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June 14, 1967

TO: Members of the Food, Drug, and Cosmetic Chemicals Committee

SUBJECT: PMA Paper

Gentlemen:

I am enclosing a copy of Dr. Lehman's paper given at last fall's PMA meeting. It has been suggested that you will be interested in it.

Sincerely yours,

Morgan M. Hoover

MMH:sjg

Enclosure

THE PHILOSOPHY BEHIND PRE-CLINICAL TOXICITY STUDIES

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The underlying principles involved in animal experimentation for predicting safety of drugs, intentional and unintentional food additives, and other chemicals to which man may be exposed, can be of great value. However laudable studies in animals may be, the approach to the problem is often questionable. Twenty or thirty years ago, animal data served fairly well for assessing the kind of toxic responses that might occur in man. The chemicals tested were substances to which the human race had been exposed for many years. Thus, the toxic responses could be extrapolated to man with a fair degree of certainty. The rapid development in food and drug technology has changed all this. Introduction of hundreds of new synthetic chemicals has brought with it a host of new problems. The impact of these new substances with which man had no previous experience began to be recognized by the lack of correlation of animal data with data obtained in humans. As the apprehensiveness of safety testing grew, the toxicologist increased the numbers of animals used in his investigations and prolonged the duration of his observations. Toxicological studies tended to assume a standard minimum one-shot deal. This eventually led to what some regarded as playing a numbers game. Since it is not feasible to monitor large groups of animals with any degree in depth, reliance was placed on results of prolonged toxicity trials. Readily detectable changes were recorded, such as effects on weight, growth, and mortality rates, occasional urinalysis and hemograms, followed by autopsy

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and histopathology at termination of the experiment. Actually, these parameters cannot be assured to have much relevance nor can they be directly transposed to man.

The ease with which this once-and-for-all type of toxicity trial can be carried out is probably responsible for the predominent interest in this procedure. However, modern scientific technology is rapidly changing our concept of what constitutes toxicology. Since continuous and rapid change is inevitable and normal, the task is to remain open-minded to accommodate these rapid changes. With present-day technology, it is now possible to correlate functional change with morphological state as an early indication of prospective, not retrospective events. A careful study of alterations in physiological functions in the living animal greatly strengthens the validity of toxicity trials. The need to innovate and develop new approaches to narrow the gap between the predictive value of animal data and experience in man was pointed out recently by the President's Science Advisory Committee[1]. The National Academy of Sciences' Subcommittee on"Some Considerations in the Use of Human Subjects in Safety Evaluation of Pesticides and Food Chemicals"[2] also emphasized the need for more certain methods for translating animal data to man. The World Health Organizations' Scientific Group on Principles for Preclinical Testing of Drug Safety[3] will issue a report laying great stress on biochemical studies.

Very few systematic studies have been designed to meet these objectives. Present methodology has been criticized as empirical, mechanical,

and even pointless, and not based on any fundamental scientific principles. It is imperative then that animal testing procedures be modified and thoroughly developed so that their technical performance will yield results reflecting more closely human characteristics and reactions. This sophistication in methodology, having its beginning about 10 years ago, is now well on the way.

Modern safety evaluation of drugs and other chemicals in man's environment calls for studies of specific organs and tissues. This involves a multidisciplinary approach utilizing the principles of biochemistry, pharmacology, and pathology. New instrumentation and technics are employed to give an insight into early alterations in structure and function at the subcellular level. The major aim is to develop clearly defined biochemical or pharmacological responses in the live, intact, unanesthetized, animal by analytical procedures applicable to man.

Much has been written on the subject of species variation. However, when the interpretative stage of a safety test is reached, the animal data are extrapolated to man, while quite frequently the investigator is fully aware such application is not completely reliable in predicting responses in humans because of interspecies differences. At the same time, the well-documented pharmacological phenomenon of species specificity is rigidly applied in any evaluation of efficacy. Only evidence in man is acceptable.

Modern toxicologists are fully aware of species difference and have learned a great deal about how chemicals, whether drugs, pesticides, or food additives, are handled in the body. Thus, the conventional practice of slavish devotion to old procedures is being rapidly supplanted by not

concentrating on rats and dogs but intensively studying the test substance in four or five species and developing evidence of the events occurring at the subcellular level. The objective is to seek measurable parameters which may be useful for a logical prediction of toxicity in man. Since the most useful species for further testing will be that species which has predicted certain events in man, it is desirable to develop as early as possible how the substance is handled in man. The early and cautious clinical trial data will make the choice of the proper animal species for further study less difficult. The toxicologist now has a greater assurance that the responses in the species he has chosen bear a reasonable resemblance to responses in man.

In the initial clinical trial of a new drug, the patient or volunteer is treated as an individual and great attention is given to details. The average hospital employs 242 persons to care for every 100 patients. In most hospitals only 20 per cent of space is allotted to beds. The remaining 80 per cent is occupied by equipment for tests and procedures, administration, engineering, and maintenance departments necessary to keep the hospital functioning 24 hours a day[4]. An animal on experiment is performing a very important function. If anything more than a few routine responses are to be recorded, the animal, too, should receive the full attention of the investigator. Dr. Karl Beyer once stated that he would not entrust a study to a toxicologist whose only other qualifications were a compound and a roomful of animals.

