MUTATION, CANCER, AND MALFORMATION

Edited by
Ernest H. Y. Chu

and
Walderico M. Generoso

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Ernest H. Y. Chu

University of Michigan Medical School
Ann Arbor, Michigan

and
Walderico M. Generoso

Oak Ridge National Laboratory
Oak Ridge, Tennessee

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FOREWORD

During the early 1930s, when I was a graduate student and later a post-doctoral researcher at the National Research Council for the University of Wisconsin at Madison, we had the opportunity to get acquainted with many graduate students from China who were sent to the University for training in modern basic sciences as well as social sciences. The University of Wisconsin continues to graduate a large number of Chinese students.

Economic conditions in the 1930s were very precarious for the United States and other parts of the world. Many of us students grew closer together because we were living on similarly tight budgets. As a matter of fact, we subleased a part of our apartment in Madison to some Chinese graduate students. This was a very nice opportunity for us to learn about the scientific and cultural background of our Chinese friends. Many of them came from the interior of China and had had very little opportunity to become acquainted with people from a western culture. Living with these students was a very pleasant and educational experience which gave us a good picture of the cultural life and educational system of China at that time--an intimate picture that one normally would not see without travelling in that country. Many Chinese students were also anxious to make friends, to get an idea of how westerners lived, to learn our cultural arts, our family structure, and our general philosophy of life. It was especially instructive for us, because the Chinese students came from a considerably longer historical tradition than we, as most of us had migrated to the United States only a few years before entering the University.

Many of the friendships established during those years lasted for many thereafter, until the political upheaval in China made it impossible to stay in contact; it is very difficult for us now to trace many of the colleagues with whom we were so closely associated.

Since the early 1930s, a number of political upheavals, World War II, and other changes have not only influenced the economic development of China and of our country but have also had a profound

influence on the development and direction or scientific issues.

My interest in molecular structure and physical chemistry has shifted to basic biology and genetics, since so many interesting developments have taken place in the field of biology in the last 20 to 30 years, and even more intensely in the last 10 to 15 years. It was for this reason that I was most pleased to have an opportunity, at the meeting arranged by the Carnegie Institution of Washington, to become acquainted again with Dr. C. C. Tan, with whose work I was very familiar. Through conversations and letters, we set up a cooperative effort. He was especially interested in bringing to China more up-to-date discussions on the recognition of environmental mutagens, carcinogens, and teratogens. These are areas that we have developed through workshops in the United States and in many countries abroad, such as Latin America, India, Egypt, the Philippines, and others.

The Council for Research Planning in Biological Sciences, Inc. was incorporated in 1981, and one of its major objectives is to help our colleagues in developing countries to become proficient with the techniques of the quickly developing field of environmental mutagenesis. Since there is great overlap between environmental mutagenesis, carcinogenesis, and teratogenesis, it was a natural development to consider all three together. Dr. Tan received the suggestion with great enthusiasm, and promised to initiate activity on the proposal as soon as he returned to China. We were also most fortunate to have been working in close cooperation with Dr. Ernest Chu, whose fatherland is China, and who has been lecturing and teaching as a guest professor at a number of universities in China. Also very closely associated with us has been Dr. Walderico Generoso, from the Biology Division of the Oak Ridge National Laboratory, who has worked with us on a number of the training programs and workshops that we developed in environmental mutagenesis. The leadership in developing this cooperation with Dr. Tan was taken by Drs. Chu and Generoso and led to these proceedings which I, unfortunately, was not able to attend.

Judging from all the reports I have received from scientists who attended the workshop, it turned out most successfully with the additional international participation of European and Japanese geneticists, who are also interested in this field. Much of the workshop's success was due to the very generous support of the National Cancer Institute, the World Health Organization, the Exxon Corporation, the Genetics Society of China, and the China Association for Science and Technology.

As a result of this meeting, the Chinese Environmental Mutagen Society has been founded, a group which we hope will continue to provide leadership in developing the fields of environmental mutagenesis, carcinogenesis, and teratogenesis.

This volume was prepared under the editorship of Drs. Chu and Generoso who should be commended for the preparation of this very comprehensive work, since it covers many areas that will be beneficial to developing countries. It will also be a valuable text for individuals who are just starting their training in the areas of environmental mutagenesis, carcinogenesis, and teratogenesis.

These are very quickly developing fields, and it is not yet possible to determine which aspects will become most important in the future. Exposure to chemical mutagens and carcinogens is unavoidable in our environment, in our food, and in the air we breathe. Many of us believe that these important fields require much further investigation. We are therefore most pleased to have the cooperation of our Chinese colleagues in this effort.

