Chapter 21. Drug Metabolism

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Introduction - In this chapter selected highlights of the recent literature on drug metabolism will be presented. The material discussed will focus on compounds with pharmacological, toxicological, or biological activity, with the objective of providing information germane to medicinal chemists. It is important to point out that the state-of-the-art in this field is changing rapidly due to rapid advances in instrumentation, new methodology and the discovery of new aspects of drug disposition.[1,2]

Recent Books - There have been several books published on various aspects of drug metabolism. Drug Metabolism Concepts, the publication of an ACS symposium, discusses the enzyme systems responsible for most drug biotransformations (cytochrome P-450's, P-448's, epoxide hydrase(s), etc.), their stereospecificity and role in metabolic activation.[3] The second volume of the series on Progress in Drug Metabolism has been published.[4] An excellent volume on Interactions of Drugs of Abuse contains many excellent chapters including one by Gillette on factors affecting drug interactions.[5] Drug Interactions is the second book by the same title published in the last few years.[6,7] The third volume of The Fate of Drugs in the Organism is now available.[8] The initial volume of a new series entitled Drug Fate and Metabolism, Methods and Techniques, has also appeared.[9] Other books on more limited subjects within the field deal with pharmacokinetics in disease states,[10] metabolic activation in mutagenesis testing,[11] mass spectrometry applications,[12] and monitoring of drug levels.[13]

Methodology - One of the more active areas of methods development is the pursuit of new or improved assays for drugs and their metabolites in biological samples. This is exemplified by the increasing use of radioimmunoassay (RIA), high performance (or high pressure) liquid chromatography (HPLC), quantitative thin-layer chromatography (QTLC), and combined gas chromatography-mass spectrometry (GC/MS). GC/MS systems are often equipped with dedicated computer data systems. Chemical ionization (CI), and selected ion monitoring (SIM) procedures may provide enhanced sensitivity and specificity, and are frequently employed in the development of GC/MS assays.

A method has been reported for the simultaneous determination of d- and l-amphetamine in human plasma using gas chromatography-chemical ionization mass spectrometry (GC/CIMS) of diastereoisomer derivatives.[14] The use of stable isotope-labeled drug modifications as internal standards for the determination of drugs in biological fluids by GC/CIMS can permit quantitation in the picogram region, as shown for methadone.[15] A new technique has been reported in which ^{13}C-labeled and unlabeled drug were administered in a known ratio, and metabolites in urine were detected by a combination of gas chromatography/combustion/mass fragmentography.[16] GC/MS has been applied to studies of free organic acids extracted from human saliva.[17] Autoradiography continues to be a useful tool for studying the

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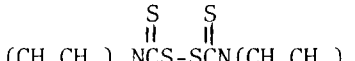
distribution of drug-related entities and is being used increasingly in the study of feto-placental transfer.[18] QTLC continues to be a popular choice for multiple simultaneous assays,[19] and sensitivity down to 8 ng/ml of plasma has been attained for codeine phosphate, and 1 ng/ml for chlorpheniramine maleate by suitable derivatization and spectrophotometric detection.[20] The method involves nitration, reduction and diazo coupling with N-(1-naphthyl)ethylenediamine directly on thin-layer plates, and should be applicable to the analysis of other aromatic compounds.

A radioassay has been developed for the quantitation of disulfiram (1) and several of its metabolites (diethyldithiocarbamate, diethyldithiocarbamate glucuronide, inorganic sulfate and a protein bound ^{35}S fraction) from a single sample of plasma, urine, or tissue obtained after administration of disulfiram-^{35}S to laboratory animals.[21] Although the method is unusually complex, it nevertheless is a fine example of the potential for the development of specific assays for drugs and metabolites utilizing radiolabeled drugs.



