Introduction to Genetic Toxicology

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Preface

Introduction to Genetic Toxicology is a survey of the problems posed in environmental toxicology, considered from a genetic point of view. It is an intricate matter in a rapidly growing field. However, since the author tries to impart his own knowledge, sprinkled with original ideas about various subjects, the reading becomes easy and attractive, being intended for students as well as for persons such as administrators or politicians who are concerned with environmental problems. A choice of judiciously selected references allows the reader who wishes to go deeper into specific scientific details to go back to the sources.

This is the reason why, after reading through the book, I am pleased to endorse the wish of Dr. Moutschen in making some personal statements derived from the text about the risks of environmental mutagens for man.

The original French issue of this monograph was provided with two prefaces, the first by Dr. A. Lafontaine, then Director of the Institute of Hygiene and Epidemiology, Brussels, and the second by myself. Having agreed to provide the present English version of the book with a translation of my preface, I feel it pertinent also to consider a few of Dr. Lafontaine's important and elegantly formulated viewpoints.

With regard to the risks run by man (and his compatriots in the animal and plant kingdoms), Dr. Lafontaine remarks that, whereas we see today a rapid progress of the evaluation of threats concerned with carcinogenesis and also teratogenesis, there has been a much slower development of sensitive methods for systematic studies of heritable damage with regard to consequences to the future chances of today's species.

This state of affairs certainly reflects the fact that, as far as man is concerned, it is easier to establish cause–effect relationships for somatic than for heritable consequences of damage to the genetic material of cells. But the level of knowledge in a scientific field is always related to priorities made in the definition of the 'research front', and these priorities are coupled to sources of funds which are in their turn closely correlated with the values of politicians and of the opinions supporting their power. And politicians are generally operating on a short-term perspective — one or two election periods. As far as health

hazards are concerned, this short-term perspective of politicians coincides with the electors' egotistic fear of cancer and other diseases in their own generation, including the fear of begetting malformed children. In contrast, it is difficult to arouse a similar interest in the possibility that what we call technological progress may lead to an impairment of the health of our descendants in a remote future.

Partly due to imperfect epidemiological techniques and lack of adequate 'translation factors' from laboratory organisms to man, knowledge of the magnitude of such heritable damage is in fact so incomplete that we are unable to rule out the possibility of a genetic catastrophe. And those future citizens of the world, who will maybe accuse twentieth-century generations by saying, 'See what they did to us!', are left unheard in the setting of today's priorities.

In particular, the egotistic favouring of the interests of our generation may have severe consequences for future man when, at periods of economic crisis, first priority is given to productivity and security of employment, risks of late health effects becoming a matter of secondary importance.

Considering other species, the future of man, in his interplay with the environment, is still more threatened by the now ongoing loss of variation (i.e. of genetic information developed during multimillion-year-long evolution) caused by technological progress in a much broader sense than activities leading to the emission of mutagens.

The author discusses in a stimulating way various efforts to quantify risks of heritable harm. In this context he does not forget to touch upon the ethical problems involved in decisions about the permissibility of exposures and of the danger involved in man's intervention with his own species.

Being a valuable introduction, for students of science as well as for laymen, to known and desirable facts about the interaction of environmental factors with the genetic material of the species, Dr. Moutschen's monograph will at the same time create a sound background of taking responsibility in the difficult problems concerned.

LARS EHRENBERG

Introduction

During the last decades, alarming ideas arose because of the increased presence in our environment of new chemicals, and also from the appraisal of new techniques. Already in 1957, Ehrenberg and Gustafsson (see Ehrenberg and Gustafsson, 1970) drew the attention of the Swedish medical authorities to the urgent necessity to investigate in detail the potential mutagenic and carcinogenic effects of several chemicals to provide against these hazards.

In 1968, Epstein (cited by Sanders, 1969) stated: 'I believe the risks from mutagenicity may well transcend those of cancer. It is incomprehensible to me that some 10 to 20 years after radiation hazards have been at least partially assessed, we have no firm knowledge of the mutagenic hazards of chemicals in the human environment.'