Alexander Hollaender

Council for Research Planning in
Biological Sciences, Inc.
1717 Massachusetts Avenue, N.W.
Suite 600
Washington, D.C. 200036-2077 U.S.A.

PREFACE

Protection of the environment in the interest of human health and well-being is a global concern. National efforts and international cooperation are essential to bring about a better world to live in. In the People's Republic of China, where one-fourth of the world's population live, the health impact of the rapid technological and industrial modernization that are currently underway can be great, not only at the national level but also worldwide. Fortunately, there has been increasing emphasis on research and training in the areas of carcinogenesis, mutagenesis and teratogenesis, and in the various aspects of toxicology and epidemiology in the People's Republic of China. However, although some glimpses on the organization of research programs and scientific accomplishments in China have been reported by recent visitors, the information is sporadic and not widely available. In the meantime, Chinese scientists have been eager to establish contacts with colleagues in other countries; they have been keenly interested in learning about recent advances in science and technology and have expressed willingness to share their own experiences and discoveries.

In response to a Chinese initiative under the leadership of Professor Chia-Chen Tan of Fudan University, an International Workshop on the Principles of Environmental Mutagenesis, Carcinogenesis and Teratogenesis was organized, with the dual purpose of introducing to the Chinese colleagues the latest information in these areas in an organized manner, while affording the opportunity for the visitors, and thereby the world scientific community, to learn about the progress that is being made in the host country. It was hoped that the Workshop would provide a forum for information exchange on both the scientific basis of toxicology and the methodologies and experiences in dealing with the human risk problems in different countries.

The Workshop was held in Shanghai from May 25 to June 1, 1983. It was cosponsored by the Genetics Society of China, the Shanghai Association for Science and Technology, the International Association of Environmental Mutagen Societies and the

International Programme on Chemical Safety (World Health Organiation/International Labor Organization/United Nations Environmental Programme). This Workshop is also Number 5 of the IPCS Joint Symposia Series. Financial assistance came from the United States Public Health Service (grant CA 34165), the International Programme on Chemical Safety, the China Association for Science Technology and the EXXON Corporation, to support the travel of participants and the cost of the preparation of these proceedings. In addition, governmental, industrial and private sources of support from various countries including Japan, the United Kingdom, Italy, West Germany, Switzerland and the U.S. enabled the participation of several speakers in the Workshop.

In addition to some 45 formal presentations, a poster session was held consisting of Chinese contributed papers. Thirty-seven full length papers and 41 selected abstracts have been included in this volume. The Workshop was attended by 35 scientists from 11 countries and more than 200 scientists representing over 130 research and educational institutions in China. One important outcome of the Workshop was the founding of the Chinese Society of Environmental Mutagens. Thus the Workshop more than accomplished the expected goal of information exchange at the international level.

The Organizing Committee consisted of Alexander Hollaender (Chairman), E. H. Y. Chu, W. M. Generoso, C. Ramel, C. C. Tan and Y. Tazima. It is our pleasure to acknowledge the invaluable advice from numerous individuals in many countries. We are particularly grateful to Wang Xinnan (the Shanghai Association for Science and Technology), Xue Shouzhen (Shanghai First Medical College), Xue Jinlun (Fudan University) and their colleagues and coworkers for their unfailing efforts leading to a smooth and successful conclusion of the Workshop. In addition to all scientific contributors, we thank Mrs. Mhairi Gehlhar for copy-editing, Ms. Lisa Campeau and Mrs. Mary Kellogg for typing, and the publisher for bringing out the present volume in a relatively short time.

E. H. Y. Chu

W. M. Generoso

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ENVIRONMENTAL MUTAGENESIS AND DISEASE IN HUMAN POPULATIONS[1]

Arno G. Motulsky

Departments of Medicine and Genetics, and Center for Inherited Diseases, University of Washington
Seattle, Washington 98195

SUMMARY

Environmental chemicals can affect the genetic material and cause a variety of different mutations. Mutations in somatic tissues can lead to cancer while germinal mutations can cause various genetic diseases. The impact of germinal mutations on health will depend upon their frequency; their nature (point mutation vs. chromosomal change, dominant vs. recessive); and upon the mechanisms maintaining a given mutation in the population. Mutations causing early prenatal lethality have fewer public health effects than genetic diseases associated with prolonged medical and social problems. Differences between and within species in metabolism of environmental chemicals and in DNA repair make mutational estimates in humans imprecise. Results on mutation frequency in somatic cells cannot be readily transferred to conclusions regarding germinal mutations until appropriate comparisons have been made. Studies on atom bomb survivors suggest an increased mutational frequency but such results failed to reach conventional statistical significance. Current estimates of the role of induced germinal mutation in human populations have wide confidence limits. An accurate assessment of the potential hazards of environmental human mutagenesis requires better fundamental understanding of human genetics and continued attention to studies on humans and their tissues and fluids. Crash programs on environmental mutagenesis at the expense of other biomedical research appear unwarranted.