1

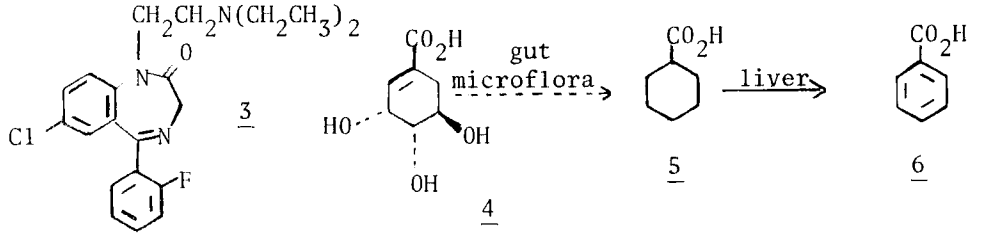
Hepatic Microsomal Enzymes - Research into the nature, specificity and inducibility of drug metabolizing enzymes is making steady progress. A kinetic method for the determination of multiple forms of microsomal cytochrome P-450 has been reported.[22] Further support has also been obtained for the hypothesis that drug metabolism in isolated hepatocytes correlates better with metabolism *in vivo* than does metabolism in subcellular hepatic microsomal fractions.[23] Metabolism studies in isolated rat liver parenchymal and nonparenchymal cells showed that parenchymal rat liver cells metabolized (acetylated) sulfadimidine, sulfanilamide, p-benzoic acid, and isoniazid (2), but nonparenchymal cells only metabolized isonaizid to an appreciable extent.[24] Steric factors affecting the inhibition of microsomal enzymes by imidazoles have been investigated.[25] Spectral studies suggest that cytochrome P-450 content in rat hepatic microsomes is decreased by high concentrations ($\geq$ 0.14 M) of acetaminophen, a known hepatotoxin.[26] Two differentially inducible UDP-glucuronyl transferases present in rat liver have been separated and partially purified.[27]

An *in vitro* study of epoxide hydrase activity in human liver indicated that hydration of the 11 epoxides studied was catalyzed either by a single enzyme, or by a group of enzymes under the same regulatory control.[28] In another *in vitro* study using human liver, the intersubject variability found in the hydroxylation of a series of substrates suggests that multiple mono-oxygenases may be present in man.[29]

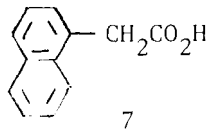
2

Extrahepatic Drug Biotransformation - Although the liver is the primary site of biotransformation of most drugs, it is not the only site in many cases. Depending upon the structure of a drug, its route of administration, and its distribution in tissues, biotransformation may occur at such extrahepatic sites as kidney, lung, skin, intestine, and blood. Recent publications on extrahepatic metabolism include a minireview on pulmonary metabolism,[30] and papers on oxidative metabolism in rat small intestine[31] and drug biotransformation in the skin.[32] The metabolism of flurazepam (3) by the small intestine was studied in man,[33] and evidence of oxidative N-dealkylation was obtained. This appears to be the first demonstration of metabolism of a benzodiazepine by human intestine *in vivo*. However, the contribution of metabolism at this site to total metabolism is not clear, since no quantitative determinations were made. The authors suggested that imiprimine might also undergo N-dealkylation in human intestine, and raised the general question as to the relative roles of the small intestines and the liver in the metabolism of drugs. Further studies are indicated.

Intestinal microflora may also be responsible for extrahepatic biotransformations, and evidence has been presented that the unusual aromatization of orally administered shikimic acid (4) to benzoic acid (6), may involve microbial formation of cyclohexane carboxylic acid (5) as an intermediate.[34] Such findings suggest that species differences in drug metabolism may sometimes be due to differences in gastro-intestinal microflora.



Interspecies Comparative Metabolism - Knowledge of species differences is important in evaluating the relevance of safety and efficacy data obtained in animals to man. The comparative metabolism of two arylacetic acids, 1-naphthylacetic acid (7)[35] and hydratropic acid (8)[36] has been studied, complementing earlier studies on the metabolism of phenylacetic acid (9), p-chlorophenylacetic acid (10) and indolylacetic acid (11). For these compounds qualitative and quantitative differences were observed among the species studied with regard to the percentage of the dose accounted for in urine as glucuronide, glycine, glutamine and taurine conjugates. Among the numerous papers published on the comparative metabolism of specific drugs, one, on metoclopramide (12) metabolism, is particularly noteworthy, since eight metabolites were identified from rat, dog and human urine, but only one (13) was common to all three species.[37]



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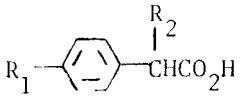
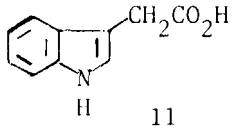


8 R_1 = H, R_2 = CH_3
9 R_1 = H, R_2 = H
10 R_1 = Cl, R_2 = H



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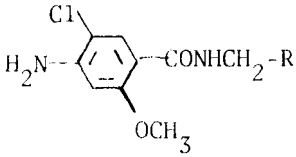
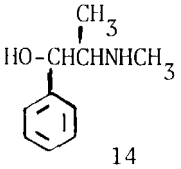


