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**PRINCIPLES
FOR PRE-CLINICAL TESTING
OF DRUG SAFETY**

Report of a WHO Scientific Group

	Page
1. Introduction	3
2. General considerations	4
3. Biochemical studies	7
4. Pharmacological and toxicological studies	11
5. Relationship between animal and human studies	18
6. Concluding remarks	20

WORLD HEALTH ORGANIZATION

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SCIENTIFIC GROUP ON PRINCIPLES FOR PRE-CLINICAL TESTING
OF DRUG SAFETY

Geneva, 21-26 March 1966

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PRINCIPLES FOR PRE-CLINICAL TESTING OF DRUG SAFETY

Report of a WHO Scientific Group

The WHO Scientific Group on Principles for the Pre-Clinical Testing of Drug Safety met in Geneva from 21 to 26 March 1966. The meeting was opened, on behalf of the Director-General, by Dr P. M. Kaul, Assistant Director-General. He outlined the terms of reference for the present meeting as part of the WHO programme for the promotion of drug safety.

Dr B. N. Halpern was elected Chairman, Dr W. Koll Vice-Chairman, and Dr J. J. Burns and Dr A. Wilson Rapporteurs.

1. INTRODUCTION

The governing bodies of WHO and its Advisory Committee on Medical Research, assisted by advisory scientific groups, have explored ways and means by which WHO can contribute to the promotion of the therapeutic safety of drugs. As a result, the World Health Assembly¹ decided, *inter alia*, that generally acceptable principles and requirements for the evaluation of the safety and efficacy of drugs should be formulated under the authority of WHO. The WHO Symposium on the Toxicology of Drugs,² considering it inadvisable to establish and prescribe rigidly formulated regulations specifying in detail the tests to be performed, recommended that guidelines for the toxicological and pharmacological evaluation of safety and efficacy should be developed under scientific authority.

Agreement on the basic principles governing the testing of drugs for therapeutic safety would, indeed, appear to be all that is possible and desirable at this juncture; requirements, with their inherent tendency to be specific, detailed, and conducive to rigid application, would have little chance of being generally accepted.

¹ See Resolution WHA 17.39 (*Off. Rec. Wld Hlth Org.*, 1964, 135, 17).

² *Symposium on the Toxicology of Drugs*, 1964, Copenhagen, WHO Regional Office for Europe (mimeographed; a limited number of copies is available for persons officially or professionally concerned in this field of study on request to the WHO Regional Office for Europe, Copenhagen).

When formulating the principles for the pre-clinical testing of drug safety the Group took into account the report of a preceding WHO meeting of experts on the rationale of pre-clinical safety studies. The Group wished to acknowledge the important contributions to its work made by the preceding group and to express its appreciation to the members¹ of that group.

2. GENERAL CONSIDERATIONS

In recent years widespread concern about the safety of drugs has been developing. WHO has therefore convened groups of experts to consider this problem and, if possible, make recommendations leading to the greater safety of new drugs. It is essential, however, to stress that in this context there can be no absolute safety. The administration of biologically active substances to human beings must always be accompanied by some element of risk that cannot be avoided by the most careful and exhaustive scientific study of the drug before it is introduced.

Any situation, including the introduction of new drugs, that may involve some hazard to an individual or to a community should be judged from an evaluation of the balance between benefit and risk. This balance implies that the therapeutic aims of the drug be considered in relation to the possible risks demonstrated by the early studies to be discussed in this report. Two aspects of the intended therapeutic effects of the drug must be considered. First and most important, the laboratory studies of the efficacy of the drug must be such as to demonstrate that there is a real therapeutic interest sufficient to justify the trial of the drug in man. Second, the intended purpose of the drug is also important, since the possibility of toxic effects may be acceptable in a drug for treatment of a severe disease whereas the same potential toxic effects would prevent the trial of a drug for treatment of a relatively minor condition or one for which other drugs of greater safety already exist. Beyond these general points, however, this report does not refer to the therapeutic actions of the drug except insofar as they may be immediately relevant to the planning of effective animal studies or their better interpretation.

The best safeguard is to place the investigation of a new drug in the hands of those experienced in pharmacology and toxicology. Any attempt to lay down a rigid plan of testing is not likely to increase the adequacy of

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this safeguard. A free and intelligent approach to the toxicological problems of a new drug, as a scientific investigation in its own right, is necessary and should not be inhibited by any formal recommendations.

In considering the problems involved in evaluating the safety of a drug the Group was able to make certain divisions based on the methods and disciplines involved. The various sections of the report follow this breakdown. Each section begins with a general consideration of the problems of that area. The Group believes that its most valuable contribution may be in this general consideration of the problems, particularly where this indicates the direction in which evaluation of drug safety must evolve. Each section contains, as a summary of these general considerations, "Recommendations" which may be used as guide-lines for the rational planning of pre-clinical safety studies and as a stimulus for improvement of methods and procedures. General outlines of methods practised at present¹ and which can be regarded as useful are listed later under the heading "Recognized Procedures". These should be interpreted in the light of the preceding general considerations.

