METHODS IN TOXICOLOGY

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EDITED BY

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Preface

The practice of toxicology is not a discipline in its own right but comprises many different disciplines, ranging from physiology to pathology and requiring very different technical skills. No one individual can be equally familiar with all the skills required nor can most organizations afford to employ individuals of the necessary diversity of skills. It is always desirable, of course, for an individual in charge of toxicity experiments to consult those having particular skills that may become relevant to the toxicological experiments. However, this is not always possible and in any event an expert in a particular discipline may not always have considered how his discipline might be applied to the study of toxicity.

This book is intended to provide an expert opinion in many areas that are particularly relevant to toxicology as to how a particular discipline may best be applied to that study. It is hoped that these expert views will serve to guide experts from other disciplines in their use of skills with which they are not familiar and to give them some insight into what can be achieved in particular fields and to indicate the various complexities that surround the subject. Each chapter is intended to be complete in itself and there is, therefore, necessarily some repetition of material covering common points as, for example, the types and numbers of animals used in various experiments. It is hoped that this repetition will enable a particular expert's views to be consulted without the necessity of frequent cross-reference to other parts of the book.

For the most part the chapters are in the form of practical advice on the actual conduct of experiments. In some cases the subjects covered are too complex for such detailed advice and there, guidance is given to literature sources of experimental details.

All the authors are experts and are in day-to-day practice of their subjects. The book is, therefore, addressed primarily to the scientific worker in toxicology rather than to the administrator or regulator

with only a peripheral interest in the subject. It is hoped that the chapters will be interpreted as guidance rather than as strict rules that should not be transgressed. Guidance is surely frequently needed by anybody engaged in this subject whereas strict rules would strangle any possibility of further development in it.

G.E.P.

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CHAPTER 1

The Design and Interpretation of Toxicity Tests

G.E. PAGET

The history of the invention of new drugs is one of increasing technological complexity. The reasons for this are, of course, many, but from every aspect a major cause of this complexity is the realization by those who are concerned with the discovery and use of drugs that the issues involved are far more complicated than was realized by the earliest workers in the field. In no area has this increasing complexity been more evident than in that which is the concern of this volume, namely the examination of the potential toxicity of new drugs intended for trial and use in man.

The earliest investigators were content with simple, brief, and superficial examinations of this question and everybody in the field will be aware of the limitations of the studies of such drugs as digitalis, salvarsan, and the earliest sulphonamides. The increasing clinical recognition of the potentiality of new drugs to cause undesirable reactions has led to the development of a more sophisticated attitude to such studies in the laboratories of firms discovering and developing new drugs. The growth of this attitude has been materially hastened by the occurrence of major and widely reported mishaps in the use of drugs, which, it was generally felt, might have been avoided if the study of drug toxicity had been more extensively developed or more widely employed.

Unfortunately a number of difficulties beset this laudable desire to determine the potential toxicity of a drug in advance of its use. The most fundamental difficulty is that the study of drug toxicity is not a technical discipline in its own right. The toxicity of potential drugs must be studied by a large number of different techniques, all or any one of which may be relevant to a particular situation.

Thus, the drug toxicologist is but a scientist of another discipline masquerading under a professional title because of the use to which he puts his primary discipline. Even in the largest organizations concerned with the study of the undesirable effects of drugs, it will prove impossible to assemble sufficient experts of sufficient diverse disciplines to design the necessary experiments from a basis of expertness in all those disciplines. One purpose of the present volume is to ensure that the individual who must design experiments in a particular field that would be relevant to the study of the toxicity of a drug and who does not have the advantage of expert advice in that field, may have a work to which he may refer and from which he will obtain advice equivalent to what a local expert might have given him. It is felt that although this process cannot substitute for the working collaboration of an expert in a particular field, it will certainly be an improvement over the solitary investigator developing methods for himself from scratch.