Although conventional safety testing methods have been useful, there has been too much trading on convention to the point of losing sight of the serious purpose of animal studies. An inherent problem in older

toxicological investigations has been an over-dependence on animal tests. A few examples will point this out.

The LD-50

This procedure has developed into an empirical screening test for toxicity. Very little attention is given as to whether or not solvent, vehicle, or suspending agent bears any relation to the ultimate formulation in which the test substance may be administered to man. At this early stage of testing, probably little is known about the physical state of the drug, less about its biochemistry, and many other circumstances which are different between animal experimentation and clinical trial. Data are usually obtained in a standardized animal as against an unstandardized human. Animals are in good health, whereas the drug may be tried first in ill patients. This does not mean that tests should be done in mongrels or sick animals. It does point out, however, the limitations that can be placed on a statistically calculated LD-50 as a measure of safety in man. Since animals on an LD-50 test are often kept under observation for a few days to a few weeks, much can be learned about absorption, transport, metabolism, distribution, and excretion during the observation period. Instead, for all practical purposes, only death is taken as the endpoint. Absolute toxicity of any compound is of relatively little interest. An LD-50 offers little more than a guide for dosage to be employed in a multiple-dose test.

Long-Term Studies

Rats are almost routinely chosen for this purpose. Dogs are frequently used. In either case there is usually little or no knowledge

as to whether there is any close resemblance to man in the species response to the test substance. The large groups of animals on experiment preclude any detailed studies in depth, and thus criteria of effects are limited to gross observations. A prodigious mass of data results, and great difficulty is often encountered in assessing its value. Interspecies differences are apt to complicate assessment. Even if some detailed studies were incorporated into the design, this effort would be a waste of time and biological material without knowledge of similarities and differences between the rat, dog, and man. If the investigator decides it is necessary to conduct a long-term study, then the importance of choosing the species closely resembling man becomes obvious. There may be some justification for long-term studies in testing for carcinogenicity. However, the predictive value of animal data has been and still is the subject of much discussion[5].

Histopathology

Although the design of a long-term test and its validity in predicting toxicity in man has been frequently discussed, surprisingly little attention has been given to the value of histopathological examination of tissues. As a rule, histopathological reports are presented in great detail, listing ratios of organ weight to body weight, naturally occurring diseases and senility changes, statistical calculations of significance or insignificance of spontaneous or other neoplasms, and some variations from normal which the pathologist cannot ascribe to treatment. The relevance of these observations, many of which may have been produced by large and possibly overwhelming doses, is not mentioned. The highest dose level

producing no histopathology is generally taken as the "no-effect" or safety level. Histopathology is important and certainly has its place in final evaluation of safety; nevertheless, the purpose of a toxicological investigation is to offer some predictive evidence of possible harm. This retrospective approach seldom meets the objective. Even in clinical practice the value of the autopsy is a controversial issue[6,7].

Margin of Safety

An arbitrary mathematical calculation has been used to assess safety of a chemical. The "100-fold" margin of safety has been widely employed and extensively applied to food additives and pesticides. Two definitions are applied to "100-fold margin of safety." When applied to a food additive, it should be possible to feed to animals, without effect, 100 times the amount proposed for the human diet. For pesticides, the safe intake for humans is one-hundredth of the dose producing no effect in animals. Neither the animal species nor the criteria for "no-effect" are specifically named or defined. This irrational application of a mathematically calculated safe dose is exemplified by variations which have been introduced. These variations range all the way from about 10 to 500. Obviously, mathematical rules for margins of safety cannot be applied to drugs. It is more appropriate to assign a safety index, the ratio between minimal effective dose and the maximal tolerated dose based on clinical experience.

Simple Methods

Expressions such as "the simple laboratory test," "the easy and simple test," or "a simple screening method" are rather common in laboratory parlance. No doubt an investigator, in attempting to monitor a large

group of animals, is faced with the fact that the conventional indices usually applied offer little proof of safety of the chemical being investigated. He may attempt to fill the gap by using "quick and easy" tests for added confidence in his final conclusions. A chromatographic paper has been developed and described as "an accurate, simple, reproducible micro method for the determination of urea nitrogen in either serum or plasma." The accuracy and precision of these paper strips were carefully evaluated by comparison with two acceptable quantitative procedures. Results showed that some strips were not accurate, lacked precision, and gave no or only partial migration. The migration process was also quite temperature-sensitive, and the strips were not stable even when stored in accordance with the manufacturer's instructions(8). Hemoglobinometry, introduced almost 90 years ago, has been and still is an important clinical laboratory test. The test appears simple enough to perform. Many methods have been devised to improve accuracy and uniformity of results, and yet surveys have shown discrepancies and inaccuracies in this"simple" analytical procedure(9). An editorial commenting on the bromsulphalein test lists many pitfalls in carrying out this "extremely simple" procedure(10). Use of certain detergents in cleaning glassware has been suspect, and even method of transportation of specimens from bedside to laboratory has created problems. If a simple laboratory test actually exists, it must be an extremely elusive one.

