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MECHANISMS OF ENVIRONMENTAL MUTAGENESIS-CARCINOGENESIS

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PREFACE

The 19th annual meeting of the European Environmental Mutagen Society was held in Rhodes, Greece, from October 21st to 26th, 1989.

The programme was chosen to explore what is currently known about the mechanisms of mutagenesis and carcinogenesis, induced by environmental agents, and the questions regarding the relationship of these two processes. Recent findings, techniques and methodologies in the area of biomonitoring of humans exposed to environmental mutagens-carcinogens were presented and considerable attention was also paid to the aspects and issues of collaborative environmental policy. Researchers from all over the world contributed to the programme of the meeting with posters and oral presentations, providing a variety of new data and interesting scientific approaches.

A number of outstanding scientists were invited to present the results of their work. It is only their presentations which are included in this book, covering the following topics: Mutations and carcinogenesis; mechanisms of chemically-induced genetic effects on molecular, chromosomal and cell division level; adaptability and repair mechanisms; chemical carcinogenesis and oncogenes; structure and metabolism of mutagens-carcinogens; biomonitoring and epidemiology of humans exposed to environmental mutagens-carcinogens.

For the sake of evaluating and controlling the mutagenic and carcinogenic potential of our environment it is indispensable to understand the mechanisms and processes by which chemicals act on the genetic material, causing either hereditary disease or cancer. The publication of these proceedings will hopefully contribute to this task.

A. Kappas

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MUTATIONS AND CARCINOGENICITY

MUTATION SPECTRUM IN CARCINOGENICITY

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ABSTRACT

The correlation between mutagenicity and carcinogenicity has been a major approach in genetic toxicology. Although the critical importance of changes in DNA in cancer induction is beyond any reasonable doubts, the actual relationship between mutagenic and carcinogenic properties of chemicals is more complex than previously conceived. A primary reason for this complexity is the multistep nature of cancer induction, which implies both genetic and non genetic events. A wealth of data shows that more than one mutational event is generally required for tumour formation. In fact the progression stage of carcinogenicity implies a cascade of mutational events, indicating a stress induced instability of the genetic machinery, ending up in a variety of genetic lesions. The endpoints involved in cancer induction do not only include conventional mutations like point mutations and chromosomal aberrations, but also genetic changes, which are rarely taken into consideration in short-term assays for carcinogenicity. The genetic endopoints, involved or suspected to be involved in cancer induction, comprise insertion mutations, recombination events, gene amplification, methylation of 5-cytosin, mitochondrial mutations and different indirect mutagenic effects. The present paper focuses on these "unconventional" genetic endpoints and attempts to give an overview of their possible role and their mechanism of action in carcinogenicity. It is emphasized that the testing strategy for carcinogenicity has to take into account these genetic endpoints as well as the rapidly growing knowledge of the molecular mechanism behind neoplastic changes.

INTRODUCTION

I feel deeply honoured and grateful to have been elected for this prestigious award by the European Environmental Mutagen Society. I have chosen as a title of my presentation

*The author of this article was awarded the 1989 EEMS Award.

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"Mutation spectrum in carcinogenicity" for several reasons. Justified or not, it is a fact that genetic toxicology has focused the attention on the relationship between mutagenicity and carcinogenicity in order to identify environmental chemicals, which may imply a carcinogenic hazard. The present concept of the role of mutational changes in the development of cancer furthermore is a suitable subject for some retrospective look on the development of this area of basic and applied research and practical applications. I think that this development constitutes an important chapter in the history of natural sciences. But the connection between mutation and cancer is and will also be in the future a crucial area from both practical and theoretical viewpoints. Relevant questions in this connection concern the present and future position and direction of environmental mutagenesis and genetic toxicology. The use of short-term tests for mutagenicity has been under particular scrutiny during the last few years and it has to adapt to the rapid increase in the knowledge of the mechanism of cancer induction in order to survive.

I will therefore take the opportunity not only to discuss the actual scientific questions related to the mechanism of carcinogenicity but also to look backward to the historical background and forward to the future of environmental mutagenesis with special consideration to cancer induction.

