ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

VOLUME 179

July 6, 1971

DRUG METABOLISM IN MAN

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

VOLUME 179

July 6, 1971

DRUG METABOLISM IN MAN*

Editor and Conference Chairman
ELLIOT S. VESELL

CONTENTS

Dedication to BERNARD B. BRODIE . . . . . .

Introductory Remarks. *By* JAMES A. SHANNON . . . . . .

PART I. FACTORS AFFECTING DRUG METABOLISM IN MAN

Drug Metabolism in Man: Past, Present, and Future. *By* B. B. BRODIE, A. K. CHO, G. KRISHNA, AND W. D. REID 11

Pharmacokinetic Aspects of Drug Metabolism. *By* MILO GIBALDI 19

Drug Biotransformation Interactions in Man: Nonnarcotic Analgesics. *By* GERHARD LEVY 32

Factors Affecting Drug Metabolism. *By* JAMES R. GILLETTE 43

Physiological and Physicochemical Bases of Drug Interactions in Man. *By* PETER G. DAYTON AND JAMES M. PEREL 67

Interaction between Phenobarbital and Diphenylhydantoin in Animals and in Epileptic Patients. *By* P. L. MORSELLI, M. RIZZO, AND S. GARATTINI . . . 88

Differences in the Metabolism of Drugs, Depending upon their Routes of Administration. *By* C. T. DOLLERY, D. S. DAVIES, AND M. E. CONOLLY 108

Pharmacokinetics of Distribution and Metabolism of Ataractic Drugs and an Evaluation of the Site of Antianxiety Activity. *By* E. VAN DER KLEIJN . . . 115

PART II. STUDIES OF DRUG METABOLISM IN MAN

Metabolism and Absorption of L-3,4 Dihydroxyphenylalanine in Patients with Parkinson's Disease. *By* JOSEPH R. BIANCHINE, LEONOR R. CALIMLIM, JOHN P. MORGAN, CARLOS A. DUJUVNE, AND LOUIS LASAGNA 126

The Metabolism of Certain Drugs and Food Chemicals in Man. *By* R. T. WILLIAMS 141

Effects of Environmental Chemicals on the Metabolism of Drugs, Carcinogens, and Normal Body Constituents in Man. *By* A. H. CONNEY, R. WELCH, R. KUNTZMAN, A. POLAND, P. J. POPPERS, M. FINSTER, J. A. WOLFF, A. D. MUNRO-FAURE, A. W. PECK, A. BYE, R. CHANG, AND M. JACOBSON 155

Impact of Aspirin and Chlorthalidone on the Pharmacodynamics of Oral Anticoagulant Drugs in Man. *By* ROBERT A. O'REILLY, MERVYN A. SAHUD, AND PAUL M. AGGELER 173

The Kinetics of Heparin. *By* J. WORTH ESTES. 187

The Metabolic Fate of Warfarin: Studies on the Metabolites in Plasma. *By* RICHARD J. LEWIS AND WILLIAM F. TRAGER 205

*This series of papers is the result of a conference entitled Drug Metabolism in Man, held by The New York Academy of Sciences on June 29–July 1, 1970.

Kinetics and Clinical Importance of Displacement of Warfarin from Albumin by Acidic Drugs. *By* EDWARD M. SELLERS AND JAN KOCH-WESER 213

Species Differences in the Effect of Chloral Hydrate on Coumarin Anticoagulants. *By* MURRAY WEINER 226

A Review of Current Advances in Metabolism of Inhalation and Anesthetics. *By* BURNELL R. BROWN, JR., AND LEROY D. VANDAM 235

Halothane Biotransformation in Man. *By* H. F. CASCORBI, E. S. VESELL, D. A. BLAKE, AND M. HELRICH 244

Salicylate Metabolism in Man. *By* CLARKE DAVISON 249

Influence of Plasma Levels and Metabolism on Pharmacological Activity: Pentazocine. *By* BARRY BERKOWITZ 269

The Role of Microsomes in the Hepatic Metabolism of Ethanol. *By* E. A. CARTER AND K. J. ISSELBACHER 282

Arene Oxide Intermediates in Enzymatic Hydroxylation and Their Significance with Respect to Drug Toxicity. *By* SIDNEY UDENFRIEND 295

Distribution of Guanidinium Antihypertensives—Mechanism of their Selective Action. *By* JOHN A. OATES, JERRY R. MITCHELL, O. THOMAS FEAGIN, JOHN S. KAUFMANN, AND DAVID G. SHAND 302