Many pharmacodynamic effects carry over from animals to man, and animal studies have a relatively high predictive value for such effects. Many toxic effects may also be predicted from observations made in animals, but, in the present state of knowledge, there are some important toxic effects that are not predictable from animal studies, and this is their main limitation. Nevertheless, increasing knowledge is steadily, if slowly, improving the value of animal and other basic studies as a mean of predicting toxic effects in man. Every effort should be made to build up knowledge of species differences and similarities in toxicological responses.

In the investigation of a new drug it is useful to establish, on a quantitative basis if possible, all the biological effects observed in treated animals

¹ Descriptions of such methods will be found in the following publications:

Association of the British Pharmaceutical Industry (1964) *First Report of the Expert Committee on Drug Toxicity*. . . 24 July 1963, London.

Deutsche Pharmakologische Gesellschaft (1963) Mitteilung des Vorstandes der Deutschen Pharmakologischen Gesellschaft und der Kommission zur Aufstellung von Richtlinien für die Prüfung neuer Arzneimittel. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmac.*, **245**, 20-31.

Division of Pharmacology, US Food and Drug Administration (1959) *Appraisal of the safety of chemicals in foods, drugs and cosmetics*, Association of Food and Drug Officials of the United States, Baltimore, Md.

European Society for the Study of Drug Toxicity (1965) *The study of the toxicity of a potential drug—basic principles*, Amsterdam, Excerpta Medica Foundation.

Food and Drug Directorate (1965) *Guide for completing preclinical submissions on investigational drugs*, Ottawa, Department of National Health and Welfare.

Interpharma (1964) *Recommendations for the pharmacological/toxicological examination of new drugs in animal tests*, Basle.

Office intercantonal de contrôle des médicaments (1964) *Directives de l'OICM concernant les documents requis pour de nouvelles substances actives (du 7 janvier 1963)*. In: *Rapport de gestion 1963*, Berne, pp. 7-8.

and, where it is feasible, to define these effects in biochemical, physiological, or other appropriate terms. This requires close integration between the pharmacological, toxicological, and biochemical studies. The report lays great stress on biochemical studies of absorption, distribution, and metabolism of drugs. Studies of this sort are particularly useful in allowing a rational choice of species to be made.

In many other areas also, new techniques and new ideas should in the next few years have an impact on the way in which the toxicity of a drug is studied. Thus, histopathology will come to be supplemented by histochemistry and it is probable that many problems at the moment inaccessible to morphological study will be clarified by the wider application of electron microscopy.

It will be clear from the various sections of the report that in many areas there is need for further research; in particular the choice of animal species for toxicity tests requires such research. It is unlikely that any one species will always be suitable for all types of investigation. The present need is for a wider investigation of the way in which species not commonly used in toxicity tests may react to drugs; in particular, research on primates is to be encouraged, although present knowledge is insufficient to allow clear recommendations to be made about the use of primates.

This report does not consider any of the problems involved in establishing the safety and efficacy of drugs by extensive clinical trial, nor problems of drugs already in wide use. It has been felt proper, however, to discuss the first administration of a drug to man and it is emphasized that the results of these early investigations are of great assistance in planning further laboratory study of the drug before extensive clinical trials. Although these early investigations may also study efficacy, many of the data obtained at this stage can be considered as a part of the pre-clinical evaluation of its safety. In the evaluation of experimental data the ultimate use of the drug in man must always be borne in mind.

Some important topics have been excluded completely or only covered in brief in this report. Particular examples are the effects of drugs on the foetus, genetic effects, carcinogenic effects, drug dependence, and the whole question of sensitization to drugs. In some cases the problems have already been, or may shortly be, considered by expert groups convened by WHO; in others the need for research is so pressing and present knowledge so inadequate that no useful consideration of the topic by the Group was possible.

There are many definitions of "a drug" and one was put forward by a WHO Study Group on the Use of Specifications for Pharmaceutical Preparations.¹ However, this definition was mainly designed for discussion

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1957, 138, 14.

of pharmaceutical and legal problems. The following definition of "a drug" is considered more suitable for this report :

" A drug is any substance or product that is used or intended to be used to modify or explore physiological systems or pathological states for the benefit of the recipient. "

The term " new drug " has also been used extensively in this report. It is recognized that this term has legal or regulatory significance in some countries. It is not, however, used in that sense here but is only meant to imply that the drug has not been extensively investigated clinically.

3. BIOCHEMICAL STUDIES

The biochemical studies discussed in this section include absorption, distribution, excretion, and metabolism of a drug. Knowledge of these factors controlling drug action is of fundamental importance for proper evaluation of toxicity.