One hazard of collecting a compendium such as the present volume is that the mere assembly of methods that may in appropriate circumstances be applied to a particular problem, may give rise to the attitude that all those methods must be applied to every problem. This danger is particularly severe since the climate of thought about the application of toxicity tests seems to be moving in the direction of comprehensive testing rather than of selective experiment, although the latter is surely to be preferred.

The reasons for this disquieting development are easy to see. A number of people are concerned in determining whether a compound has been demonstrated as being safe enough to investigate in man or to exploit more widely. Unfortunately, but inevitably, only a minority of these people have any expertise in the techniques by which compounds are evaluated from this point of view, and indeed a majority of these individuals may well be laymen in this respect. All of them are, however, aware of the public scrutiny that must necessarily follow a failure in the evaluation of the safety of a drug. In these circumstances it is not surprising that the mere existence of a test will suggest to them that the test should be applied, not so much to determine whether the drug does or does not possess a particular property, but rather to provide a climate of assurance, either political or legal, should future events bring their actions into question. This is so much the case that a number of regulatory agencies are now known or thought to have checklists

of tests that should, in their estimation, have been applied to a drug, and should the checklist be incomplete, the drug is rejected without enquiry being made as to why a particular group of tests was omitted. It is additionally difficult to sustain a contrary attitude, since many of the tests that are applied have been developed in response to a particular need. Instances of this are many and obvious, as for example, the extensive use of tests involving the pregnant female developed in response to the thalidomide episode or the considerable interest taken in ocular toxicity following experience with Mer-29. No doubt other specific tests will be developed following calamities we have not yet encountered.

Nevertheless, despite these facts, any thinking scientist must argue strongly against this attitude of mind, although the counter-arguments are more subtle than those in favour of completely comprehensive testing. Perhaps the most obvious counter-argument concerns the amount of effort which it is sensible to put into the safety evaluation of drugs. It is the case that 20 years ago a drug firm that employed one or two graduates and a handful of technicians on safety evaluation was considerably in advance of its time and was regarded as being highly advanced in this aspect of drug development. Nowadays, the largest firms may have enormous departments of drug toxicology and may devote to that subject as much as one third of their total development resources. This growth is obviously continuing and must in the present climate of opinion continue and even increase. This is because no test once described and found to be generally useful is ever dropped but is applied to an increasing range of substances, while new tests and techniques are developed at an increasing rate. To carry the process to absurdity, one could imagine the situation where all the resources available were devoted to safety testing and none to the discovery of new entities. This would plainly be a ridiculous and untenable situation. Somewhere considerably short of that situation a balance must be struck between the discovery of new drugs and the evaluation of their safety. It must be generally recognised that if new drugs are to be developed and if mankind is to have the benefit of those drugs, some hazard is inescapable and no proliferation of safety testing will ever remove it.

The second point arises from the fact that the scientific basis of safety testing is dubious, since all such tests are designed to demonstrate a negative, which, by definition, cannot be achieved. Never-

theless a point is readily reached where the size of the operation involved, the number of tests that have been performed, and their complexity give rise to a feeling of assurance in everyone concerned that safety has truly been demonstrated. The inertia of this process may be such that those whose responsibility is the evaluation of safety may be convinced that they have, in fact, achieved a demonstration of it. It is commonly remarked, in fact, that the toxicity tests that had been carefully carried out on thalidomide without exception had demonstrated it to be an almost uniquely safe compound. What was required in this instance, and I think it true to say in many other instances, was not a further proliferation of tests but rather an awareness in those concerned that situations might exist in which hazards might arise that were not covered by the extensive tests they had performed. Such awareness can only arise when the mind of the investigator is open to the possibility and when individual investigators have sufficient time to consider the whole of the problem rather than being overwhelmed by its numerous and substantial parts.