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[1]Supported by grant GM 15253 from the U.S. National Institutes of Health.

INTRODUCTION

Modern environments differ from those of earlier times in human exposure to many different manmade contaminants. Pesticides, fungicides, food additives, synthetic drugs, and atmospheric and water pollutants did not exist until the advent of the industrial age some five human generations ago. 70,000 of such substances exist and more than 25,000 are in common use in the United States. More compounds are being synthesized every year and human exposure to them is increasing. Many chemicals have been shown to be mutagenic in lower species and concern regarding the implications of chemicals on human health is therefore warranted. A detailed consideration of environmental mutagenesis in man has been provided recently in comprehensive publications (7,14,18).

CANCER AND MUTATIONS (4)

Mutations of the genetic material of somatic cells can lead to malignant neoplasms. Such carcinogenic effects are much delayed between the initial mutation and the actual diagnosis of a clinical cancer. The latency period ranges from about 5 years for certain chronic leukemias to 15–20 years for solid cancers. It will, therefore, take many years before carcinogenic effects are apparent. A variety of environmental chemicals have already been clearly implicated in cancer development [e.g., asbestos: lung and pleural cancer; benzene: marrow cancers; vinyl chloride: certain liver malignancies and others (4)]. A major environmental hazard concerns tobacco. The evidence relating cigarette smoking to cancer of the lung is overwhelming. Mortality continues to increase in the United States and pulmonary cancer is now the most frequent lethal malignancy in males (20). Mortality from lung cancer in women, whose widespread smoking began at a later date, has also risen continuously over the past 15 years. No other common cancers have shown such trends in either sex. Age-specific mortality has remained at similar rates for the past 30 years for common cancers such as carcinoma of the breast in women and of the colon and rectum in men (20).[2] Considering the long latent period between exposure and development of clinical cancers, these data suggest that these cancers are not related to novel environmental carcinogens introduced between 1930 and 1960. It is conceivable that new chemical substances introduced since the 1960s could raise the frequency of these cancers and other mutants in the next few years. Continued careful monitoring of the mortality of different cancers is therefore essential.

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[2] Carcinoma of the colon and rectum in women has slightly decreased in frequency.

Another noteworthy epidemiologic trend bearing on environmental carcinogenesis is the continued decline in cancer of the stomach in developed countries (20). This cancer was the most common lethal malignancy 50 years ago and now has become much less frequent. While the exact cause of this decline has not been elucidated, it is likely that better refrigeration and possibly the addition of food preservatives has reduced the frequency of food contaminated with carcinogens acting on the gastric mucosa. Thus, the choice of a pre-industrial life style with emphasis on organic and natural foods may not necessarily be "healthier" than eating a modern diet!

The facts of increasing rates of lung cancer due to tobacco, decreasing rates of stomach cancer, and fairly steady rates for most other cancers need to be recalled in the formulation of public health policies for cancer control. The overwhelming role of tobacco in current lung cancer mortality must be squarely faced. A very large number of lives could be saved by intensive public health education to discourage smoking. The data for the other common cancers suggest that a new mass endemic of these malignancies is unlikely. Crash programs to detect ubiquitous novel carcinogens are therefore not required. At the same time, careful monitoring of various groups of individuals who may have high exposure to one or another potential carcinogenic chemical needs to be encouraged. Collaborative studies need to be established to collect a sufficiently large number of cases that allow a meaningful assessment of the data. Many such studies are unreliable because of the vagaries of small numbers. An increase in a given cancer in a small occupational grouping would not be detected by broad registries of the general population.

GERMINAL MUTATIONS IN HUMANS

Germ cell mutations, i.e., those affecting eggs and sperm, are more insidious in humans. A cancer due to a somatic cell mutation will kill a single individual but the original mutation will be extinguished with the death of the patient. In contrast, germinal mutations may remain hidden for many generations. Thus, recessive mutations require the homozygous state for clinical expression and many induced mutations are likely to be recessive in nature. The phenotypic effects of mutations affecting genes in polygenic systems are difficult to predict and no definite statements regarding such mutations can be made until better understanding of the various genes underlying such phenotypes becomes available. Only dominant mutations and certain chromosomal defects will have clinical effects in the first generation. The persistence of mutations depends upon the biologic fitness of the phenotype. If carriers of a given mutation have no offspring because of lethality or infertility, the mutation will only persist for one generation.