Quantifying Metabolic Effects of Antihypertensive and Other Drugs at the Sympathetic Neuron Level: Clinical and Basic Correlations. *By* W. A. PETTINGER AND W. D. HORST 310

The Clinical Value of Serum Digitalis Glycoside Concentrations in the Evaluation of Drug Toxicity. *By* THOMAS W. SMITH AND EDGAR HABER 322

Some Aspects of the Distribution and Disposition of Digitoxin in Man. *By* DANIEL S. LUKAS 338

Interactions between Digitoxin and Other Drugs *In Vitro* and *In Vivo*. *By* HARVEY M. SOLOMON, STEVEN REICH, NENA SPIRIT, AND WILLIAM B. ABRAMS 362

Pharmacokinetics of Procainamide in Man. *By* JAN KOCH-WESER 370

Disposition Kinetics of Lidocaine in Normal Subjects. *By* MALCOLM ROWLAND, PATE D. THOMSON, ANTHONY GUICHARD, AND KENNETH L. MELMON 383

The Metabolism of Probenecid in Man. *By* PETER G. DAYTON AND JAMES M. PEREL 399

The Action of Drugs on Bilirubin Metabolism in Man. *By* BARBARA H. BILLING AND MARTIN BLACK 403

Mechanism of Hepatic Drug Oxidation and Its Relationship to Individual Differences in Rates of Oxidation in Man. *By* DONALD S. DAVIES AND S. S. THORGEIRSSON 411

Clinical Implications of Enzyme Induction. *By* A. BRECKENRIDGE AND M. ORME 421

Metabolism of Anticancer Agents in Man. *By* RICHARD H. ADAMSON 432

Factors Influencing the Rate of Metabolism of Steroid Hormones in Man. *By* M. B. LIPSETT 442

Parathyroid Hormone and Thyrocalcitonin: Their Mode of Action and Regulation. *By* CHARLES Y. C. PAK 450

Hypolipidemic Drugs and Hyperlipoproteinemia. *By* ROBERT I. LEVY AND TERRY LANGER 475

Penicillamine and Analogous Chelating Agents. *By* H. VASKEN APOSHIAN 481

Biotransformation of Prazepam in Man. *By* FREDERICK J. DICARLO, JEAN-PAUL VIAU, JOYCE E. EPPS, AND LLOYD J. HAYNES 487

Effects of Urinary pH on Amphetamine Metabolism. *By* JOHN M. DAVIS, IRWIN J. KOPIN, LOUIS LEMBERGER, AND JULIUS AXELROD 493

Some Historical Reflections on Drug Metabolism. *By* THOMAS C. BUTLER . . . 502

Deficiencies in our Present Protocol for Chemical Evaluation and Possible Remedies. *By* MARVIN S. LEGATOR 508

PART III. GENETIC ASPECTS OF DRUG METABOLISM IN MAN

Gene Therapy Systems: The Need, Experimental Approach, and Implications. *By* JOSEPH V. OSTERMAN, ANNA WADDELL, AND H. VASKEN APOSHIAN . . . 514

Bilirubin: A Model for Studies of Drug Metabolism in Man. *By* H. REYES, A. J. LEVI, R. LEVINE, Z. GATMAITAN, AND I. M. ARIAS 520

Studies on the Mechanism of Aryl Hydrocarbon Hydroxylase Induction and Its Role in Cytotoxicity and Tumorigenicity. *By* H. V. GELBOIN AND F. J. WIEBEL 529

Molecular Models for Pharmacological Tolerance and Addiction. *By* ROBERT S. KROOTH 548

Aryl Hydrocarbon Hydroxylase Induction in Cell Culture as a Function of Gene Expression. *By* D. W. NEBERT AND L. L. BAUSSERMAN 561

Corrective Factors for Inborn Errors of Mucopolysaccharide Metabolism. *By* ELIZABETH F. NEUFELD AND MICHAEL J. CANTZ 580

Allopurinol and Oxipurinol: Alteration of Purine and Pyrimidine Metabolism in Cell Culture. *By* WILLIAM N. KELLEY, IRVING H. FOX, THOMAS D. BEARDMORE, AND JEAN C. MEADE 588

Early Events in Hormonal Induction of Alkaline Phosphatase in Human Cell Culture: Hormone-Cell Interaction and its Dependence on DNA Replication. *By* RODY P. COX 596