Typical experiments on new drugs involve the administration of single doses by various routes to animals and measurement of drug concentrations in body fluids and tissues. The purpose of these studies is to estimate the rate and degree of absorption, rate of disappearance from the body or body fluids, renal excretion and localization in tissues. In many instances, simple linear relationships can provide estimates of these and other parameters.

The value of quantitative studies of this type has been established. This information facilitates extrapolation of animal data to man, discloses metabolic products with therapeutic or toxic effects, and provides the rationale for development of suitable dosage regimens.

3.1 Method

The studies discussed here require methods for the assay of the drug in biological fluids and tissues. Most drugs can be assayed by a relatively few procedures such as spectrofluorescence, chemical coupling, ultraviolet absorption, and complex formation with dyes. For some drugs, the use of isotopic tracer methods may be necessary.

The specificity of the method must be known and may be established by techniques such as gas chromatography, thin layer chromatography, paper chromatography, and countercurrent distribution. In some instances, methods of low specificity may give useful information in the preliminary study of a new drug.

3.2 Absorption, distribution and excretion

It is essential to demonstrate whether the drug is absorbed after administration by the route and in the species intended to be used for the toxicity study. The extent of absorption may sometimes be conveniently estimated by comparison of blood levels after oral administration with those after parenteral administration, or by determination of the drug in the faeces. The extent and rate of absorption of a drug will depend upon a number of factors, including the lipid/water partition, the physical state in which the drug is administered, the solubility of the drug in the body constituents with which it comes into contact, and the blood flow through the area of absorption.

Any drug will be distributed in the body fluids and tissues in a particular pattern that may vary with time and dose. Drugs vary in their ability to enter cells, to cross membranes and to pass barriers, e.g., the blood-brain barrier or the placenta. A further point of great potential toxicological importance is the binding of drugs to proteins or other macromolecules in blood or tissues. Drugs may compete with each other or with body constituents for the same binding sites on a protein. Thus, the administration of a drug may interfere with the binding of some body constituent, such as bilirubin, or it may cause displacement of some other drug from a protein to yield an unbound active form which may give rise to toxic effects. Qualitative and quantitative differences in protein binding also occur from species to species and such differences may account for variation in pharmacological or toxicological effects when different animal species are studied.

The relation between the systemic activity of a drug and its concentration in plasma should be determined. Absence of such a relationship would suggest the need to consider, for example, whether activity was due to a metabolite or whether the drug has a persistent effect resulting from irreversible binding to tissue components.

It is of considerable importance to know the route by which a drug is excreted. The drug may be excreted unchanged or in the form of conjugates or metabolites. The most important organs for the excretion of drugs are the kidneys, liver and lungs. The extent and rate of excretion of a drug in urine and bile may depend on such factors as lipid/water partition, acid or base dissociation, or molecular weight.

3.3 Metabolism

The term metabolism in this report is taken to include any alteration of the drug in the body. Metabolism of the drug may affect not only activity but also toxicity, and either property may be enhanced or diminished. The importance of drug metabolism can be illustrated by two examples. First, a drug may be so rapidly metabolized in the body that an

effective level cannot be attained ; secondly, a drug may be so slowly metabolized that it is not readily eliminated and therefore is accumulated in the body.

Drug-metabolizing enzymes catalyse processes such as oxidation, reduction or hydrolysis. Conjugation of drugs or metabolic products of drugs may occur in a variety of ways. Although the process of drug metabolism generally yields products that are more readily eliminated than the parent drug, there are also instances where metabolites accumulate in the body. An attempt to account for the total administered dose of a drug and its metabolites in the tissues and the excreta may be of value in revealing accumulation of the drug.

3.4 Factors controlling drug metabolism

3.4.1 Species and individual differences

The rate and pattern of metabolism of a drug may vary between species and this may make prediction of effects in man difficult. When these rates and patterns have been obtained in man and compared with information from other species, extrapolation of toxicological and pharmacological data to man is facilitated. Differences in drug metabolism also occur within the same species, which may explain individual differences in response to drugs. Genetic factors appear to be important in determining these differences.

3.4.2 Enzyme induction

The administration of a drug can stimulate its own metabolism or that of a subsequent dose of another drug. Drugs can exert this action by increasing the amount of drug-metabolizing enzymes in the liver and this is referred to as enzyme induction. This effect occurs in all species that have been investigated, including man. It has been shown to occur with many drugs of widely different chemical structure and pharmacological activity, including barbiturates and other hypnotics, tranquilizers, analgesics, anti-histamines, oral antidiabetics and uricosuric agents. Enzyme induction will have a profound influence on long-term toxicity studies, especially when the drug is administered at a fixed daily level. If this phenomenon is not recognized, misleading results may be obtained, since the level of active drug in the body may be much lower at the end of the test than at the start.

