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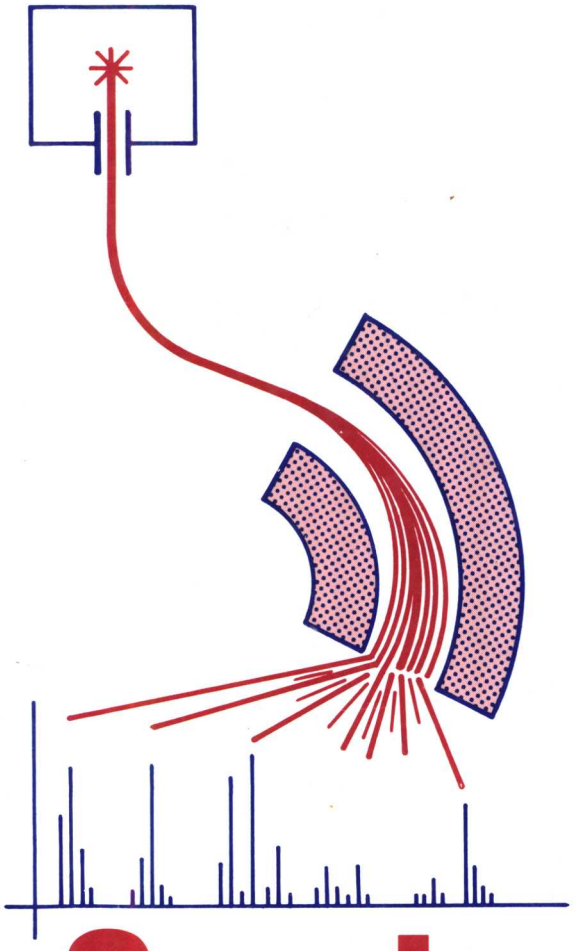

Mass Spectrometry

Part B

edited by Charles Merritt, Jr. • Charles N. McEwen

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(in two parts) **Part B**

edited by Charles Merritt, Jr.

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PREFACE

This book is Part B of the Mass Spectrometry volume of Practical Spectroscopy and, like its predecessor, Part A, and companion volumes, aims to bring together a number of topics of practical value. In this part, the application of mass spectrometry to studies of drug metabolism by Reinhold and Costello and the use of chemical derivatization by P. Vouros are treated in detail and with comprehension by authors whose experience and involvement with their subjects well qualify them to write. As in Part A, a chapter on collisional activation by Bente and McLafferty has been included which deals with newly developing techniques in which practical applications may soon be realized. Finally, potentially powerful methods of negative ion chemical ionization are described in a chapter organized by J. R. Hass. This last chapter reflects a culmination of current practice, since it has been organized from a collection of papers presented at a workshop on the subject held at NIEHS in Research Triangle Park, N.C. The authors and editors wish, in particular, to acknowledge the contributions of those participants whose work is reported herein.

The editors and publisher wish especially in this preface to acknowledge the contribution of the cover design by Dr. Berndt Soltmann, and apologize for this omission in the preface to Part A.

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CONTENTS OF PART A

CONTENTS

Chapter 1

MASS SPECTRAL APPROACHES TO THE STUDY OF DRUG METABOLISM

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I. INTRODUCTION

The qualitative and quantitative analysis of drug metabolites is a task for which mass spectrometry is particularly well suited. The sensitivity and specificity of the technique make possible the determination of metabolites even in the presence of similar entities--the original drug and other metabolites--and of the many other components of biological extracts. The success of the mass spectrometric approach requires an understanding of the routes of drug disposition, metabolism, and excretion, and of the chemical manipulations that are most effective for recovery of the metabolic products. Equally important is an understanding of the range of analytical procedures made possible by the utilization of mass spectrometry and its adjunct techniques.

With the consideration that the analysis of drug metabolites is an interdisciplinary problem we have summarized in this review the pharmacology of administered drugs. We have done this to aid the analysts in selecting the body fluid or tissue to be examined for the presence of metabolites and to assist them in predicting the likely

chemical modifications of drugs by the living system. Particular attention is focused on the contributions that mass spectrometric techniques have made in the elucidation of these pathways. All literature references cited are those where mass spectrometry has played a major role in the elucidation of structure. In addition, a survey of the chemical and instrumental techniques presented in the recent literature that are suitable for this type of analysis is included in order to help the mass spectrometrist to make a judicious choice among them or, perhaps, to improve upon them.

The application of mass spectrometry to drug metabolism studies is a rapidly expanding area of research. For this review we have drawn upon original sources and our own experimental results and have included references primarily from 1973 to the present. Earlier work is summarized in the review articles cited and these are grouped with the general methods. Publications that deal with the analysis of specific drugs and their metabolites have been classed according to the type of drug involved (with some poetic license). Those publications concerned with methods development are assigned according to the approach that they describe. We hope this organization will permit the reader quickly to survey area(s) of particular interest.