I have noted above that the scientific basis of the study of drug safety is dubious. Most of the tests that have been developed and that are described in this volume were originated as rule-of-thumb methods that were intuitively thought to possess some validity but which it would be difficult to substantiate from any basis of scientific principle. An essential point in the development of more satisfactory methods, and one which is demonstrated in all the contributions to this volume, is a research approach to the problems. On many occasions this will demand going outside accepted methods in order to develop new and more satisfactory ones. This can only be possible if time and facilities are available to the investigator and if the management of pharmaceutical firms, and even more the civil servants in regulatory agencies, are sufficiently flexible to see that new methods may be more relevant and more informative than the blind application of old ones. The mere existence of those new methods, however, should not suggest to the regulator that they must forthwith be applied across the board, since this would be to compound the fault which I have already criticized.

Similarly, regulatory agencies must recognize that often the application of a new method or experimental technique will give rise to information which may not be easily evaluated when it is obtained.

whatever its ultimate use may be. A real and powerful reason for the failure of many pharmaceutical firms to mount investigations of problems of toxicity employing techniques new to this field is that, should they obtain information of which the significance cannot be evaluated, they anticipate severe difficulties with the regulatory agency to which that information must be disclosed. They are often, therefore, placed in the anomalous and scientifically ridiculous situation of preferring not to obtain new information in case they are saddled with impossible requirements by a regulatory agency as a result. This attitude has certainly materially held up the development of techniques that undoubtedly in the long run will prove to be of use.

From all the foregoing, it will be clear that I believe that the evaluation of the potential toxicity of a drug is a scientific problem that is not to be resolved by the blind application of a set of standard tests, and it is not the intention of this volume to recommend the use of such standard tests. When it becomes relevant to determine whether a chemical compound may be tested in man after it has been demonstrated that it may possess useful properties, a number of questions should arise in the mind of the responsible investigator long before a single test is carried out. These concern the mode of action by which it is presumed to bring about its desired effects. Consideration of this mechanism of action, even when it is only partly understood, should enable many predictions about the safety of the drug in use in man to be made. In the case of novel mechanisms of action, some consideration of the total biological situation and how it is affected by the new drug may give rise to apprehensions about its safety in particular respects. Drugs that are analogous to known drugs are an easier problem, and here too specific circumstances of hazard may be defined.

A number of questions will remain and answers to these questions must be sought before it is sensible to commence designing elaborate, long-continued, large scale tests. In their very nature such tests are to be carried out in animals, and it is, therefore, important to decide what species of laboratory animal is to be used for the test. This question is perhaps the most fundamental of all those that must be answered before experiments are designed. The considerations that affect this choice are discussed in Dr Benitz' chapter. Often enough the question of the ideal species must be left unresolved because of inadequate information. There is, however,

a growing tendency to believe that procedures permitting the choice of a species, which in some respect or other may be thought to resemble man more closely than others that might be used, are desirable. Perhaps this point is one on which major advances are to be expected.

Another point that must be determined before major experiments are designed is what aspects of the action of the drug warrant particular attention during the definitive and prolonged toxicity experiments. Often if it is known that a particular organ or system is liable to be damaged by a drug, particular observation of that organ during the course of other experiments will be desirable. There is much to recommend a short, preliminary experiment using small numbers of animals as a method of determining how the main experiment is to be monitored.

It is increasingly possible to measure in animals some parameter to which drug effects are likely to be more closely related than they are to such simple measures as the dose actually administered. The tendency is, of course, to believe that effects are more clearly related to blood level of the agent than to any other measurement. While this is often true, it is by no means exclusively true and the measurement of blood levels, although desirable, should not be allowed to obscure the fact that other measures are sometimes more relevant, such as the concentration of the material in a particular tissue or the concentration in blood or tissues of some material related to the action of the compound. It may be, for example, that the compound exerts its action by depleting or increasing some normal tissue constituent and a measure of this may well be more relevant than a measure of the actual compound. A particular case of this is, of course, compounds which inhibit particular enzyme systems where the toxic effects are often related to the degree of inhibition rather than to the actual level of the compound present.