Re-use of Animals

An animal in which various normal baselines have been established represents a considerable expenditure of time and effort. This investment

increases as the animal is placed on test. Modern toxicology makes every attempt to avoid death, since efforts are directed towards developing evidence of early signs and symptoms of toxicity without sacrifice or death of animals. Previous reference to the need for individual monitoring indicates that only a few, generally no more than six of larger species, may be placed on experiment. An animal or two may be lost during an experiment, but the majority may survive. A well-designed experiment should have developed a fairly comprehensive index of effects of the test substance. There is actually no need to sacrifice the survivors for an exhaustive histopathological examination. When baselines have returned to normal, or have been re-established, there is no reason why these valuable animals cannot be re-used for other toxicity trials. This has its counterpart in clinical practice. A patient may have several illnesses in his lifetime and be treated with a variety of drugs. In every instance appropriate baselines are established before any new treatment is begun.

The Placating Petition

Certain features of toxicity trials for evaluating safety of food additives are somewhat different than for drugs. A drug is a medicament developed for a specific use. Eventually the drug will be subjected to a controlled clinical trial. Thus, clinical experience supports evidence of safety. Although the ultimate consumer rarely objects, he does have the right to choose if he does not wish to take the drug. The consuming public does not have this choice with food additives, and only on rare occasions has there been any attempt to obtain clinical data on safety. The toxicologist relies almost entirely on animal work for this evidence. He fully realizes the pitfalls in transposing animal data to man, but even

knowing this, he makes every effort to support his conclusions by testing the additive for months or years in two and even three species, different strains of the same species, and short-term studies in other species. The large numbers of animals--as many as 2400 have been used--are reported in great detail, species differences, strain differences, and details of certain tests and other observations, which hold no allegiance to any species except the ones used. The ponderous report seems to instill a placating effect on the toxicologist that safety has been established at some level of use for man. More assurance may be attempted by invoking a mathematical margin of safety. Even further security may be sought by comparing the results of the test substance with an imitative compound whose safety has been reasonably established--for instance, applying safety data on formic acid and ethyl alcohol to ethyl formate on the theoretical basis that ethyl formate hydrolyzes to ethyl alcohol and formic acid in the body. Obviously this is not justified. The toxicologist's apprehensiveness also becomes apparent in his final assessment, which states that the level of proposed use in foods is safe. Any reference to human safety is omitted because human trial is rarely attempted or even considered. The creation of an exhaustive compilation of charts, graphs, tables, and written material, without knowledge of the extent to which these may support the conclusions, has been blamed on the legislator[11], some administrative body[12], bureaucrats[13], authors of marrow-curdling books[14], and so on. There is but one answer to the placating petition--a full knowledge of the compound's toxicity. New methods of modern toxicology must be applied to learn how to deal with a possible hazard or how to avoid it.

If the toxicologist does not keep in tune with developments in modern toxicology, the opportunity is apt to be grasped by someone else who may incorporate features which are most undesirable. The toxicologist cannot afford to be remiss in his responsibilities.

Summary

Toxicological evaluation of a substance intended for human use must be based on methods which are adaptable to the first human clinical trials. They must be prospective and not retrospective in purpose, or else the concept of preclinical toxicity studies becomes a distorted effort. It is conceded that evidence on absorption, blood levels, distribution, metabolism, and excretion may be difficult to attain, and for this reason is generally neglected. Nevertheless, greater use should be made of the molecular biological approach employing histochemical, cytochemical, ultrastructural and immunohistochemical methods. The specific purpose is not to determine cumulative anatomical changes, but cumulative physiological effects correlated with ultrastructural changes where the effects are taking place--at the cellular level. By such methods, early warning signs of toxicity should be demonstrable. The test substance should be capable of inducing similar changes in man if the animal model is to serve a useful purpose.

Since there are more than 200 analytical procedures of prospective value, it is unreasonable to expect to apply all of these tests in every toxicity trial. The appropriate choice of parameters, not the number, must be left to the experienced investigator. Obviously, the number will vary with the stage of the investigation. In the beginning phase, horizontal coverage of a few animals representing 4 or 5 species may be required

to uncover leads. The more observations the investigator makes on one subject, the more confidence he has in his conclusions. Choice of species and parameters for studies in greater depth rests largely on results from early clinical trial. Finally, the investigations should be placed in the hands of an experienced toxicologist who should be free to design, conduct, and interpret the toxicological problem. Although there is a lack of methods for detecting responses, and for differentiating a beneficial effect from an injurious one, nevertheless, if we are inept enough not to incorporate more precise indicators into the research program, we deserve the consequences.

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WHO Publications (Ref. 3) may be obtained from Columbia University Press, International Document Service, 2960 Broadway, N.Y.C. 10027.

UICC Publications may be obtained from Prof. R. Truhaut, University of Paris, Facultie de Pharmacie, 4 Avenue de l'Observatoire, Paris, France (Ref. 5)