Since the reproductive performance of the carriers of recessive mutations in the heterozygote state will be similar to that of normals, such mutations will persist for many generations.

Considerable problems in recognition of environmentally-induced mutations are posed by the high probability that new mutations will present with phenotypes similar to those that exist in the populations because of spontaneous mutations. It will, therefore, be difficult to recognize slight to moderate increases in human diseases caused by mutations induced by environmental agents. Background frequencies of existing tumors and genetic diseases are such that marked increases of a given phenotype need to occur before a causal relationship to an environmental mutagen is apparent. Careful epidemiologic monitoring of the existing genetic disease load is therefore necessary to detect moderate increases. Etiologic heterogeneity, i.e., different genes or nongenetic factors causing the same disease phenotype, is a further complication. Even if an increase of a given disease entity is found by epidemiologic monitoring, the underlying cause for the increase will not necessarily be obvious and will require detection of the offending agent. The search for induced mutations in human populations is thus very difficult.

It should be recognized that current quantitative assessments of human mutational risks are based on inferences with wide confidence limits (1,7,14). In fact, there is as yet no direct evidence for any induced germinal mutation having caused genetic disease in humans. Not even the extensive studies on atomic bomb survivors in Japan have furnished unambiguous evidence for a clearcut increase in radiation-induced mutations. There were no statistically significant effects between irradiated populations and controls in untoward pregnancy outcomes, survival through childhood, X chromosomal aneuploidy, and electrophoretically detectable novel biochemical variants (16). However, the differences between the irradiated and control groups were all in the expected direction of increased mutations as is consistent with data from lower organisms.

GENETIC VARIABILITY IN MUTAGEN METABOLISM (12,17)

Considerable species differences exist in the metabolism of many xenobiotics. Such differences may affect quantitative rates of metabolism as well as qualitative routes of metabolism. Thus, different quantities of a given mutagen may be found following administration of a standard dose in animals of different species. Furthermore, a given mutagenic chemical may be detected in one but not in another species because of qualitative differences in metabolism between species.

Metabolic variation occurs frequently within one species. Human twin studies have shown a high heritability for the metabolism of most xenobiotics suggesting strong genetic determinants in biochemical makeup involved in the metabolism of chemicals. In some instances, specific enzyme differences under monogenic control have been detected. Some of these enzyme deficiencies affect the action of specific drugs and cause unexpected drug reactions. The study of such reactions evolved into the field of pharmacogenetics (8). The realization that not only therapeutic drugs but the metabolism and response to any kind of foreign chemical might be under genetic control led to the concept of ecogenetics, i.e., the role of genetic factors in response to various environmental agents (9). The demonstration in recent years that about 5-8% of populations of European origin are homozygotes for a recessive mutation affecting a specific subcomponent of the P450 system that causes marked differences in oxidation of many different drugs is of great interest (6). This system is currently being studied to assess its significance for differences in susceptibilities to cancer of the lung from smoking (Idle, personal communication). Earlier studies with other components of the P450 system in humans failed to demonstrate monogenic effects although undefined genetic factors have usually been demonstrated (17).

The implications of pharmacogenetic and ecogenetic data are far-reaching for human mutagenesis. Inert substances may become mutagenic following metabolic activation and in vitro testing may give a false sense of security. Conversely, mutagenic substances may become "detoxified" so that they no longer are mutagenic. If a monogenic trait with low frequency mediates a critical metabolic step, a small proportion of the population may be at higher risk. Racial and ethnic differences in gene frequencies are common and some populations may be at higher danger of mutational injury than others (12). The results from one population cannot necessarily be transferred to another. Alleles with different functional capacity (isoalleles) may also exist at certain genetic loci. Those individuals within the normal range who, because of isoallelic variation, carry out a given biochemical reaction less efficiently may be at higher risk. If multiple genes are involved in a given metabolic reaction, those individuals at the extremes of the distribution curve may be in significantly greater jeopardy (10). Additional sources of variation in the human population relate to potential differences in DNA repair. Some persons may be heterozygotes for DNA repair enzyme deficiencies that in the homozygote state cause certain diseases such as Bloom's syndrome and others. Isoallelic variation of DNA repair (as described above) within the normal range may also exist. Differences in oncogene genotypes may also contribute to cancer susceptibility and are currently under intense study (23).