Some important events in the development of genetic toxicology and the relationship between chemical mutagenesis and carcinogenesis is illustrated in Fig. 1. The genetic hazards from chemicals did not become any generally recognized issue until the 1960ies in spite of the fact that the ability of chemicals to induce mutations has been demonstrated two decades before by Auerbach, Robson and others. Previously the issue of induced mutations and genetic hazard had been brought up with Muller's discovery 1927 of radiation induced mutations and almost all discussions of genetic hazards were confined to this source of mutations. The atom bomb 1945 and the subsequent use of nuclear power emphasized that approach to the hazards of mutations.

When chemical mutagenesis was brought into the discussion of human genetic hazards the concern was mainly directed towards effects on germ cells and hereditary hazards. This was in fact the situation when the American and European Environmental Mutagen Societies were founded 1969 and 1970 respectively.

Chemical carcinogenesis dates back much further; it usually is referred to the discovery of scrotum cancer among chimney sweepers by Sir Percival Pott (1775). Other milestones in that field were the recognition of multistep carcinogenicity by Berenblum (Berenblum, 1941; Berenblum and Shubik, 1947) and the metabolic activation of chemical carcinogens in the 1960ies by the Miller's (see Miller and Miller, 1971).

Gradually data accumulated, which pointed to a connection between chemical mutagenicity and carcinogenicity and the two areas became united in the beginning of the 1970ies. It was manifested by the introduction of the term "genotoxic", which Druckrey suggested at a conference in Stockholm 1972 (Ramel,

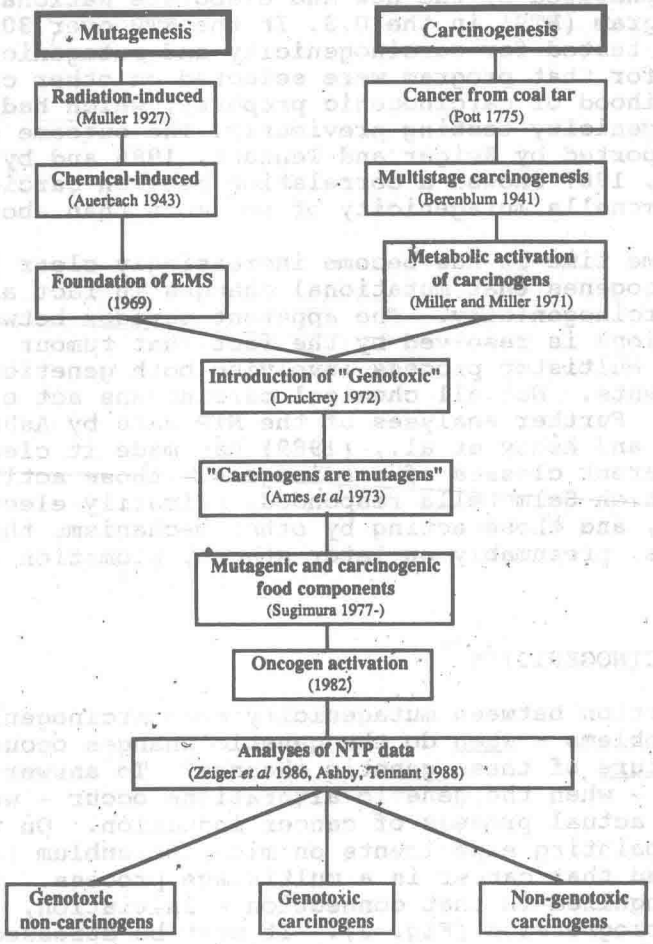

Fig. 1. An overview of the development of chemical mutagenesis and carcinogenesis.