What is responsible for this situation? First, it should be pointed out that people have been long to realize the occurrence of such hazards. Also the information in this field has for a long time been practically non-existent. Finally, it should be remembered that the initial aim of the geneticists in the field of mutagenesis was to use the most powerful mutagens for plant and animal improvements or as a tool for genetic analysis (Auerbach, 1976, in Gen. Refs.).

Beside this somewhat positive aspect of mutagenesis, it must be recognized at once that mutagens can also exert deleterious effects in man, by inducing hereditary diseases. After a long maturation period the idea was fully formulated that mutagens were actually increasingly present in the environment, and that it was urgent to prevent noxious effects.

Scientific societies progressively evolved in several countries. First, Hollaender created the Environmental Mutagen Society in the USA. Similar societies, efficiently working together, have evolved in Europe, Japan and India. These societies are not confined to geneticists but also include toxicologists, hygienists, ecologists, manufacturers and physicians; namely, all persons interested in one way or another in the future of man and in the preservation of the environment. Since their creation, these societies have regularly organized international meetings as well as workshops in conjunction with industry and the medical authorities of various countries. Local sections of these societies

are now active. Beyond the basic problems, one task is to investigate the specific problems of each country.

In 1969, in the United States scientists were chiefly interested in mutations produced by pesticides, for which a committee of 'Health Education and Welfare' was created. In 1972, a conference on the human environment was held in Stockholm, a part of which, following the tradition of this country, aimed to evaluate genetic hazards (Ramel, 1973). In 1973, the first international conference on environmental mutagens was held in Asilomar in California, a second in Edinburgh in 1977, and the last in Japan in 1981. This field is still expanding. Even the legal aspects of the problems are currently being explored. In the United States, laws have been proposed by congressmen to extend the present legislation to chemical mutagens (Public Law 94–469, 11th October, 1976, 94th Congress 1978) and also more recently in the EEC (Loprieno, 1983). Mutagenicity is now identified as a hazard for public health.

Before coming to the core of the matter, we should first explain what is meant by genetic toxicology.

The potential toxicity of chemicals occurring in the environment is a familiar concept. In everyday life, when the parameters are generally well defined, it is almost routine work. If the toxicity of a chemical is to be evaluated, it is first given to a number of animals, generally a mammal, then after a suitably selected time, the number of survivors is counted. The dose of the chemical that kills half the animals treated within a given time is called the lethal dose 50% or LD_{50}*. After fixing this parameter, and in some cases after comparing the sensitivity of different species, it is required to describe the effects of the chemicals by investigating in detail the functional modifications of the organism and of each organ in particular, i.e. the physiopathological and anatomopathological reactions.

Finally, the last step should attempt to elucidate the mechanism of action at the molecular level. For pharmaceuticals, toxicological data selected from the indexes of pharmacology allow the doses required to be ascribed and the toxicity defined.

Results from epidemiological investigations of industrial pollutants allow confidence limits to be fixed and adequate action to be taken. Except in very special cases, methods used in classical toxicology take little or no account of the so-called long-term effects. These effects are essentially of three kinds:

First, substances absorbed at doses considered as showing little toxicity on the basis of classical criteria can act preferentially on the embryo at specific stages of its development, and therefore induce an anomaly resulting in a monstrosity. This is the *teratogenic* effect.

Independently from this effect, small amounts of various substances that by

* It is sometimes justified to define a dose which kills 37% of treated organisms (or LD_{37}) for agents which act by a one-hit mechanism. In this case, the proportion of survivors $A/A_0 = e^{-\alpha D}$ where α is the probability of hitting the target and D the dose. Thus, when $\alpha D = 1$, $A/A_0 = e^{-1} = 0.37$.

themselves do not produce detectable toxic effects can eventually result in a malignant tumour. This is the *carcinogenic* effect.

Finally, independently from any teratogenic or carcinogenic activity, and in the longer term, various substances can, at concentrations sometimes extraordinarily small, irreversibly damage the genetic system of an organism producing effects that will only appear in further generations. This is the *mutagenic* effect.

The present handbook will deal almost exclusively with this latter aspect of toxicity.