Abnormal Steroid Hormone Metabolism in the Genetic Liver Disease Acute Intermittent Porphyria. *By* ATTALLAH KAPPAS, H. LEON BRADLOW, PETER N. GILLETTE, AND T. F. GALLAGHER 611

The Effects of Certain Drugs on the Hexose Monophosphate Shunt of Human Red Cells. *By* SELMAN I. WELT, ELIZABETH H. JACKSON, HENRY N. KIRKMAN, AND JOHN C. PARKER 625

Variants of Glucose-6-Phosphate Dehydrogenase. *By* A. G. MOTULSKY, A. YOSHIDA, AND G. STAMATOYANNOPOULOS 636

Effects of Some Metabolites of Hemolytic Drugs on Young and Old, Normal and G-6-PD-Deficient Human Erythrocytes. *By* I. M. FRASER, B. E. TILTON, AND E. S. VESELL 644

Topics in Pharmacogenetics. *By* WERNER KALOW 654

Dapsone Acetylation in Man: Another Example of Polymorphic Acetylation. *By* JOHN H. PETERS AND LOUIS LEVY 660

The Fate of Atropine in Man. *By* SARAH CHINN KALSER. 667

Plasma Esterase Activity and the Metabolism of Drugs with Ester Groups. *By* BERT LA DU 684

Suxamethonium Sensitivity. *By* H. WERNER GOEDDE AND KLAUS ALTLAND 695

Biochemical and Genetic Factors Regulating Dilantin Metabolism in Man. *By* HENN KUTT 704

Plasma Protein Binding, Plasma Concentrations, and Effects of Diphenylhydantoin in Man. *By* LARS LUND, PER KNUT LUNDE, ANDERS RANE, OLOF BORGA, AND FOLKE SJÖQVIST 723

The Role of Genetic Factors and Serum Protein Binding in Determining Drug Response as Revealed by Comprehensive Drug Surveillance. *By* GEORGE P. LEWIS, HERSHEL JICK, DENNIS SLONE, AND SAMUEL SHAPIRO 729

Individual Differences in the Pharmacokinetics of Monomethylated Tricyclic Antidepressants: Role of Genetic and Environmental Factors and Clinical Importance. *By* BALZAR ALEXANDERSON AND FOLKE SJÖQVIST 739

Genetic Control of Drug Levels and of the Induction of Drug-Metabolizing Enzymes in Man: Individual Variability in the Extent of Allopurinol and Nortriptyline Inhibition of Drug Metabolism. *By* ELLIOT S. VESELL, G. THOMAS PASSANANTI, FRANK E. GREENE, AND JOHN G. PAGE 752

Publication of this monograph was aided by contributions from:

- ABBOTT LABORATORIES, SCIENTIFIC DIVISION
- A. H. ROBINS COMPANY
- BAXTER LABORATORIES, INC.
- BURROUGHS-WELLCOME & CO. (U.S.A.) INC.
- CHAS. PFIZER & CO. INC.
- CIBA PHARMACEUTICAL COMPANY
- LILLY RESEARCH LABORATORIES
- FARMACEUTICI ITALIA
- G. D. SEARLE & CO.
- HOECHST PHARMACEUTICAL CO.
- JOHNSON & JOHNSON
- MERCK INSTITUTE FOR THERAPEUTIC RESEARCH
- NATIONAL INSTITUTE OF GENERAL MEDICAL SCIENCES
- S. B. PENICK FOUNDATION
- SMITH KLINE & FRENCH LABORATORIES
- SCHERING CORPORATION
- STERLING DRUG INC.
- WALLACE LABORATORIES

原书缺页

原书缺页

原书缺页

原书缺页

INTRODUCTORY REMARKS

James A. Shannon, M.D.

Rockefeller University
New York, N.Y.

It was a great pleasure for many of us to participate in the conference that is the basis of this volume. The pleasure was derived from many circumstances that surrounded the occasion, not the least of which was the quality of the presentations and their relevance to contemporary problems in pharmacology. But there are additional reasons.

First, I suppose, is that such a conference could not have taken place as short a time ago as a decade.

During the fifties, too few brilliant young people were entering the field for a critical mass of information to be available for the presentations and the discussions that would characterize such a meeting. More importantly, the substantive content of the investigations of the fifties, despite the large number of therapeutic agents that were evolving by empirical methods, carried much too little scientific interest to attract the attention of scientists in collateral fields such as would permit the definitive examination of the interaction of chemicals and biological systems. Too many of the activities of pharmacologists then addressing themselves to the development of therapeutic agents were viewed as the shallow efforts of a group of "drug testers," a damning but probably well-earned accolade in reverse.

