews by Peacocke and Drysdale (2) and Sadgopal (3)] but we will deal with certain key aspects.

What indirect evidence exists is based on a correlation between the properties exhibited by DNA and that required or expected of genetic information. Obviously, if genes are composed of DNA, the distribution of the latter should

parallel that of the genes in terms of cellular location and quantity. This is the case: DNA is common to all those entities that carry genetic information, i.e., germ cells, nuclei, chromosomes, mitochondria, and viruses (again, with the exception of ribonucleic acid viruses). As originally demonstrated by Vendrely and Vendrely (4), the DNA content of cells of higher organisms is fairly constant from one tissue to another except for germ cells which contain only half the number of chromosomes (haploid set of chromosomes) and half the quantity of DNA. Just as impressive are the observations showing a direct correlation between the quantity of DNA in yeast cells and the ploidy of the organism (number of chromosomal sets per cell) (5).

Another characteristic of genetic material is its metabolic stability. Once formed, genetic material persists until the cell dies. This feature is satisfied by DNA whose metabolic stability has been demonstrated in many tissues and organisms (6, 7). There are, however, some notable exceptions (8, 9), but these are not relevant to our discussion.

Assuming all cells of an organism contain the same genetic information, the chemical composition of the genetic material should be identical from one tissue to another within the same organism. In terms of this concept, the relative proportion of the purine and pyrimidine bases of which DNA is composed should be constant for all tissue. In fact, no significant differences have been found among DNA samples of different organs of the same organism [with the exception of certain germ cells which amass large quantities of special genes (10)]. This constancy is particularly meaningful when we consider how variable the base composition of DNA is from species to species (11).

By definition, genetic material must be able to self-replicate, that is, synthesize a copy of itself so that all the genetic information of a parental cell may be conferred on daughter cells. Recently, this has been demonstrated by Kornberg and colleagues (12) who synthesized the infectious DNA of a bacteriophage virus *in vitro* from the chemical constituents of DNA, polymerizing and rejoining enzymes, and a viral DNA template which was prepared *in vitro*. The demonstration constitutes both indirect and direct evidence for the relation between DNA and genetic material. Indirect, in the sense that it demonstrates the ability of DNA to direct its own synthesis; direct, in the sense that the DNA prepared *in vitro* was infectious, possessing all the genetic information necessary for making new virus.

A series of experiments have shown how DNA could serve as genetic information and how it can carry the "code" for specifying the spectrum of enzymes and other proteins which characterize an organism (13, 14). This will be considered more fully.

The observation that isolated, highly purified DNA can impart (transform) new genetic character to bacterial cells (15, 16) and cells of higher organisms (17) is taken as direct evidence that genes are composed of DNA, as are the

observations which show that viral DNA alone is capable of producing new viral particles (18) whereas other viral constituents are not (again, with the exception of certain ribonucleic acid viruses).

The validity of the concept is further consolidated by the congruity between the structure and composition of DNA (18) and what genetic analyses (i.e., genetic maps) demand of the structure of the genetic material. For example, certain bacteriophages and bacteria were shown, by genetic analyses, to possess a circular chromosome, while subsequent investigation showed that their DNA was circular (19).

II. Chemical Composition and Structure

DNA is composed of heterocyclic bases (purines and pyrimidines) which are attached to the C-1 position of 2-deoxy-D-ribose through a β-glycosidic bond. The base-sugar units are termed nucleosides. Upon phosphorylation of the ribose ring they are converted to nucleotides (Fig. 2.1). DNA is simply

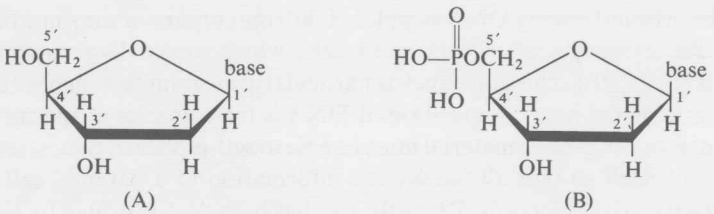

FIG. 2.1. Structure of (A) nucleoside and (B) nucleotide.

a long phosphodiester polymer of nucleotides in which the phosphate attached to the 5′-sugar position is further esterified to the 3′-sugar position of its nearest neighbor (Fig. 2.2).

Depending upon the organism and the quantity of genetic material, there may be many thousands of nucleotides in a single DNA molecule. For instance, the genome (total genetic information) of *E. coli* appears to consist of a single DNA molecule (20) containing an estimated 10^7 nucleotides (21). In higher organisms, it is not clear whether each chromosome represents one or many molecules of DNA.

The commonly occurring bases are adenine (A), guanine (G), cytosine (C), and thymine (T) (Fig. 2.3). With some exceptions, these bases predominate in the majority of DNA's, though 5-methylcytosine has been found in both