Enzyme induction may also explain some unusual effects that are observed when two drugs are given. For example, treatment of animals with phenobarbital for several days enhances the metabolism of a variety of drugs with resulting reduction in their pharmacological activity. If a metabolite has more activity than the parent drug, enzyme induction may enhance the drug's action.

Not only drugs but certain substances present in the environment, such as insecticides and polycyclic hydrocarbons, can cause enzyme induction. Exposure of several animal species, including monkeys, to insecticides such as chlordane and DDT stimulates the metabolism of a variety of drugs. These observations must be considered in the care of animals and in the design of experiments.

3.4.3 *Enzyme inhibition*

A drug may also inhibit the metabolism of another and thus intensify and prolong the pharmacological action of the latter. For instance, inhibitors of cholinesterase can enhance the action of some parasympathomimetics and other esters. Monoamine oxidase inhibitors block the metabolism of certain sympathomimetic amines, and hypertensive crises have occurred in patients receiving monoamine oxidase inhibitors who were given sympathomimetic drugs or who have eaten cheese with a high tyramine content. The action of coumarin anticoagulants is potentiated when their metabolism is inhibited by some drugs.

3.4.4 *Age of the animal*

The activity of some drug-metabolizing enzymes may be very low in the new-born and young animals of many species. When drugs that are normally detoxified by metabolism in adults are given to such young animals, unexpected and severe toxic effects may be encountered.

3.4.5 *Sex of the animal*

Sex differences in the rate of metabolism of drugs occur in several species. For example, some drugs are metabolized more rapidly in male than in female rats and this difference may account for the greater sensitivity of the female to the action of such drugs.

3.4.6 *Pathological state of the animal*

The existence of pathological conditions in the major organs of drug metabolism or excretion may markedly alter the degree or duration of drug action, and cause the appearance of toxic effects. However, pathological changes do not necessarily imply that the metabolism or excretion of drugs will be altered.

RECOMMENDATIONS

(a) The general intention of the biochemical studies considered in this section is to determine those parameters, such as plasma concentration, biological half-life, drug distribution and metabolism, that have an impor-

tant relationship to drug effects. The correct interpretation of this work will enable many aspects of the investigation of toxicity to be more rationally based. Thus, such studies should always be considered and whenever possible they should be carried out.

(b) The type of biochemical study and the aspects to be emphasized will depend on the drug and on the nature and stage of the investigation.

(c) These studies are most useful when they are closely related to, and integrated with, all other phases of drug safety evaluation.

(d) Biochemical studies can be successfully carried out only by an investigator who is experienced in this work and is well aware of their relevance and limitations.

4. PHARMACOLOGICAL AND TOXICOLOGICAL STUDIES

4.1 Pharmacological studies

In every case, a detailed study of the properties of a drug is a prerequisite for the evaluation of its safety. In general, these studies have two purposes: first, to define the general pharmacological actions of the drug and, second, to estimate its intended therapeutic properties. The methods will not be discussed since they differ according to the class of drug.

4.2 Toxicological studies

As already stated in section 2, the detailed planning, execution and interpretation of toxicological studies should be left to the investigator. However, there are some points that call for special comment. These will be briefly discussed under three headings: the drug, the animals used, and the procedures employed.

Procedures fall into two classes: those using single administration (acute toxicity studies) and those using repeated administration, which include short-term or subacute studies (less than three months); long-term or chronic studies (three to six months); and life-span toxicity studies.

4.2.1 *The drug*

4.2.1.1 *Specifications*

Adequate specifications should be available before toxicological studies are made. These specifications should identify the drug, define its stability, and establish limits for impurities. If the specifications are altered for one reason or another, for example by a modification in the method of preparation, it is necessary to assess these changes in relation to the toxicological studies carried out on the original drug preparation.

4.2.1.2 *Physical state of the drug*

The physical characteristics of the drug, for example, solubility and particle size, have an important influence on activity. The nature of the vehicle is also relevant.

4.2.2 *Animals used*

4.2.2.1 *Choice of animal*

Animal species differ widely in their response to drugs, and the way in which the drugs are absorbed, distributed, metabolized and excreted. The rat and the dog are frequently used, especially for chronic toxicity studies, and have the advantage that their reactions are well documented. However, the evaluation of species other than those conventionally used should be encouraged in an attempt to select species that absorb and metabolize drugs in ways as similar as possible to man. Differences in the response to drugs are also observed between strains. Many of the well-known strains, such as Wistar or Sprague Dawley rats, have undergone great changes in different laboratories, so that these strain names have little meaning. Except in special cases, there is no need to use highly inbred strains.