Second, despite this rather unauspicious scientific beginning, the field of drug development itself was generally characterized by highly productive work. But the productivity of the field was the result of highly ingenious organic chemists who developed a variety of chemicals with quite novel biological activities. The rapid decay in the development of new therapeutic agents in the sixties must, at least in part, reflect the self-sterilizing influence of the lack of balanced attention to the two components of the system; i.e., the chemical substances and the biological systems. But this deficiency is now obviously being remedied, for there have now evolved a number of broad fields of biological endeavor that, contrary to the fifties, are self-sustaining, that are increasingly characterized by scientific excellence, and that provide a striking increase in scientific breadth and depth of penetration in the substantive aspects of both biological and chemical studies.

The general field is no longer self-limiting. Rather, it is one out of which new approaches to an understanding of the interaction of chemical substances and biological systems are proving to be intriguing, exciting, and intellectually rewarding, as well as highly productive of information with great utility in the satisfaction of needful information directly applicable to important medical problems. These form the combination of characteristics that will no doubt sustain the growth of these fields during the coming decade. Fortunately, it is a field that now clearly spans the fundamental, the applied, and the developmental. It further has the potential to develop its own general principles which will facilitate the more rational evolution of therapeutic agents—with much firmer information on both their utility and their limitations. It is quite clear, too, that pursuit of these types of studies will greatly clarify the fundamental nature of a number of important biological systems.

But for this to come about—and indeed for the field of pharmacology to come

fully of age—it is important that more scientists concern themselves with the establishment of sets of the broad biological generalizations that are now only becoming visible. This will not be easy, although the feasibility of developing such generalizations seems quite predictable: in part because of the information already available, even though much of this is qualitative in nature, and in part because of the availability of tools, which will permit the conversion of qualitative to quantitative information.

This comment on the past accomplishments of pharmacology, its achievement of scientific respectability, and its capability for even more definitive contributions in the future is quite pertinent to the career of the scientist to whom this volume is dedicated, Bernard B. Brodie.

Dr. Brodie reflects in his own career the progress of these sciences. He has indeed been responsible for a surprising amount of the progress, and his concepts for the future development of pharmacology are, as always, intriguing.

Starting his career with a Ph.D. in organic chemistry from New York University in the thirties, Dr. Brodie moved into biology through quantitative studies on the physiological handling of halogens. He expanded his views on the interaction of chemicals with complex biological systems incidental to his studies, which provided a quantitative base for the rational development of antimalarials during World War II. He has moved through a series of subspecialties in pharmacology and related physiology in a highly productive fashion since that time. These included the fields of analgesics, antiarthritic and antiarrythmic agents, and the explorations of drugs with actions on the CNS and the exploration of the control of CNS function, to mention a few. His activities have resulted in a profound increase in our understanding of drug action, but, more importantly, have modified our views on what could or indeed should be done to clarify the complexities of a broad field of concern common to all of us. But then Dr. Brodie's contributions to medicine, and its scientific base, is reflected less in his direct contributions than in the careers of the many scientists who have been affected by his work.

It is quite appropriate, then, that the initial presentation at the conference and the first contribution in this volume is by Dr. Bernard B. Brodie, Head of The Laboratory of Chemical Pharmacology, National Heart and Lung Institute, The National Institutes of Health. It is also appropriate that this contribution is concerned with yet another novel concept.

DRUG METABOLISM IN MAN: PAST, PRESENT, AND FUTURE

B. B. Brodie, Ph.D., A. K. Cho, Ph.D., G. Krishna, Ph.D., and W. D. Reid, M.D.

Laboratory of Chemical Pharmacology, National Heart and Lung Institute, Bethesda, Md.

Dr. Shannon neglected to state that he is really the father of clinical pharmacology. During the war, I had the privilege of working on the clinical screening of antimalarials at Goldwater Memorial Hospital, New York, headed by this gifted investigator. I advise clinical pharmacologists to read his concepts on clinical pharmacology, published in the Harvey Lectures, 1945–46, Vol. 42. Despite the emergency nature of the antimalarial project, Dr. Shannon realized how quickly a screening program could become sterile unless it was associated with problems of a fundamental nature. As a matter of fact, the important discoveries in this program were not the development of new compounds, but the development of new principles in drug screening. Actually, the most important single finding was the development of an effective dosage schedule for an old drug, atabrine.