1973). The introduction of the Salmonella microsomal test by Ames 1973 in his paper with the provocative title "Carcinogens are mutagens" (Ames et al., 1973) was another step in the same direction. The subsequent research on chemical mutagenesis by Ames, Sugimura and others indicated a very high correlation between short term tests for mutagenicity and animal carcinogenicity. The hope that simple tests on bacteria would provide a reliable method to identify carcinogenic chemicals in our environment came to an end, however. In 1979 Rinkus and Legator pointed out that some categories of chemical carcinogens did not exhibit any correlation with mutagenicity and this was further emphasized by the new and elaborate National Toxicology Program (NTP) in the U.S. In the NTP over 300 new chemicals were tested for carcinogenicity and mutagenicity. The chemicals for that program were selected on other criteria than the likelihood of carcinogenic property, which had guided animal carcinogenicity testing previously. The outcome of the NTP work as reported by Zeiger and Tennant, 1986 and by Tennant et al., 1987 showed a correlation between carcinogenicity and Salmonella mutagenicity of not more than about 50%.

At the same time it has become increasingly clear through research on oncogenes that mutational changes in fact are critical in carcinogenicity. The apparent paradox between these observations is resolved by the fact that tumour induction is a multistep process involving both genetic and non genetic events. Not all chemical carcinogens act on genetic steps. Further analyses of the NTP data by Ashby and Tennant (1988) and Ashby et al., (1989) has made it clear that there are different classes of carcinogens - those acting as mutagens, to which Salmonella responded, primarily electrophilic compounds, and those acting by other mechanisms than point mutations, presumably on later stages, promotion and progression.

MULTISTAGE CARCINOGENICITY

The connection between mutagenicity and carcinogenicity implies two problems - <u>when</u> do the genetic changes occur and <u>what is the nature</u> of these genetic changes? To answer the first question - when the genetic alterations occur - we have to look at the actual process of cancer induction. On the basis of skin painting experiments on mice, Berenblum (op. cit.) recognized that cancer is a multistage process. Three stages are recognized in that connection - initiation, promotion and progression (Fig. 2). It must be stressed, however, that the course of events in cancer induction is not rigidly determined. There are no precise dividing lines between the stages and evidently there are wide variations in the processes, depending on tissues, inducing agents, differentiation and so forth. It is, however, a useful model and appropriate for our purpose to discuss the variety of genetic and other mechanisms in cancer induction.

When it comes to the initiation of cancer a wealth of data strongly indicates that it is an irreversible process based on mutations. In accordance with the mutational nature of initiation no threshold can be expected.