4.2.2.2 *Management*

Healthy animals are required for toxicological work and animals obtained from uncontrolled sources should be avoided. In recent years, animals largely free of pathogens, called specific-pathogen-free (SPF) or pathogen-controlled animals, have become available in some centres. Evidence is accumulating that for many purposes, and particularly for long-term studies, these animals are superior to conventionally reared animals, and for such studies their use is to be encouraged. At present only rats and mice are commonly available, but the provision of other pathogen-controlled species should also be encouraged. The drug response of animals is influenced by a variety of factors, such as diet, season and environmental temperature; the use of insecticides or anthelmintics may also alter some drug responses. Institutions concerned with the breeding, care and study of laboratory animals already exist in some countries and their establishment in others should be promoted.

4.2.2.3 *Numbers used*

Experiments will often be evaluated statistically and the numbers of animals used must be in accord with statistical requirements. It is recognized, however, that for a variety of purposes useful information may be obtained from experiments using very small numbers of animals.

4.2.2.4 *Physiological and pathological state of the animal*

Many physiological factors may influence the effects of drugs, such as sex, endocrine activity and state of nutrition of the animals, as well as the administration of the drug before, with, or after food. The relevance of these and other factors must be borne in mind by the investigator.

It has been suggested that toxicity studies should include investigations in immature animals. In our present state of knowledge the relevance of these studies to man remains to be assessed, because the stage of development of the new-born animal varies greatly from species to species. A fruitful approach might be to establish in greater detail in man and animals the patterns of enzyme and organ development that are relevant to drug metabolism and toxicity. Information of this nature might enable better prediction of toxicity in infants to be made.

Pathological states may modify the toxicity of drugs. The use of animals with induced, spontaneous or genetically determined disease cannot yet be generally recommended for toxicity studies since the relevance of such states requires further study.

4.2.3 *Procedures employed*

4.2.3.1 *Single administration*

The aims of acute toxicity studies are to define the range of lethal dose and the effects on important functions such as locomotion, behaviour and respiration. These signs often furnish information on the cause of death and may be supplemented by dissection and sometimes by histological examination. The time course of these events may vary considerably between drugs. Furthermore the use of several routes of administration provides important though preliminary data on absorption and distribution.

The volume of solution administered is important, as is also the rate at which intravenous injections or infusions are given.

4.2.3.2 *Repeated administration*

The aims of toxicity studies using repeated administration are to reveal untoward effects that occur when a drug is used over a period of time and to show how these effects are related to dose. Attempts have been made to relate the duration of these tests to that of the therapeutic administration. However, experience has shown that all relevant information can be obtained from a three to six months' study. Tests of shorter duration may be appropriate before the preliminary study of the drug in man (see section 5); their exact duration must be left to the judgement of the investigator. Short-term studies may also be useful in elucidating such problems as cumulative toxicity, tolerance, and enzyme induction phenomena. Tests of longer duration are mandatory for the assessment of carcinogenicity. Throughout

the experiments valuable information can be obtained from detailed clinical observations of the animals, including urine and blood analysis and, where appropriate, the examination of biopsy material.

It is conventional to record the weight of many organs, but this may be restricted to those that are likely to provide useful information. Histo-pathology is a reliable and sensitive method for detecting toxic changes in the tissues. New histochemical techniques and electron microscopy are likely to make a great impact in this area in the future.

4.2.3.3 *Carcinogenicity, teratogenicity and other special problems*

Assessment of a drug for carcinogenicity requires prolonged, detailed and exacting studies, the results of which are not always conclusive. Nevertheless, special attention should be given to carcinogenic assessment of compounds which are structurally related to known or suspected carcinogens and of drugs that affect mitosis or may be taken for a long time or are likely to be retained in the tissues.

The assessment of carcinogenic risk in connexion with the toxicology of food additives has been considered in a report of the Joint FAO/WHO Expert Committee on Food Additives,¹ which should be consulted for appropriate methods.

Although teratogenicity tests are generally done on all new drugs, the predictive value of such tests is open to question. Other special problems concern the effects of drugs on fertility and reproduction and on hereditary mechanisms, as well as those toxic effects that are not at present predictable from animal experiments, e.g., some types of blood dyscrasia, drug-induced jaundice, neurotoxicity, and sensitization reactions.

Some of these problems merit special consideration, as already mentioned in section 2.

4.2.3.4 *Problems in the use of two or more drugs*

The problem of adverse reactions arising from the use of two or more drugs has given rise to much concern. When two or more drugs are combined in a formulation, interactions may occur that may modify the activity and toxicity of the components. Furthermore, two or more drugs are frequently administered concurrently and interaction may even result from the presence of a long-lasting drug administered previously. Certain dietary components and alcohol may also interact with drugs. Some of these interactions may be revealed by appropriate pharmacological study.

One aspect of drug interaction is connected with competition and displacement phenomena occurring at different binding sites. These include blood proteins, metabolizing enzymes, transport and excretion mechanisms, and receptor sites.