In these studies, Dr. Shannon insisted that responses to drugs should be related to the plasma level. His arguments were simple. First, there was a shortage of patients and time, hence an inactive drug must be discarded as quickly and with as few patients as possible. Second, once a drug showed promising activity in man, a thorough study of its physiological disposition was essential in order to obtain information that could be used in devising a rational regime of therapy. These studies provided insight into the large variability in drug metabolism, which is recognized today as one of the most important factors in the clinical pharmacology of lipid-soluble drugs.

I shall not discuss the past any further, neither shall I dwell on the present status of clinical pharmacology, since this will be thoroughly reviewed by the many distinguished participants of this conference. Instead, I should like to discuss the future—a new kind of drug metabolism that may be of considerable importance—the formation of small amounts of toxic metabolites that produce pathological changes in liver, kidney, and other tissues.

During a recent trip to Australia, I traveled throughout the country to see the various species of marsupials. As a side trip, I visited a sheep station, a name given to a vast expanse of inhospitable territory containing as many as 100,000 or more sheep. There I became aware of a problem of some concern to the sheep ranchers. At various times, the sheep are dewormed by administering CCl_4 in amounts nontoxic to the majority of the animals. Occasionally, however, a group of sheep would succumb to the drug. On autopsy, these sheep were found to have suffered massive liver necrosis. I was well aware of the toxic effects of CCl_4 on the liver and kidney, but for the first time it struck me that CCl_4 is actually exceedingly inert in the test tube, reacting with other chemicals only under very extreme conditions. I met Dr. A. A. Seawright, a pathologist from Brisbane, who described his studies showing that pretreatment of sheep with phenobarbital greatly increased the liver toxicity of CCl_4 so that "therapeutic" doses of CCl_4 almost invariably produce massive liver damage,[1] thus suggesting that CCl_4 is converted by hepatic microsomal enzymes to a toxic metabolite. Similar results have been obtained in rats.[2]

Discussion of these incidents with my co-workers triggered a thought. We recollected that a considerable number of compounds, especially halogenated aromatic hydrocarbons used as industrial solvents or chemical intermediates, were even more chemically inert than CCl_4 in the test tube but were also relatively specific in producing hepatic and renal necrosis.

This led us to the question "Why do many therapeutic agents that are chemically relatively inactive produce in the occasional patient tissue lesions including liver and kidney damage, bone marrow depression, and various kinds of skin disorders, some of which are ascribed to allergic responses?" This laboratory had previously postulated that in order for a drug to produce cel-

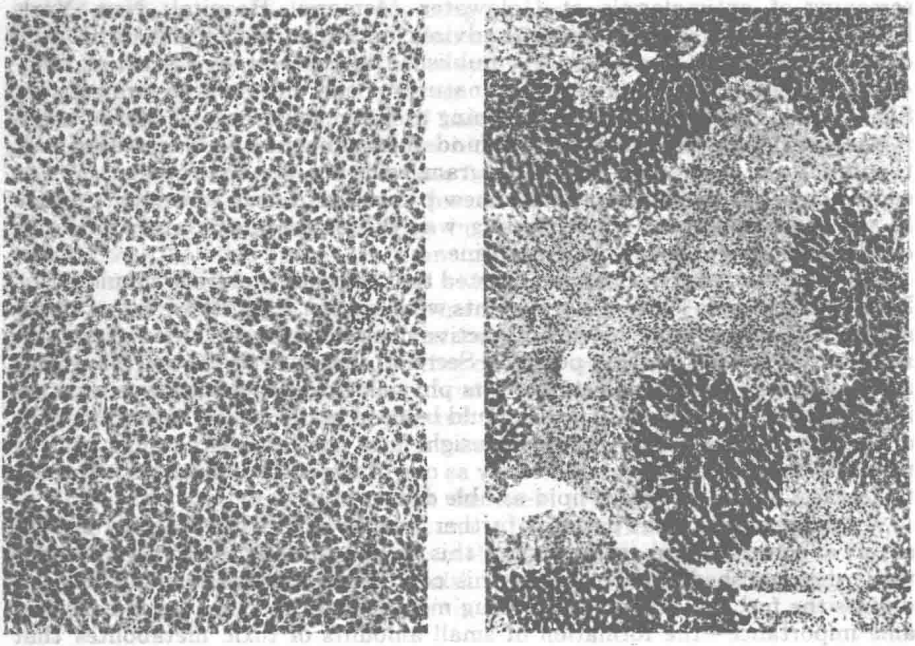


FIGURE 1. Paraffin sections from livers of Sprague-Dawley rats (male, 180 g); PAS stain; × 30. *Left:* Normal liver of control animal killed 24 hours after injection of 1 ml sesame oil. *Right:* Extensive centrolobular necrosis in rat killed 24 hours after administration of 1 ml/kg bromobenzene in sesame oil, i.p.

lular damage it must first form a covalent linkage with various cellular components, either directly or through a metabolite.[3,11] To test this view, Reid and co-workers[11] studied a series of halogenated hydrocarbons, many of which reproducibly cause hepatic necrosis in various animal species.[4] By disclosing the toxic mechanisms of these compounds, we hoped to obtain some insight into the tissue lesions caused by therapeutic drugs.

