Drug Fate & Metabolism

Edited by Edward R. Garrett and Jean L. Hirtz

Methods and Techniques Volume I

DRUG FATE AND METABOLISM

Methods and Techniques

VOLUME 1

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PREFACE

Historically, the major emphasis in drug development was on the isolation and synthesis of active principles and the evaluation of their safety and efficacy in animals and man. The fate of drugs in the body, which includes their absorption, distribution, metabolism, and elimination, was not emphasized. Systematic studies on the fate of drugs in the body have been conducted only within the last several decades.

Such studies were inhibited by the inadequacies of analytical techniques and methods to isolate, identify, and assay the drugs and their metabolites in the biological tissues and fluids of the organism. Drug metabolism studies were performed as long as a century ago on quinine (1869), salicylic acid (1877), and morphine (1883) with the simple techniques then available. However, such studies were infrequently done and in limited depth until B. B. Brodie elaborated a general method for the discriminatory extraction of drugs and metabolites from biological fluids during World War II in connection with the United States' antimalarial screening program. At about the same time, L. C. Craig developed countercurrent distribution procedures for separation and identification purposes. By modern standards, methods of quantification then available, which included colorimetry, fluorometry, and ultraviolet absorption spectroscopy, were insensitive and in the microgram per milliliter range.

Pharmacokinetics, the study of the time course of a drug's absorption, distribution, metabolism, and elimination, is another aspect in the fate of drugs and was of even later vintage. Its maturation also depended on the development of sensitive and reliable assays in biological fluids. Probably the first publication in this field of adequate sophistication was on ethanol in 1922. The basic principles of pharmacokinetics were elaborated by Torsten Teorell of Uppsala in 1937, and the first book on the subject was published by F. H. Dost of Berlin, later of Giessen, in 1953. However, this field did not truly flower until the 1960s, and its initial blossoming

was observed at the first international conference on the subject in 1962 held under the initiative of Ekkehard Krueger-Thiemer at Borstel Forschungsinstitut in Germany.

The burgeoning of these studies on drug fate in the organism was fertilized by the development of radiometric techniques when radiolabeled drugs became available. Gas-liquid chromatography, now the most widely used method, provided a simple and inexpensive technique to separate and quantify drugs and their metabolites. Sensitive detectors were developed to provide picogram monitoring of nonlabeled materials. Other analytical and separative methods of high sensitivity and precision became commonplace in the laboratory. Instrumentation became available and less expensive for detection (NMR, spectrofluorimetry, infrared, gas and mass spectrometry, immunoassay) and for separation (thin-layer and high pressure liquid chromatography, etc.).

Today, it can be stated that the sensitivity of analytical detection no longer limits investigation into the fate of drugs. Separation and purification still is a rate-determining factor in assay development and demands a multidisciplined expert in biological, physical, organic, and analytical chemistry.

Similarly, the theoretical bases of pharmacokinetics and the technology of its applications have been expanded and refined within the last two decades. The generalized use of computers has permitted quantification of the models used to describe the totality of processes contributing to the time course of the drug in the body and to relate this time course to that of observed pharmacodynamics and pharmacological and toxicological action. The foundations of a modern pharmacology have been laid down, upon which structure personalized dosage regimens can be predicted for individualized optimum treatment with minimum toxicities; it is upon these premises that action and toxicities in one species can be predicted from studies performed on another.

The insights gained into the mechanisms of drug action provide clues to molecular modification that can best embody the active principal of action. Metabolic engineering can be construed as that practice which modifies the design of the molecule to take advantage of extant metabolic pathways to prolcng or shorten the time of drug presence in the body. The clinical awareness that the rate and extent of drug release from a dosage form can perturb the availability and delivery of therapeutic agents has led to the necessity of establishing standards for bioequivalences of formulations. Pharmacokinetics now serves as a basis for these biopharmaceutical necessities.

It is therefore not suprising that the study of drug absorption, distribution, metabolism, and excretion constitutes a large part of the modern research for new and more efficient therapeutic agents. Governmental

regulatory agencies in various countries now require precise data on the fate of new drugs and their formulations in animals and man and are increasingly insistent on stricter compliance.