The promotion stage is more complex and the data indi-

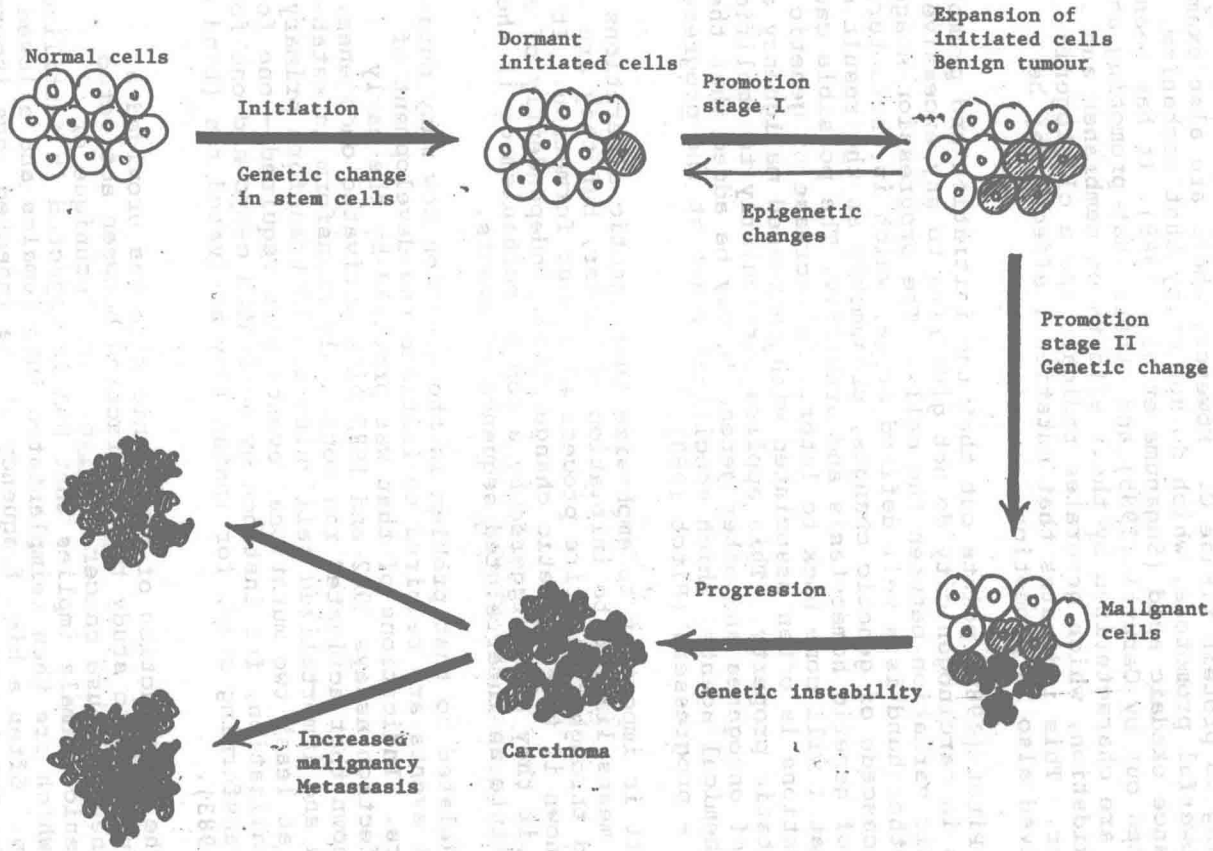

Fig. 2. Multistage carcinogenesis.

cates that more than one step is involved. In skin carcinogenesis the formation of benign tumours is a reversible and evidently epigenetic process, which is caused by promoting agents like phorbol esters, while the further development to malignant tumours responds to mutagenic agents (Hennings et al., 1983). At the molecular level the classical promoting agents such as phorbol esters act on cell proliferation by binding to protein kinase C. However, there are also examples of powerful promotors, which do not ct by that mechanism, for instance okadaic acid (Suganuma et al., 1988). It has been pointed out by Cerutti (1985) and others that promoting agents also are characterized by their effects on membranes and lipid peroxidation, which generates radicals and a clastogenic factor. This indicates that mutational effects may be involved also in promotion.

Pitot (1989) points out that the initiation and promotion steps in carcinogenicity do not give rise to any excessive genetic variation between the cells. The progression stage on the other hand is a well defined stage, which is characterized by a cascade of genetic changes, presumably as the result of a loss of genetic homeostasis and stability. The possible cause of that I will come back to later. An increase of genetic alterations is often associated with increased malignancy and metastatic property. This applies particularly to amplification of oncogenes and other genes. It may be added that there are chemical agents, which specifically act at the progressive stage - progressers (Pitot 1989).

It is important to emphasize that genetic alterations are by no means limited to initiation of cancer, but they are spread through the entire process of tumour formation. It is not known if these genetic changes occur independently of each other,if they are triggered by a common mechanism or if they constitute an interrelated sequence of events.

Related to that problem is the question how many mutational events are required to initiate the development of tumours. Indications of that was provided by the early transfection assays 1982 and 1983 with activated oncogenes. It was shown that activated ras could only transform an established and immortalized cell culture. To transform primary cells at least two mutational events were required – one for immortalization, for instance by activated c-myc and one for the transforming step, for instance by activated ras (Land et al., 1983).

The introduction of transgenic mice has provided a powerful tool to study the interaction between activated oncogenes to cause cancer in vivo. The technique of transgenic animals implies that DNA is injected in fertilized eggs, which are then reimplantated into females and allowed to develop. Often a high frequency of the injected eggs incorporate the DNA in the genome and develop a new generation with this hybrid DNA piece actively transcribed.

The technique has developed rapidly and several research groups have studied transgenic mice with activated oncogenes. Leder's group in the U.S. found that transgenic mice with activated ras or myc oncogenes developed hyperplasia, but incorporated together they gave rise to carcinomas (Sinn et