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1961, 220.

Although no general principles can yet be established for all drugs, considerable advances in the understanding of these problems have already been made and further research in this field should be actively pursued.

4.2.3.5 *Drug interaction*

The effects of drug interaction should be studied when the new drug is formulated together with another drug, or when the initial trial in man requires the new drug to be given in addition to concurrent therapy. Such studies may vary from a pharmacological appraisal to a complete toxicological evaluation.

4.3 **Recognized procedures**

From the review conducted by this Group it is clear that many procedures are widely used and generally recognized. Some of the more important are the following.

4.3.1 *Pharmacological studies*

(i) The effects of all drugs should be studied on the various systems, such as the cardiovascular, respiratory, central and autonomic nervous systems, and on neuromuscular functions.

(ii) In appropriate cases evidence should be obtained from experiments that have been devised to resemble the conditions in which the drug will be used for clinical purposes.

4.3.2 *Toxicological studies*

4.3.2.1 *General*

(i) Toxicity should be studied in several species, three or more in acute experiments and two or more in long-term investigations. One of the species should be non-rodent. Both sexes should be studied. In at least one of the species selected, the drug should, if possible, have an activity related to the expected therapeutic effect.

(ii) The investigation should if possible include experiments conducted with the drug in the vehicle intended for therapeutic application. Control experiments should be done with the vehicle alone. A new vehicle requires a toxicological study in its own right.

(iii) For drugs intended for topical application, the possibility of toxic reactions following systemic absorption should be explored.

4.3.2.2 *Acute toxicity*

(i) Three or more routes of administration should be used in at least one of the three or more species mentioned above. Studies should include

administration by those routes that are intended for clinical use and at least one should be systemic.

(ii) Several dose levels, spaced logarithmically, should be used. The doses should range if possible from that which causes no significant effect to that which kills nearly all the animals.

(iii) Observations should be extended over the period during which signs are present or may be expected ; at least one week after administration of the drug is necessary. The number of deaths and the time of their occurrence must be recorded.

(iv) When the number of animals permits statistical evaluation the method used for estimating the lethal dose should at least provide for the expression of the LD₅₀ with stated limits of error. If the toxicity of the drug is too low for a defined lethal dose to be established the highest dose administered should be recorded.

4.3.2.3 *Chronic toxicity*

(i) For long-term toxicity studies the selection of species should be guided by evidence obtained from acute toxicity tests and from metabolic studies. Thus, whenever possible, species should be chosen that are sensitive as well as those that have a metabolic pattern resembling that of man.

(ii) Studies should include administration by those routes that are intended for clinical use and at least one should be systemic.

(iii) More than two dose levels should be used. At least one experimental group should receive a dose that is toxic and kills some of the animals. Other dose levels should be included that are related to the proposed clinical dose.

(iv) Detailed observations should be made throughout the course of the experiment on pertinent parameters, such as changes in appearance and behaviour and in rate of body-weight gain ; urine and blood analysis, including haematology, should be performed.

(v) Detailed *post mortem* studies should include comprehensive histological examination. The local response of the tissues at the site of drug administration should also be recorded.

RECOMMENDATIONS

(i) Pharmacological studies and acute and short-term toxicity tests must always be carried out before the early administration to man recommended in this report. Long-term toxicity tests and extensive studies will be carried out before extensive clinical trial. Procedures for the conduct of such investigations are included in documents drawn up by many national groups and these should be consulted for details of such methods. The following points are thought to merit special comment in this report.

(ii) A knowledge of the pharmacological actions of all potential drugs of any kind should be sought, since it is essential for understanding and predicting possible toxic reactions in man. The most frequent undesired reactions to drugs arise from processes that are an extension of the main pharmacological action or of a secondary action.

(iii) In experiments using large numbers of animals statistical analysis is essential. However, it should be stressed that careful and detailed observation of small numbers of animals can give valuable results.

(iv) Knowledge of the pharmacological properties of the drug and the reaction of different species to it may guide the choice of species for toxicity studies. Comparative biochemical studies of absorption, distribution, metabolism and excretion may also be used for this purpose and are of great value. The limitations of biochemical studies of this sort must be understood before this approach can be used intelligently.

(v) The duration of toxicity tests may be difficult to decide upon on a wholly rational basis, although some ideas at present under investigation in various centres may ultimately provide such a basis. Experience suggests that it is not necessary to exceed six months except for certain special studies, like that of carcinogenicity.

(vi) It is difficult to specify exactly what studies should be made on animals during toxicity tests since different observations will be relevant for different species and different drugs. Attention should be drawn to investigations that give information about toxic actions that are not otherwise easily detected. Functional or structural abnormalities of the special sense organs are difficult to detect in toxicity tests and this point requires further research. However, ophthalmoscopy and slit-lamp examinations of the eye are often carried out in larger animals and give valuable information. Electrocardiographic and other dynamic studies of the cardiovascular system are generally desirable and sometimes essential. Biopsy of important organs during the course of a test may be invaluable. Elaborate behavioural studies, although sometimes recommended, are of rather doubtful relevance. Experienced investigators will be aware of many other types of investigation that sometimes give useful information and many discussions of this subject have been published.