Single doses of chloro-, bromo-, or iodobenzene (1 ml/kg, intraperitoneally, in sesame oil) were administered to rats, and within 24 hours produced massive necrosis in the centrolobular regions of the liver. For example, FIGURE 1 contrasts the histology of a normal liver (left) with that of a necrotic liver

(right) from a rat treated with bromobenzene. Similar results were obtained with the α- and β-chlorinated naphthalenes. The effects of all these agents were markedly potentiated by pretreatment of the rats for three days with phenobarbital (80 mg/kg, i.p.), a drug known to stimulate markedly the microsomal drug-metabolizing enzymes in the liver. For example, doses of bromobenzene (0.15 ml/kg, i.p.) that produced minimal changes in the livers of control rats (FIGURE 2, left) elicited massive necrosis in livers of animals pretreated with phenobarbital (right). These studies suggested that microsomal enzymes convert aromatic hydrocarbons to a toxic derivative responsi-

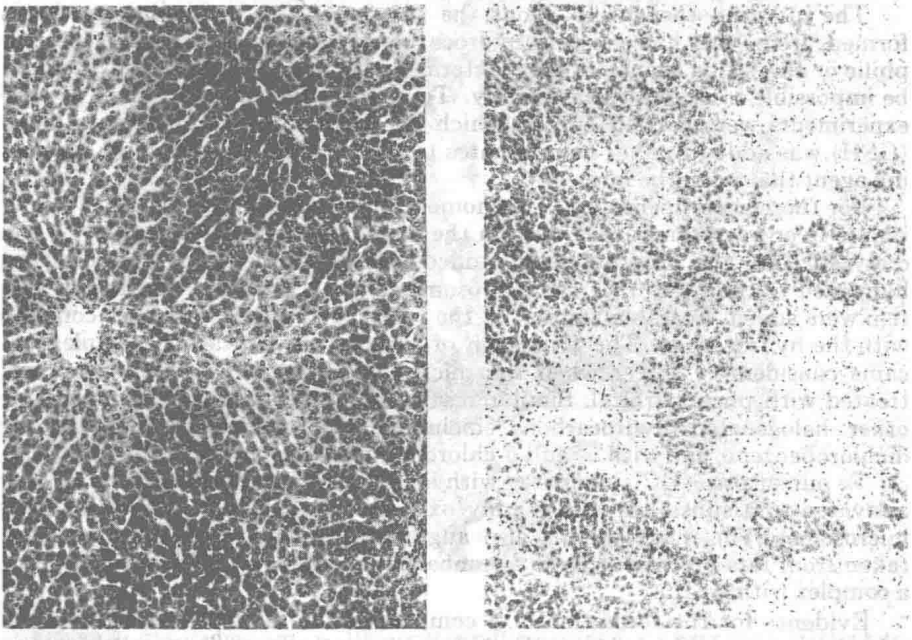


FIGURE 2. Paraffin sections of liver; PAS stain; × 30. Left: Liver from rat 24 hours after administration of 0.15 ml/kg bromobenzene. The centrolobular areas are decreased in glycogen and have some small patches of round cell infiltration but relatively little necrosis of hepatocytes. Right: Extensive centrolobular necrosis 24 hours after administration of bromobenzene (0.15 ml/kg) in rats pretreated with phenobarbital (80 mg/kg, i.p., for three days) in order to induce hepatic microsomal enzymes.

ble for the hepatic necrosis. The centrolobular distribution of the lesions may be explained by the observation that phenobarbital-induced changes in the smooth endoplasmic reticulum (that is known to contain microsomal enzymes) are localized to the central zone of the liver lobules.[5]