II. PRINCIPLES OF DRUG DISTRIBUTION

A. Absorption

To gain access to body compartments drugs must cross one or more barriers and this transfer is usually accomplished by either passive diffusion (a simple process leading to equilibrium), facilitated diffusion, active transport, or pinocytosis. It is frequently observed that passive diffusion depends on the degree of lipid solubility, and that compounds that are highly soluble in lipids diffuse rapidly whereas those that are relatively lipid-insoluble diffuse more slowly. Many drugs, especially hydrophilic molecules, show peculiarities in their migration through a membrane. For some drugs the rate of penetration is greater than would be expected given either limited lipid solubility, the presence of a charge, or, in

some cases, the differences between optical isomers. This unexpectedly facile permeation has been defined as facilitated diffusion and is driven by a concentration gradient as with passive diffusion. In accounting for this absorption, a hypothetical receptor or carrier concept has been introduced in which binding to this carrier facilitates transmembrane movements. Active transport, on the other hand, is an energy-requiring process and is defined as such when drugs are observed to move against a concentration gradient. The rate of transport is dependent on the binding capacity of the solute to its hypothetical carrier and is limited by the availability of that carrier. Pinocytosis is the mechanism whereby drugs are absorbed even though they are not in a true solution. Droplets and solid particles are engulfed by processes comparable to phagocytosis. This occurs, for example, from the lumen of the intestinal tract across the intestinal epithelium into the lymphatic or venous capillaries. Other mechanisms of absorption have been recently delineated, such as convective (small molecules through pores) and ion-pair (transmembrane movement of electrochemically neutral complexes) absorption to account for passive diffusion of small water-soluble molecules and quaternary ammonium and sufonic acid absorption respectively. Various transport mechanisms are available in different organs and tissues and a drug may be absorbed by one or more of these mechanisms.

B. Disposition

The sequence of movement of a drug in the process of its distribution is the reverse of that involved in its absorption. Frequently, distribution occurs throughout the body; the drug is carried by the aqueous phase of the blood plasma and reaches tissue compartments at a rate determined by the blood flow through that organ. Arrival at the site of action thus assumes the drug, or its metabolite, has successfully passed through the surface epithelia, the capillary endothelia, and the plasma and intracellular membranes. These loci, the binding sites within and between these barriers, the size of the body's fluid compartments, and the overall physiochemical properties

of the molecule itself are major factors accounting for the drug's ultimate distribution.

The extent of drug distribution is frequently expressed in terms of the water content of the body compartments, which are anatomically and functionally distinct. Thus, assuming a drug to be unbound to any tissue component, unmetabolized, not excreted, and found only in the extracellular fluid, its volume of distribution would be about 9-12 liters for adults. In the absence of active transport (assuming cell membrane permeability), transfer will proceed until equilibrium, thereby diluting the concentration of the drug equal to the total intracellular water space, approximately 28 liters. Consequently, a drug that can pass readily all biological barriers will have a volume of distribution equal to the total body water, about 40 liters (60% of body weight). Many lipid-soluble drugs have this volume of distribution. This oversimplified drug dilution ignores many known variables but does permit approximations of molecule concentration from the circulation. In many pharmacological studies the volume of distribution for certain drugs has been known to exceed the total body water, thus providing an indication that the drug either goes specifically to "deep" tissue in peripheral compartments or is stored or pooled in a peripheral compartment, such as fat.

C. Excretion

The processes of excretion, metabolism, and storage are the three mecahnisms whereby drugs are ultimately removed from their sites of action. Excretion at the kidneys, biliary system, intestine, and sometimes the lungs accounts for most drug elimination, and renal excretion is by far the most important of these routes.

The kidney is admirably suited to the task of drug elimination. About 130 ml of plasma water is filtered each minute through the glomerular membranes. Of this volume, only a small proportion is excreted as urine; the remainder is resorbed in the renal tubules. The actual site where elimination (and resorption) take place is the nephron. Each kidney has approximately 1 million nephrons, each

consisting of a long, unbranched, tortuous tubule originating in the kidney cortex as a closed-ended structure known as Bowman's capsule. It has been estimated that the total length of the tubules of both kidneys is of the order of 75 miles. This huge surface area and the thinness of the barrier separating the tubular lumen from the internal environment make it obvious that the kidneys are ideally structured to provide maximum contact between the external and internal environments of the body. As blood flows through the Bowman's capsule (glomerulus), any drug that is free in the plasma will be filtered as will be other constituents. Only drugs bound to protein or drugs of excessively large molecular size will be retained in the bloodstream. The rate at which a drug will be eliminated in the urine is the net result of three renal processes: glomerular filtration, tubular secretion, and tubular readsorption. Glomerular filtration and tubular secretion are dependent on the concentration of drug in the plasma whereas resorption by the tubules is dependent on its concentration in the urine.

