TOXICOLOGY

Mechanisms and Analytical Methods

Edited by

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FOREWORD

Each year there is a sharp increase in the number of potentially poisonous substances to which man is exposed. They are met with in the factories, in the fields, and in the homes. They often contaminate the air, may be present in food or beverage, and are contained in most medicines.

The number of instances of illness and death caused by poisoning as reported in the vital statistics of countries where such figures are compiled probably represents only a small fraction of those which actually occur. Thus, in one large American industrial community a total of 66 cases of lead poisoning were reported over a seven-year period. During the seventh year, the services of an analyst and the facilities of a well-equipped laboratory were made available to the physicians of the community for making urinary lead determinations. At the same time, a program of professional education concerned with the clinical recognition of lead poisoning was launched. The following year, 65 cases were recognized. Since there was no reason to believe that there had been anything like a seven-fold increase in poisoning during that particular year it was inferred that the apparent increase was due largely to better detection.

Whatever the facts may be as to the frequency with which poisoning actually occurs, it is clear that there is a need in most countries for much better facilities than now exist for securing toxicological analyses as well as for greater alertness on the part of the medical profession in the recognition of poisoning.

The many advances that have been made in the field during the past several decades are widely scattered and relatively difficult to locate. They are to be found in the files of more than a hundred scientific journals, in the many monographs that deal with special analytical methods or with special kinds of poisoning, and in chapters devoted to analytical toxicology that are to be found in various textbooks of legal medicine.

The first reaction on learning the contents of these two volumes, of one whose requirements for knowledge in the field although frequent and diversified are not such as to justify the maintenance of a large special library, is one of great relief. It is to be hoped that these volumes will stimulate a general interest in this important subject—an interest that will lead to improvement in the recognition and prevention of poisoning and in the care of the poisoned.

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June 17, 1960

Preface

The preparation of this work on toxicological analysis was undertaken with the help of a team of collaborators all of whom were thoroughly experienced in this work and were especially interested in a particular phase of it. This method was adopted because it was quickly realized by the editors that the task was beyond the capacity of one or two men, owing to the vast range and rapid growth of the diverse sciences from which the toxicologist must borrow so many of his techniques and so much of the knowledge he needs to interpret his results.

The editors' objective in organizing these volumes has been to supply first an account of the general methods of chemical analysis available to the toxicologist along with a discussion of the principles on which they are based and a survey of the material to which they are to be applied. In pursuance of this aim, an introductory chapter deals with general aspects of the work of a toxicological analyst. This is followed by a series of chapters on the mode of absorption and excretion, the distribution in the body, and the metabolic changes undergone by toxic substances. Knowledge of these subjects, or the means of acquiring it when needed, is an essential part of the armament of the toxicologist—or other medico-legal consultant—since he must know what to look for, where and when his search may be successful, and how his results are to be interpreted with respect to amount of poison administered, the time and route of administration, and other factors of enormous importance in extracting the full value of the chemical analysis. Then follow chapters on methods of extraction, identification, and quantitation, which complete the first volume. The methods of analysis available to the toxicologist are given with sufficient discussion of the principles and sufficient examples of their use to indicate extensions to other particular instances. This is necessary because, since every new drug is a potential poison, the list of substances within the purview of the toxicologist is continually growing longer. Unquestionably, each worker will modify the techniques to suit his specific purpose or study, and the approach used is designed to take cognizance of this rather than to present a series of rigidly standardized procedures.

The main part of this volume supplements the methods quoted in the first volume as specific examples of general procedures by considering in turn the various important groups of poisons, arranged approximately in their order of extraction from biological material, and bringing together the methods available for identifying and determining the members of each group. In each of these chapters there are numerous specific examples, but though it was necessary to be selective, enough guidance is given to help in the selection of tests to be tried for new drugs of known chemical composition.

Since the toxicologist should clearly understand something of the nature of toxic action even though he may be primarily an analyst and not a pharmacologist, this volume is prefaced by a short essay on the subject and this, being of general interest rather than a detailed study, is less fully documented and gives leading references for further reading instead of the full bibliographies which are an important feature of the other chapters. Similarly, because toxicologists are often asked for advice on treatment, a chapter on this subject brings the volume to a close.

It is hoped that this plan will make the treatise valuable to all who may be concerned with the identification and determination of poisons (which in practice may mean any drugs). In this group of scientists are included, besides professional toxicologists, clinical chemists who are inevitably drawn into such work from time to time, and biochemists who as collaborators in pharmacological or medical studies require help in selecting and carrying out analytical procedures.

References to the literature cited are arranged alphabetically (according to authors' names) at the end of each chapter. A separate author index has not been included in the volume since it is felt that the reader will be able to find the material he is seeking by referring to the detailed subject index and reference lists.

We gratefully acknowledge the help and cooperation of the contributors and the willingness with which they undertook their task. We also wish to thank Miss Dorothy E. Carlson and Mrs. Elizabeth Proffitt for their valuable assistance and patience. Finally we should like to express our admiration of the work done by the publishers and printers of the book; their help enormously lightened our burden.