(vii) Histopathology remains the most sensitive method of detecting tissue damage, and comprehensive studies of this sort must always form part of any toxicity test. Newer morphological methods like histochemistry and electron microscopy give much information about cell structure and function that cannot be obtained in any other way. Electron microscopy has shown that changes in the endoplasmic reticulum of liver cells may indicate that enzyme induction has occurred. In addition, changes that can be observed by conventional microscopic techniques but that cannot be interpreted when these techniques are used alone can often be elucidated

when electron microscopic or histochemical studies are made. Both electron microscopy and histochemistry require special experiments for their maximum utility and they cannot be used as screening methods in the way that conventional histopathology has been used. When used to answer special questions, however, they will be found to be an invaluable adjunct to the study of toxicity.

Valuable methods of investigation are constantly being developed. They may add information to studies of toxicity and their use should be encouraged.

(viii) Interpretation of the result of toxicity tests remains the most difficult and critical part of the study. The mere accumulation of masses of data is not a substitute for an intelligent interpretation of the work. When this requisite is lacking it is doubtful if meaningful studies can be carried out.

5. RELATIONSHIP BETWEEN ANIMAL AND HUMAN STUDIES

5.1 Timing and nature of first studies in man

Some of the animal studies recommended in this report will have been carried out without any knowledge of the absorption, distribution and metabolism of the drug in man. The best situation for the study of new drugs in laboratory animals is that in which the species used resemble man in the way the drug is absorbed, distributed, metabolized and excreted. Such species cannot be chosen until the drug has been given to man and, therefore, before laboratory studies are completed, it is important that this element of uncertainty be removed as far as possible by an investigation in man. Obviously an element of risk is involved in these studies, but it must be stressed that there is always some risk when a drug is given to man for the first time. It is believed that the procedure here recommended minimizes this risk, and this is the objective of all toxicity studies.

Before a new drug can be administered to man for this purpose it must be established by preliminary study that useful information may be obtained from the human investigation and also that the risk to the humans receiving the drug in this way is minimized. The first point requires that some measure of the way the drug is absorbed and metabolized is available. This will usually consist of methods for the determination of the drug and possibly its metabolites in blood and urine. Sometimes an easily observed and measurable specific effect may permit an estimate to be made of the degree of absorption and duration of action of the drug.

To minimize the risk in this investigation it is essential that full pharmacological study of the drug as well as acute and subacute toxicities with histopathological evaluation of the latter shall have been completed. The

duration of the subacute tests need not be as long as will be required ultimately for the drug, but should be sufficiently long for major pharmacological and toxic effects to have become evident. The tests should involve at least two species, one of which should not be a rodent.

If, bearing in mind these two requirements, it is decided to administer the drug to man at an early stage of the investigations, the main objective must be to obtain information about absorption, distribution, metabolism and excretion of the drug in man that will permit more meaningful laboratory work to be carried out in the further experimental evaluation of the drug.

However, to carry out such studies it will usually be desirable to build up from very small doses which will permit the biochemical studies to be undertaken. This preliminary dose-range study will require great care.

It may well be that the clinician carrying out such studies will at the same time be able to form some opinion as to the efficacy of the drug for the purpose for which it is intended, and for this reason, and for others, may prefer to use patients suffering from the disease in which it is believed the drug may have desirable actions. However, the information required for interpretation of the animal studies may also be obtained from healthy human beings.

When it is decided to administer the drug to patients rather than to healthy human beings, the clinician should be reminded that the illness or the other treatment that the patient is receiving may interfere with the new drug, even to the point of vitiating any observations and, of course, that the effects of the drugs already used in treatment may be modified by the new drug. The choice of subject is plainly the responsibility of the clinician conducting the investigation.

Although the responsibility belongs to the clinical investigator, it is thought proper to point out that laboratory workers must make available to him the results of all the work so far carried out with the drug, and equally that the clinical investigator must regard it as an important part of his responsibility to familiarize himself with these data. From the pharmacological and toxicological studies he should be able to determine what doses may properly be used in his investigations and what observations are relevant. This may be important for the early detection of toxic effects that may be anticipated from animal studies.

RECOMMENDATION

Facilities, attitudes and legal restrictions vary widely from country to country and therefore the precise way in which the first administration of a drug to man is carried out must be left to the investigators concerned. It is stressed that the early administration of drugs to human beings forms an essential part of the evaluation of safety and great efforts must be made to

facilitate such investigations because their results are needed for the planning of further study of the drug.