A second major route of drug excretion is the liver, which secretes 0.5 to 1.0 liter of bile daily into the duodenum through the common bile duct. However, the majority of agents reaching the small intestine in this way are not subsequently excreted in the feces. They are almost completely resorbed because their physiochemical properties are favorable for passive diffusion across the intestinal barrier. These agents then remain in the enterohepatic cycle (portobiliary circulation) until they are excreted in the urine.

Drugs may pass from the circulatory system into the gastrointestinal tract by different routes. The major pathway, as discussed above, is by biliary excretion, but drugs may also pass directly into the gastrointestinal tract or, more important, undergo salivary excretion. This transport mechanism is primarily passive diffusion of nonionized moieties.

D. Binding

When considering the distribution of many drugs in body compartments, attention should be focused on possibilities of protein binding.

Although the term "binding" lacks scientific description, it most frequently involves reversible drug-protein interactions in which several different types of bonding prevail. Since there exists a great diversity of drug and protein structural variations, the consequence of interaction can be expected to run the gamut of stability and attempts to classify these relationships physiochemically are meaningless. The most significant representation of this association must be the equilibrium constant. From kinetic studies and associated manipulations, much can be learned about the character of drug binding. Protein-bound drugs do not cross tissue boundaries (e.g., glomerulus, placenta, blood-brain barrier, renal tubular epithelium), and are not immediately available for metabolism. It should be kept in mind that binding is dynamic and rapidly reversible. Hence binding in no sense precludes further pharmacology of the drug.

E. Induction and Inhibition

Drugs and certain foreign chemicals have long been known to influence their own metabolism and that of therapeutic agents. The administration of phenobarbitol to various animal species markedly increases that animal's ability to metabolize other agents. These inducing agents likewise affect the metabolism of certain endogenous substances such as vitamins, estrogens, androgens, corticosteroids, and progestogens. Mechanisms of enzyme induction have been studied extensively and certain of these produce a proliferation of the endoplasmic reticulum, and thus increase synthesis of protein and cytochrome P-450 (see oxidations). Different types of enzyme inducers appear to act specifically but differentially on certain of the cytochrome constituents, increasing them in a selective manner. Others appear to be relatively nonspecific, affecting a wide range of enzymes that catalyze the metabolism of drugs.

Inhibitors of drug metabolism that result in potentiation of pharmacological effects have long been recognized. Most inhibitors are thought to act directly on the drug-metabolizing-enzyme system as a competitive substrate. The antischizophrenic action of chloropromazine (CPZ) may be due to the mono- and didesmethylated metabolites of

CPZ, which are excellent substrates for indolethylamine N-methyltransferases, thereby preventing the formation of psychotogenic substances [450].

Society's increasing use of tranquilizers combined with alcohol has caused concern for the additive or potentiating effect of these drugs. Ethanol is metabolized by mixed-function oxidases as is diazepam, a popular tranquilizer. It is therefore not surprising that investigators detected an inhibition of ^{14}C-diazepam metabolism when animals were pretreated with ethanol [152].

III. DRUG METABOLISM

Metabolism serves three principal purposes: (1) the supply of energy for body functions and maintenance; (2) catabolic and anabolic processes, the latter usually requiring energy; and (3) the conversion or biotransformation of foreign compounds to more polar, water-soluble, and ionized structures that can be eliminated more easily. Most drugs undergo enzyme catalyzed transformations but a few undergo spontaneous reaction when given the appropriate conditions such as pH or contact with a suitable physiological compound with which they can react. A few drugs are excreted unchanged.

The principal site of drug metabolism is the liver; less important are the kidney, muscle tissue, and gut wall. The liver consists primarily of parenchymal cells that form plates arranged in such a way that they provide walls around continuous spaces or lacunae. Within the lacunae are capillaries that differ from other capillaries in having larger pores, thus being more permeable to macromolecules. The walls of the capillaries are lined by Kupffer cells. Each cell has either one or two nuclei and a large number of mitochondria (about 2,000 per cell) and lysosomes. Metabolizing enzymes occur in the soluble, the mitochondrial, or the microsomal cell fractions. This extremely versatile group of enzymes is known collectively as the drug-metabolizing enzymes and requires as a major enzyme cofactor cytochrome P-450. Several other extrahepatic sites for drug metabolism have been identified, including the lung, intestine, and kidney,