In other experiments,[11] we administered allyl alcohol and allyl formate (0.05 ml/kg, i.p.), which produced liver necrosis mainly in the periportal region. This region contains high concentrations of the enzyme alcohol dehydrogenase, which converts these compounds to the aldehyde acrolein, a very reactive alkylating agent.[6] From these experiments, it could be presumed that

the pattern of cellular necrosis was determined by the distribution of the enzymes responsible for forming the toxic metabolite acrolein.[6] As might be expected, phenobarbital administration did not enhance the hepatoxicity of these compounds.[11]

The exclusively centrolobular damage produced by the halogenated aromatic hydrocarbons suggested that these compounds were converted to highly reactive intermediates by cells in the centrolobular region, where they destroyed the very cells that formed them. Since the toxicity was potentiated by phenobarbital, the possibility was entertained that the conversion was mediated by the NADPH-dependent drug-metabolizing enzymes in liver microsomes.

The question then arose about the nature of the active intermediates formed from the halogenated hydrocarbons, presumably highly electrophilic or alkylating agents. If these intermediates were very reactive, it would be impossible to isolate them directly. To circumvent this difficulty, *in vitro* experiments were carried out in which the active nucleophile glutathione (GSH) was added to liver homogenates to react covalently with any alkylating agent that might be formed.[7]

For these experiments, rat liver homogenates were spun at 100,000 $\times$ *g* to isolate liver microsomes that contain the drug-metabolizing enzymes. To the dialyzed 100,000 *g* supernate were added bromobenzene and GSH. Nothing happened to the GSH until the microsomes and an NADPH generating system were added. A sizable amount of the GSH now rapidly formed a complex with the hydrocarbon. The formation of the GSH hydrocarbon complex became considerably larger when the microsomes were taken from rats pretreated with phenobarbital. Similar results were obtained with a number of other halogenated hydrocarbons, including chloro- and iodobenzene, *o*-dichlorobenzene, and with α- and β-chloronaphthalene.

To our surprise, CCl_4 incubated with liver supernate, NADPH, and O_2 was activated to a substance that readily oxidized GSH in the presence of liver microsomes. This reaction was also augmented when the microsomes were taken from rats pretreated with phenobarbital. However, CCl_4 did not form a complex with GSH.

Evidence for the formation of a complex in the *in vitro* system was obtained by using ^{35}S-labeled GSH and ^{14}C-bromobenzene in parallel experiments and identifying the labeled GSH-hydrocarbon complexes on paper chromatograms.[7] The R_f of the ^{35}S-GSH-hydrocarbon complex was the same with use of either ^{35}S-GSH or ^{14}C-bromobenzene and was formed only in the presence of liver microsomes. In the case of compounds unavailable in the labeled form, the appearance of a complex with ^{35}S-GSH was also evident in the presence of liver microsomes. These results indicate that microsomal enzymes can convert the above-mentioned aromatic halogenated hydrocarbons to chemically reactive intermediates capable of forming a complex with GSH.[8] However, the substrate-dependent alkylation of GSH was not stoichiometric with the substrate-dependent disappearance of GSH. The GSH that did not form a complex with the substrate was apparently oxidized, and the mechanism of this oxidation is currently under investigation. By contrast with the aromatic compounds, CCl_4 did not form a complex with GSH.

Perhaps the most definite evidence that the bromobenzene formed a covalent bond with macromolecules in liver parenchymal cells was provided by radioautographs of mouse liver prepared 40 hours after the administration of ^{14}C-bromobenzene.[11] FIGURE 3 shows the presence of large amounts of ^{14}C in