5.2 Feedback of information on adverse reactions

There are some toxic effects that cannot be predicted from a study of the chemical and physical properties of the drug or from information obtained about its biological effects in animals. There are also some toxic effects seen in man that have a relatively low incidence, which makes it unlikely that they will be detected in clinical trials. Both these difficulties make it important that the use of any new drug should be monitored for two or three years after it has been placed on the market. It is to be hoped that both types of toxic effect will become predictable in the future from animal studies. This is most likely to occur if the toxic effects encountered in man are carefully studied. Animal experiments may usefully supplement such studies but are not a substitute for careful clinical investigation.

6. CONCLUDING REMARKS

This Scientific Group was required to formulate generally acceptable principles for the pre-clinical evaluation of drug safety ; the foregoing document is the result of its considerations. To arrive at this position the Group and its predecessor had to review the methods that are at present used to establish the safety of a drug and that are detailed in several documents prepared by many national groups, and in other publications on the subject. In the general consideration of these methods the Group has commented upon them briefly, emphasizing those that it believes to be of particular importance. As a result of this review, however, it became clear that present methods when applied intelligently and conscientiously are useful, but do also have some limitations, a view shared by many concerned professionally with safety evaluation.

The main limitation arises from the phenomenon of species variation, which makes extrapolation of results from one species to another, and particularly to man, extremely difficult. There are many factors concerned in species variation, one of which is the metabolism of drugs. Methods are becoming available by use of which the absorption and metabolic fate of drugs can be studied. This is because research in methodology has developed a number of techniques that are sufficiently sensitive to detect the small concentrations of drugs and their metabolites that are found in body fluids and tissues. Unfortunately it is not yet possible to detect all drugs and their metabolites in the body in this way. When a method can

be developed the investigator has a powerful tool for the study of many important factors controlling drug actions. This Group has therefore laid particular stress on such an approach, partly at least because in no other publication available to the Group was this approach adequately discussed. To be of maximum utility in allowing meaningful studies of drug safety to be performed, studies of drug metabolism must be carried out in man at an early stage of the drug's development. Of course, the exact stage in the study of a drug when it will be proper and desirable to give it to man for studies of its absorption and metabolism will depend on a number of factors. It cannot be laid down and must be left to the conscience and judgement of the clinical investigator, in consultation with those who have carried out the laboratory studies. There are, however, certain prerequisites that can be described in general terms, and that have been discussed in this report.

The Group therefore feels that in appropriate cases the risks associated with the introduction of a new drug will be minimized if its study follows a pattern in which activity is established in experimental animals by a thorough study of the pharmacological properties of the drug and then methods for estimation of the drug and its metabolites in biological systems are developed. Both its toxicity and metabolism will then be studied in laboratory animals, although not as exhaustively as would be the case if the drug were to be submitted to extensive clinical trial. When all concerned are convinced that sufficient has been done to minimize the risk represented by a small number of controlled administrations to human beings, then absorption, metabolism and excretion of the drug should be studied in man, to a degree sufficient to permit a comprehensive study of toxicity in animals on a more rational basis. In particular, it should then be possible to choose species that resemble man in the way in which the drug is absorbed, distributed, metabolized, and excreted.

The Group has recommended that this approach be considered, and adopted when possible. It is recognized that such an approach may not always be possible or appropriate for a variety of reasons and it may pose problems of its own. Nevertheless, when these studies are appropriate their effect on the evaluation of safety will be profoundly beneficial and will supplement what can be learned from present techniques.

The discussions have constantly stressed the need for an intelligent approach to the evaluation of the safety of a drug as a research problem in its own right, and this cannot be repeated too often. The application of all relevant new techniques to these problems must be actively encouraged even when their immediate usefulness cannot be seen clearly enough for them authoritatively to be recommended. With these ideas in mind, a discussion of the phenomenon of enzyme induction has been included. Work on this subject is relatively recent and is constantly extending our knowledge of drug metabolism. It is not yet possible to recommend pre-

cisely how this phenomenon should be studied in each case but clearly those concerned with toxicity tests should consider in their experimental plans the implications of enzyme induction.

Unfortunately, there is a great shortage of scientists trained in subjects appropriate to the study of drug toxicity and steps must be taken to attract capable investigators into this field. In the last few years, scientific societies concerned with the study of toxicity have been constituted. These societies and other national and international bodies have sponsored seminars, symposia, workshops, and large scientific gatherings devoted to various aspects of the subject. These activities have stimulated interest in this subject among scientists and are to be encouraged. The Group feels that there is room for even more activity of this kind, and in particular for workshops and seminars and special training programmes for young scientists.

Many areas considered in the report need further research and this is pointed out. Further research will be amply rewarded by decreasing meaningless work carried out because fundamental knowledge is lacking and by increasing safety.