Numerous common points exist between carcinogenesis and mutagenesis; the majority of physical and chemical agents that show mutagenicity also show carcinogenicity. It is therefore not surprising that in most treatises carcinogenicity is referred to alongside mutagenicity. This is derived from the old concept that cancers arise from somatic mutations (Bauer, 1928; Boveri, 1929, in Gen. Refs.). Nowadays, carcinogenic processes are thought to be far more complicated than a single mutation, however important that might be (Berenblum in Sutton and Harris, 1972, in Gen. Refs.) We think that, since we lack evidence about the etiological similarity of the two processes, the study of the mutagenic properties of an agent should in many respects be separate from the study of its carcinogenic effects, though these latter could in some ways serve as pilot experiments designed to evaluate mutagenic effects for the very reason that they can be detected in the exposed generation (Butterworth and Golberg, 1979, in Gen. Refs.) Cancerology — as also teratology — developed its own methods quite distinct from the genetic methodology. This is why we are excluding carcinogenic and teratogenic effects from the present handbook, without underestimating their enormous importance. We should mention, however, that during the last decade some enthusiastic ideas have emerged, attempting to link the two fields of mutagenesis and carcinogenesis despite the many question marks. It is in fact a biased approach to the problem which arose from the emergency of requiring to test thousands of substances within a short time. For this purpose, short-term tests were designed. Some carcinogenic substances were tested for mutagenicity and actually found to show positive effects in lower organisms. Therefore, if we could demonstrate that all carcinogenic substances in mammals and possibly in man have a mutagenic effect in microbes, would it not be possible that mutagenicity testing in short-term tests detects potential carcinogenicity in mammals?

Now, it must be stated that this assertion is based on no more than a correlation. It means that when we state that a carcinogenic substance for man is also mutagenic in bacteria, it has to have been previously proved by cancer methodology that it was actually carcinogenic after long-term tests in mammals. This correlation between mutagenicity and carcinogenicity can only be made at this cost. It has been found for hundreds of substances, but not for all. Therefore, if we assume that there are still classes of substances, untested in mammals, which give false results in bacteria, this makes the correlation questionable. To see how far it is possible to pursue this line of research, and

also to develop new methods to correlate mutagenicity and carcinogenicity, international organisms evolved for this very purpose. One of them is the International Commission for Protection against Environmental Mutagens and Carcinogens founded in 1976 (Sobels, 1977; summary of the first five years in Sobels and Delehanty, 1982). They specialize in the field, and the objective is not only to critically evaluate the body of data presently available from which priorities for future research can be derived, but also to make recommendations for future guidelines and regulations. There are also national organizations such as the Gene-Tox programme in the United States which are investigating such problems.

After these preliminary considerations, we are now in a position to define genetic toxicology and to place it in its context.

> *'Genetic Toxicology is the systematic investigation of the effects that all physical and chemical agents present in our environment can exert on the genetic system of man as well as of their remote genetic consequences for the future of the species.'*

In the same way as traditional toxicology, its first aim is to describe the outcome produced by toxic substances in various organisms, but only from the genetic standpoint, and to draw conclusions that can be extrapolated to man. The next aim is to investigate the mechanisms of action of the substances and, on the basis of this knowledge, to evaluate the risks for man. On the other hand, genetic toxicology takes its methods only from genetics, in such a way that some researchers have called it toxicological genetics, attempting to stress the methodological aspects. We think this name is less justified than genetic toxicology, for it should be preferable to give more importance to the aims rather than to the means to reach them. The dichotomy exists, however.

Therefore, the first part of this book deals more specifically with genetic methods applicable to genetic toxicology. As a rule, these models allow all kinds of mutations to be detected and their frequency to be assessed. However, we must emphasize that the concept of genetic toxicology largely overlaps the concept of mutations and of the risk of these mutations for man. In fact, some agents can modify the population structure considerably without necessarily increasing the mutation rate, especially by modification of the recombination frequencies or by selection processes. These effects, at least as important as mutations, are much less known, however, and are going to be investigated in the future.

Furthermore, genetic toxicology not only comprises the systematic investigation of all environmental agents which can, one way or another, modify the structure of a population — the subject of the second part of this book — but also includes investigation of all regulatory processes which tend to counteract the action of these agents, and, from a prophylactic or hygienic standpoint, the study of the consequences derived from the knowledge of the genetic risks.