Although there are several books dealing separately with drug metabolism, drug disposition, pharmacokinetics, and the like, a proper compendium has been lacking which encompasses the various fields and provides a delineation and appropriate critique of the useful methods and techniques that can be applied in them.

One of the editors (JLH) published (1968) a book on the analytical techniques (Les Méthodes Analytiques dans les Recherches sur le Métabolisme des Médicaments, Masson, Paris) which was later translated by editor ERG into English (Analytical Metabolic Chemistry of Drugs, Marcel Dekker, New York, 1971). The reception of this book was gratifying and prompted us to bring out the present more comprehensive and modernized series of volumes which includes other methods and techniques in the study of drug fate, not only analytical procedures. Since this ambitious goal exceeded the expertise of only one or a few authors, a multi-author series was projected. Experts were chosen who were highly respected in their fields. We reserved the right, and exercised it, to edit and revise to maintain a reasonable level of homogeneity in conformance with the objectives of the series. We hope we have succeeded.

The intent of these volumes is to review all the techniques, physical, chemical, biological, medical, and mathematical, which can be applied to the study of drug fate in the organism. It is addressed primarily to the research scientist and is devoted to methods, with only the minimal theory given for perspective, appreciation, and proper evaluation of results. The intent was not to compete with the many fine theoretical texts available, but to provide a broad spectrum of information that can be readily utilized by the research worker.

The practical use of these methods is explored fully. The limitations are explained. Necessary precautions and sources of error are delineated. Examples are given of applications in the study of the fate of drugs. When possible, each chapter includes tables that condense the appropriate literature on the particular topic. Each chapter has a selected, adequate, but not exhaustive, bibliography. For a more complete bibliographic survey, the reader is referred to the series edited by editor JLH (The Fate of Drugs in the Organism: A Bibliographic Survey, Marcel Dekker, New York: Vol. 1, 1974; Vol. 2, 1975; Vol. 3, 1976; Vol. 4, 1977).

It was deemed proper to include chapters on methods that would not be modern methods of choice but are of historical importance in evaluating the significance and limitations of the earlier studies in these fields. Whenever possible, a critique is provided, the future development is predicted, and the utility of a considered technique is evaluated.

It is our sincere hope that these endeavors of our dedicated authors will serve the desired purpose.

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I. INTRODUCTION

If a specimen containing a radioactive element is placed against a photographic emulsion, the emission from the element may reduce the silver halide in the emulsion in the same manner as a photon of light. This process is termed autoradiography because it produces a "self" radiograph which shows the distribution of the isotope in the specimen. The term autoradiography is preferred to radioautography for several reasons; the most important being that the product is a "self" radiograph and not a "radio" autograph or one's transmitted signature.

Autoradiographic techniques for biological specimens may be divided into two broad categories. The most commonly used is that for substances which are incorporated into large molecules such as proteins and nucleic acids. If one is studying the incorporation of amino acids or nucleosides into large molecules, the autoradiographic procedure is greatly simplified. In this case ordinary histological procedures may be used because the large molecules are not soluble in the fixing and embedding solutions after fixation; the histological solutions do not remove or translocate the compound. An entirely different situation exists for most drugs; very rarely are they incorporated into large molecules or so tightly bound that they are not removed or translocated by the solutions. In drug distribution studies one is ordinarily looking for sites of concentration due to active transport, metabolism, binding, pH gradients, specific solubilities, specific receptors, etc. In order to identify these sites one must use an autoradiographic technique which does not translocate or remove the drug or its metabolite during the process of preparing the autoradiograph. It has been well established that the only procedure which is suitable for drug metabolism studies is one that completely avoids the use of any solvents and does not allow thawing of the tissue which has been frozen while still alive.

The technique most widely used is that of placing sagittal sections of whole experimental animals against x-ray film to determine the distribution of drugs in the entire animal; the process was developed by Ullberg [1-5]. A wide variety of species have been used for this technique including mice, rats, fish, birds, monkeys, cats, dogs, and even human embryos; isolated tissues can, of course, also be used. There are two unique advantages to this technique for studying the distribution of drugs. The first is that tissues or fluids which ordinarily are not sampled can be readily analyzed. The second is that gradients of concentration within a tissue can be determined. Removal of the tissues and analysis by homogenization destroys gradients of concentration within the tissues and may completely obscure a high concentration in a few cells. We have routinely