These measurements and many others that will undoubtedly be developed offer the opportunity of increasing quantification and sophistication in the monitoring of all types of toxicity experiments while they are in progress. No measure, however quantifiable and 'scientific' will ever replace the supervision of the experiment by a skilled and experienced experimentalist. No schedule of necessary tests, no automation of numerical results of such tests, and no intervention of specialist technologists should obscure the need for inti-

mate supervision of all toxicity experiments by an individual who is concerned with the totality of the experiment and with its purpose. Only in this way will the unexpected and significant observation ever be made. The same consideration is also true of procedures used to assess the experiment at its conclusion. The various contributors to this book have pointed out the various observations that are regarded as essential to the correct assessment of toxicity tests. The most important single point is that toxicity experiments are by their nature intended to detect unexpected effects. Unexpected effects are often only evident to the prepared mind of an expert observer and this is one element of an experiment that should never be omitted.

The intimate association of pathologists with toxicity experiments, for obvious reasons, has led to some unsatisfactory attitudes in the evaluation of the experiments with which they are concerned. One suspects that this is because pathologists' training has classically been concerned with the precise diagnosis of human lesions and little with the functional assessment of the lesion and even less with its predictive significance for individuals of different species in different circumstances from those in which the lesions studied actually arose. For much human pathology the job in hand can be satisfactorily completed by a careful study of a single section conventionally stained and examined in a light microscope; indeed, the pathologist's opportunity may be confined to this classic situation. This is not the case in the pathological observation of toxicity experiments. In these circumstances a diagnosis of a lesion is merely the beginning of a process of assessment of its significance that may, and should, involve not only the skills of the modern experimental pathologist in histochemistry, electron microscopy and other specialized techniques, but also experts from other disciplines such as pharmacology and biochemistry. Only the most complete picture of the meaning of the lesion detected by the pathologist can serve as a satisfactory basis for the prediction of likely human effects from effects discovered in toxicity experiments.

This latter process is, of course, the purpose of all toxicity experiments and is the toxicologist's most difficult task. Much has been written about the basis for this prediction and many of the precepts that guide it are obvious. Thus, if a compound has been given in adequate doses to a large number of different species of animals and all have shown a particular toxic effect, it is not unreasonable to predict that such an effect will be encountered in man. Unfortunately,

the converse is not true. That is to say, if the compound has been given to a number of species and none has shown a significant effect, it does not necessarily follow that human beings, when exposed to the compound, will not show some serious toxic effect.

However, these clear-cut and simple cases, where all the species used in toxicity experiments give homogeneous and consistent results, are far from being the rule. More commonly toxic effects of one sort or another will be found in some but not all the species used in toxicity experiments. These discordant results pose the real problems in the interpretation of toxicity experiments in the prediction of likely effects in man and in deciding upon a proper course of action. Again, some conclusions are obvious. A serious effect found even in only one species should disqualify a trivial compound intended for a trivial condition or purpose from further study in man. There is a tendency, however, to extend this obvious precept to one that while appealing is not obvious and is certainly retrogressive. It is often suggested that any significant effect found in any species should disqualify any compound from further study in man. Such a doctrine, of course, makes life easy for the toxicologist and for regulatory agencies, since all risk and responsibility is removed from those making the judgement. It is the case, however, that virtually every drug commonly used in human beings can be shown in appropriate experiments in animals on some occasions in some species to produce adverse effects such that, if they had been discovered prior to the use of the drug and the experiments had been assessed on the simple precept enumerated above, would have disqualified the drug from further use and removed a valuable agent from the therapeutic armamentarium. Such a sweeping generalization cannot, therefore, be sensibly used as a basis of judgement.

Certainly in all cases where adverse effects are detected in toxicity experiments the nature of the effect must be assessed against the need for the drug and the seriousness of the condition in which it is used. Indeed, this precept has a validity beyond the field of therapeutics since it must also be true of feed additives, pesticides, and industrial chemicals. Certainly, too, caution and prudence should govern this analysis but neither should be carried to the point where all action is vitiated.

With compounds sufficiently important, both in their intended use and commercially to justify the expense, it will often be possible to determine the mechanism by which they produce a disturbing