Before approaching the study of the genetic effects of physical and chemical agents present in the environment, we emphasize two points: first, it is obvious that the knowledge of toxic molecules has taken advantage of the spectacular

progress in chemical technology. Second, in contrast, our knowledge of some genetic mechanisms especially in man has yet to be improved.

In this context, the molecular mechanisms leading to the various kinds of mutations or determining crossing-over with a somewhat mathematical precision are still poorly understood. If we consider that all the principles required to approach the study of human inheritance successfully were established at the beginning of this century, it appears that opposed ideas only could have hampered the advances in the field. These ideas possibly originated from sociocultural and political influences, i.e. from deep interactions between genetics and eugenics.

Having resolved the conflicting situation arising from divergent ideologies, human genetics is now ready to stride ahead — the gates of the future are wide open.

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Chapter 1

Methodology for Detecting Mutations at the Molecular and Cellular Levels

It is essential to be able to control the techniques that allow the risks of mutagenic agents found in the environment or liable to be generated therein to be assessed as precisely as possible.

Animals or plants used for this purpose are numerous, and it is not always easy to evaluate the real importance for man of the sometimes contradictory results obtained with the tests at present available. Originally, genetic technology, although elaborate, was not intended to detect mutagens in the environment. The majority of methods in mutagenesis were developed to measure the effects of physical and chemical agents well known for their high mutagenic efficiency, and not to check the risks of substances of generally weak activity compared with major mutagens.

As a whole, the tests can be separated into four groups. Some researchers consider that knowledge of the reactions of toxic compounds at the level of DNA molecules can provide sufficient information to suggest caution against potential mutagenicity (group I). Other researchers believe that an easier way to solve the problem is to use micro-organisms rather than more complicated higher organisms, arguing that this will increase the resolving power, thus the sensitivity, of the test. In micro-organisms, biochemical mutants are generally thought to be more convenient for this purpose compared with more complex morphological mutants (group II). Other researchers are trying to improve methods to reveal the damage directly induced by mutagenic agents at the chromosomal level (group III). Finally, other researchers prefer a less rapid but more fundamental genetic approach, that of observing the effects in the progeny of treated organisms (group IV). A set of test systems available is briefly summarized below.

Classification of tests available

Group I: Tests designed to detect lesions at the molecular level

a. In DNA of various origins – microsomal fraction added or not

Unscheduled DNA synthesis

b. In proteins, e.g. haemoglobin

Group II: Tests designed to detect mutations at the cellular level

A. Direct

a. Lower organisms

Phages

Bacteria: *Escherichia coli*, *Salmonella typhimurium*, *etc.*

Yeasts: *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*

Fungi: *Neurospora crassa* and other species, *Aspergillus nidulans*, various species of *Penicillium*, *Sordaria brevicollis*

Protozoa: Paramecium aurelia, Tetrahymena piriforme, etc.

b. Higher organisms

Plants: Maize (*waxy* mutants in pollen cells)
Barley (*waxy* mutants in pollen cells)
Tradescantia (mutations in stamen hairs)

Mammals: Rabbit (*in vitro* cultured cells)
Syrian hamster (*in vitro* cultured cells)
Chinese hamster (*in vitro* cultured cells)
Mouse (*in vitro* cultured cells, spot test)
Man (*in vitro* cultured cells, *in vivo* immunological detection – blood and sperm)

B. Indirect

Host-mediated assays

Rabbit: *Salmonella typhimurium*, *Neurospora crassa*

Mouse: *Salmonella typhimurium, Neurospora crassa, Schizosaccharomyces pombe*

Rat: *Salmonella typhimurium*, *Neurospora crassa*

Hamster: *Salmonella typhimurium*

Group III: Tests designed to estimate the induced chromosome damage (clastogenic effect)

A. *In vitro*

a. Plants: *Tradescantia* (pollen grains)
Trillium (pollen grains)
Carrot, *Nicotiana*, etc. (various diploid tissue cultures)

b. Animals: Numerous cell strains of rabbit, Syrian and Chinese hamsters, rat, mouse and man (including malignant tissues)