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CHAPTER 1

The Mode of Action of Poisons

by C. P. STEWART *and* GUSTAV J. MARTIN

I. INTRODUCTION

In a way, toxicology can be regarded as one of the oldest of the branches of the modern discipline of pharmacology. Long ago, our ancestors were interested in observing all possible therapeutic benefits to be derived from the crude medicines with which nature provided them, even though they were quite unable to elucidate the *modus operandi* of their medicaments or to provide what we should consider a rational explanation of their action. At the same time, and with the same limitations, they were interested in the deleterious and often lethal (i.e., poisonous) effects of many natural substances and in the ways of overcoming these actions. Whether the materials studied were beneficial or harmful, therapeutic agents or poisons, our ancestors were forced merely to observe the effects produced by their administration and to describe, mainly in anatomical terms or in terms prescribed by the fanciful theories of their day, the sequence and severity of the symptoms and the efficacy of apparent antidotes. Factually, of course, they accumulated a good deal of information about such things as dosage, classification of drugs according to their site of action or obvious symptomatic effect, and the like. At this stage, toxicology could properly be regarded as a pre-science, an assemblage of data about the poisonous effects of a whole range of specific substances, but without correlation.

Toxicology is, even today, largely a descriptive science. It has accumulated a vast collection of data; it utilizes the methods of modern medicine and the underlying basic sciences to investigate with increasing precision the effects of poisons, their metabolic changes, and the means of identifying them or their products, but it has not proceeded very far in organizing the data according to the basic mechanisms of action. Only recently has progress in this direction become possible, for only recently have biochemistry and physiology advanced far enough to provide more than glimmerings of what such basic action may be. Indeed, in medical science generally, anatomical or microanatomical descriptions of disease are only gradually being displaced or elucidated by descriptions based on the concept of the "biochemical lesion" (G1) and metabolic diseases are only beginning to be considered in terms of "disordered molecules" (L1). Throughout medicine and the biological sciences major changes in outlook are occurring as new techniques and increasing knowledge make it possible to probe more and more deeply into the fundamental mechanisms, at cellular and subcellular levels, of phenomena which previously could only be superficially and inadequately described.

One may ask what advantage would accrue to the practicing toxicologist from an orderly coordination of the more or less empirical facts already at his command, or how he would benefit from a study of the mechanism of poisoning as distinct from the readily observable effects. Such questions are not, of course, posed by the true scientist to whom order, correlation, and the search for basic principles are, axiomatically, the very nature of his work. But, on a purely utilitarian level, a classification of toxicological data which is based on a coordinating hypothesis of mechanism makes the material more comprehensible and more easy to memorize for the student and practitioner alike; it suggests, on a rational basis, possible methods for the investigation not only of pharmacological action but of chemical analysis; and, perhaps most important of all, it increases vastly the possibilities of searching for suitable antidotes since it is clearly easier to ascertain how a toxic effect may be overcome once the basic mechanism producing the effect is understood.

It is now possible to make progress—and indeed progress has already been made—on the basis of the postulate that the metabolic processes on which life depends, consist *in toto* of a vast number of well-organized and interlocking enzymatic reactions, interference with any one of which can produce deleterious effects (H1). In a series of such reactions, interruption at any one of a number of points (which may be brought about by different agents) may produce the same over-all abnormality. Conversely, where a group of enzyme systems covers several metabolic lines the apparent effect may depend on the particular branch interfered with.

II. Enzyme Inhibition*

A. General

So far as the toxic effects of a substance are exerted by inactivation of a particular enzyme (and there are, of course, other possibilities), they depend upon considerations of the kinetics of enzyme action. It is generally postulated that there is a formation of an enzyme-substrate complex, and the modern view expressing this is a development of the concept of Michaelis and Menton (N1). According to this, if E, S, and P represent the concentration of enzyme, substrate, and product respectively, while k_1, k_2, and k_3 are reaction constants,

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \overset{k_3}{\rightleftarrows} E + P$$

It is further believed that absolute specificity does not exist, in the sense that in no case is an enzyme (or other reactant) capable of reacting only with one unique substance. On this basis specificity is invariably relative, and one should speak rather of enzyme selectivity; this has been termed the concept of biological relativity (M1). Of course, selectivity may in another sense amount to complete specificity—thus carboxypeptidase will not catalyze the hydrolysis of fats or complex carbohydrates and in this its specificity may be considered complete, and similarly, though it is active with respect to synthetic peptides whose terminal amino acids are of the L-series, is virtually without effect on others of similar structure except that they have the D-configuration. But within the large group of peptides having the appropriate structure and configuration there are big differences in the ease with which they are hydrolyzed by the enzyme; here there is partial specificity, i.e., selectivity. But, related to the "normal" substrate or substrates there are invariably substances which also in one way or another react with the enzyme. Thus succinic dehydrogenase reacts not only with succinic acid but also with malonic acid and many other dicarboxylic acids, though these do not permit the "normal" action

* See Dixon and Webb (D1).