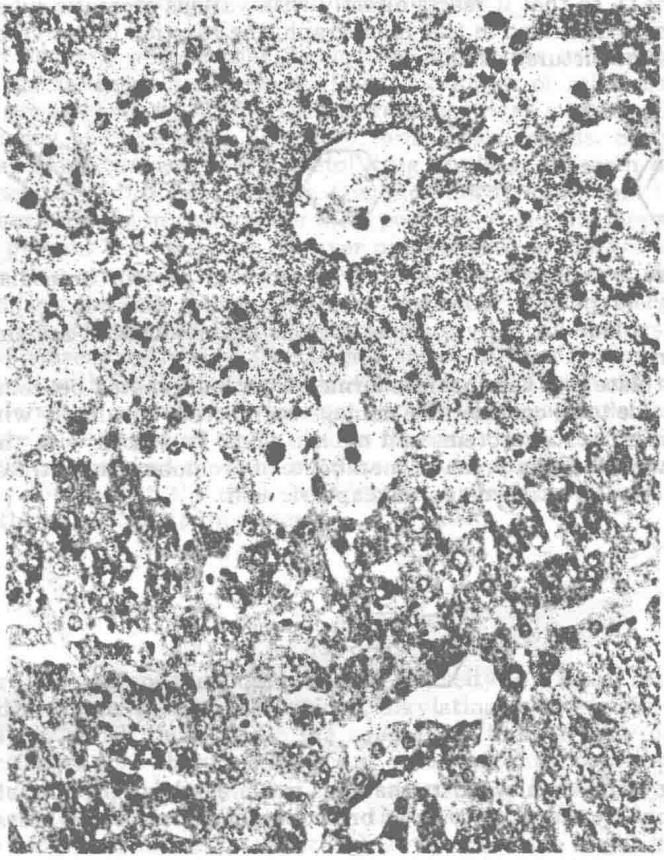


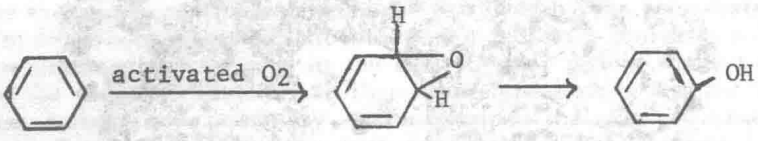
FIGURE 3. Autoradiogram (× 100) of a paraffin section from liver of an NIH white mouse (30 g; male) 40 hours after administration of ^{14}C-bromobenzene (0.685 ml/kg; 250 μc). The sections were stained with PAS, coated with Kodak NTB-2 emulsion, developed after two weeks and counterstained with haematoxylin. The ^{14}C, which is represented by the large number of very small black grains, is localized mainly in the necrotic tissue of the centrolobular region.

the necrotic centrolobular zone relative to the periportal region, where the hepatocytes appeared normal. Since the histologic slides of the liver were prepared under conditions that would probably remove most of the water-soluble and liposoluble substances, it may be presumed that the ^{14}C represents a covalent bond between bromobenzene metabolites and macromolecules of the liver cells.

On the Biochemical Mechanism

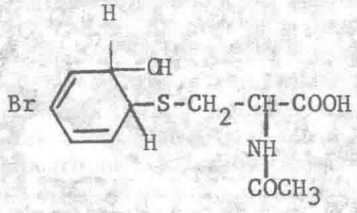
Work discussed in this monograph by Dr. Gillette shows the central role of the enzyme cytochrome P-450 in drug oxidation. This enzyme activates

atmospheric O_2 so that it reacts directly with various aromatic substrates. As described by Udenfriend in this monograph, the reaction of activated O_2 with benzene can be pictured as follows:

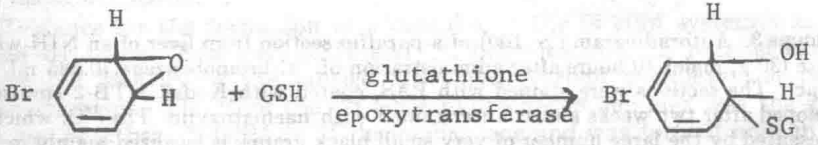


The epoxide is transient, and in the presence of protein immediately rearranges to yield phenol. However, various epoxides differ in stability, from those that explode violently to those such as heptachlor that are almost completely inert.

We postulate that the epoxides formed from halogenated benzenes are sufficiently stable to do considerable damage by reacting covalently with various nucleophilic groups in proteins and nucleic acids. In accord with this view is the well-known fact that a major metabolite of bromobenzene is the acetylated cysteinyl derivative known as a mercapturic acid:

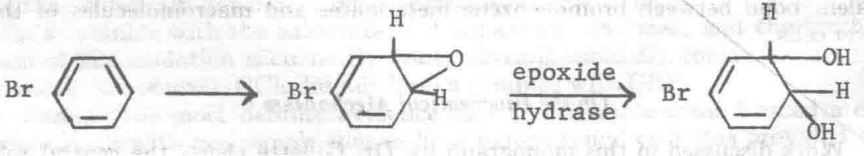


Convincing evidence now exists that this is a breakdown product of the substance formed by the interaction of bromobenzene epoxide and glutathione as follows:



The resulting GSH conjugate loses two of its amino acids and water and gains an acetyl group to yield finally mercapturic acid.[9]

Additional evidence that bromobenzene is metabolized to an epoxide stems from the finding that a metabolite of bromobenzene is the dihydrodiol derivative.



This compound is probably formed by the reaction of the bromobenzene with water in the presence of epoxide hydrase.[10]