B. *In vivo*

a. Plants: Barley (root tips)
Onion (root tips)
Broad bean (root tips)
Tradescantia (pollen grains, pollen mother cells, root tips)
Trillium (pollen grains, pollen mother cells)

b. Animals: Mouse, rat, hamster (spermatogenesis, bone marrow)

Group IV: Mutagenicity testing at the level of the whole organism

a. Plants: Barley (chlorophyll mutants, induced sterility)
Maize (chlorophyll mutants, *yg2* test)
Arabidopsis thaliana (chlorophyll mutants)
Wheat (various mutants)
Pea (various mutants)

b. Animals: *Drosophila* (recessive visible and lethal sex-linked mutants, *ClB*, *Muller-5, B In sc y, facl*
Habrobracon (recessive visible lethal autosomic mutations)
Silk worm (induced mutation in oocytes)
Mouse: specific-locus mutation test, dominant lethal mutation assays, chromosomal non-disjunction, sex-linked recessive (visible and lethal) mutations, heritable translocations (hemisterility).

These methods have been described in detail in several textbooks (Burdette, 1962, 1963a, b; Fishbein *et al.*, 1970; Vogel and Röhrborn, 1970; Hollaender, 1971a, b, 1973, 1976; Hollaender and de Serres, 1978; de Serres and Hollaender, 1980, 1982; Kilbey *et al.*, 1977, and 1984, in Gen. Refs.) In Chapters 1 and 2 only some chief principles are indicated, followed by a critical discussion in Chapter 3.

Group I

Among procedures that aim to detect chemical alterations of DNA after reaction with a mutagenic agent, Marmur's (1961) method has long been used in several laboratories. It is based on the use of the transforming factor. This factor is extracted from a wild type bacterium, then treated with the mutagenic agent for a short time. After elimination of the mutagenic agent, the transforming factor is incubated with bacterial cells of a tryptophan-dependent strain; this strain is normally unable to grow on a medium not supplemented with this amino acid. A mutation of the transforming factor in the donor DNA, located near the *trp* (tryptophan) region, will transform the bacteria so that not only will they be able to grow on this tryptophanless medium, but they will also accumulate a fluorescent precursor allowing an easy and rapid count of the mutant colonies, and hence the estimation of mutagenic events induced in the donor DNA.

Treatment with the so-called microsomal fraction has been much used with a large variety of test systems not only DNA. After suitable extraction of the microsomal fraction from a tissue or a whole organ (e.g. liver, testicle or even plant organs), homogenization and centrifugation at 9000 *g* (S 9), the potential mutagen is treated with this extract for a certain time and then transferred to the test system. This treatment is designed to check how the substance is detoxified by the active fraction of the organ or, conversely, to check if a normally non-mutagenic substance (i.e. a promutagen) can be transformed into a real mutagen (Montesano and Magee, 1970; Ames *et al.*, 1973) (Chapters 8 and 10). Human cells are also used for metabolic activation of chemicals.

More recently, the demonstration of induced DNA lesions made this molecule a valuable tool for detecting potential mutagens. It can be assessed by measuring strand breakage (Kohn and Grimek-Ewig, 1973; Lee and Zbinden, 1979) or covalent binding of the chemical with DNA (Brookes and Lawley, 1964; Lutz, 1979).

There is also another possibility for indirect measurement of the damage based on the study of excision-repair processes, namely unscheduled DNA synthesis. It can be performed *in vitro*, but it is desirable to confirm the results with *in vivo* experiments. Maturation stages of the seminiferous tubules of mouse (Sega, 1974; Sega *et al.*, 1976) or rabbit (Zbinden, 1980) are selected. After administering the test substance, generally intraperitoneally, labelled thymidine (often with tritium) is injected as a precursor of DNA synthesis. Radioactive spermatozoa are collected at regular intervals, either by sacrificing the animal (mouse) or collecting sperm (rabbit). The radioactivity is accurately measured by liquid spectrometry or by autoradiography. This has become an important test which takes into account the metabolic fate of the tested ubstances and yields information about testicular barriers.

Another method for detecting genotoxic agents at the molecular level has een developed more recently (review in Ehrenberg and Osterman-Golkar, 980) and is rapidly being improved. It is based on the fact that most genotoxic