12 R = $CH_2N(C_2H_5)_2$
13 R = CO_2H

Stereoselectivity in Drug Disposition - Differences have been reported in the metabolism of R- and S-ephedrine (14),[38] R- and S-warfarin (15),[39] R- and S-methadone (16),[40] and the R/S ratio of unchanged drug excreted after oral administration of a racemic amphetamine analog (17) to rabbits varied between 1.0 to 1.7 according to dose.[41] An R/S ratio of approximately 2 was observed in rat urine after administration of 17.[42] Racemic 17 was metabolized in vitro at a different rate than either of the individual enantiomers which were metabolized at approximately the same rate.[43] Differential disposition has also been demonstrated for the two enantiomers of cocaine in rats, and may be a partial explanation for differences in pharmacological effects.[44] These observations suggest that racemates can have pharmacological and toxicological properties different from the individual enantiomers.



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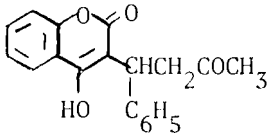

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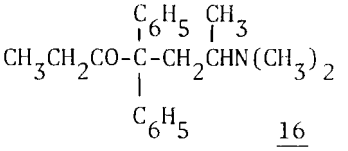

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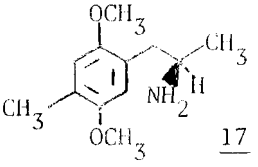
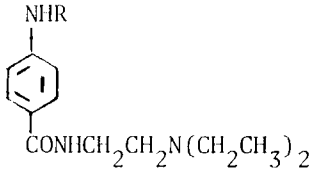


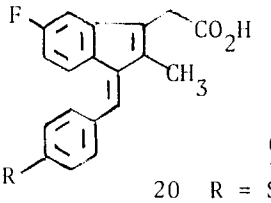
17

Biotransformation to Active Metabolites - Biotransformations may result in the formation of compounds with pharmacologic properties comparable to, or more potent than the parent compound. One such example is the antiarrhythmic drug procainamide (18), which is metabolized to an active acetyl metabolite (19).[45] The conversion of prodrugs to active substances is exemplified by sulindac (20), a nonsteroidal antiinflammatory agent which is converted in vivo to its active form 21.[46,47]



18 R = H

19 R = $\overset{O}{\overset{\|}{C}}CH_3$

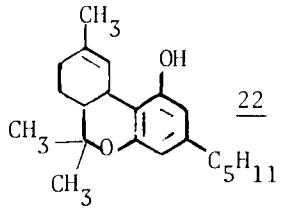


20 R = $\overset{O}{\uparrow}SCH_3$

21 R = SCH_3

Highly reactive metabolites can also be produced, and such processes are being studied with increasing intensity, since they may be responsible for serious toxic effects of drugs.[11] Formation of highly reactive metabolites has been implicated as the cause of a wide array of toxic effects, from hepatic necrosis to mutagenesis and carcinogenesis, and as an explanation of the similar toxicities observed for compounds of very dissimilar structures.[11,48,49] Reviews of the subject continue to appear in the literature. Since the liver is the most common site for biotransformation, it is not surprising that hepatic necrosis, and malignant hepatic tumors are among the more frequently observed toxic manifestations when very reactive metabolites are formed. In recent years it has become increasingly apparent that the most toxic metabolites rarely survive long enough to be excreted, and studies limited to the identification of metabolites in excreta may therefore provide misleading information. It should also be mentioned that metabolic activation is an integral part of mutagenicity testing.

The teratogenicity of Δ^9-tetrahydrocannabinol (THC) (22) could be altered by stimulation and inhibition of its metabolism, and an active metabolite may be responsible.[51] *In vitro* studies of 7,12-dimethylbenz[a]-anthracene (23), a potent carcinogen have shown it to undergo bioactivation in mouse skin homogenates from mice treated with 3-methylcholanthrene.[52] A great deal of attention is currently focused on the bioactivation of the aspirin substitute, acetaminophen (24). When overdoses are ingested, a normal biotransformation pathway (conjugation with glutathione) is saturated, and a highly reactive metabolite is formed which binds irreversibly to hepatic tissue and may result in extensive hepatic necrosis.[53,54,55,56]



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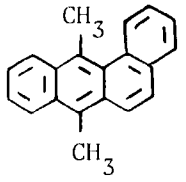

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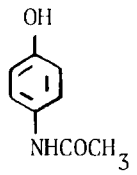
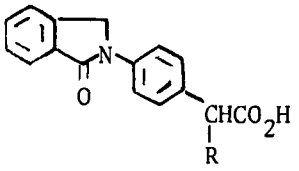


24

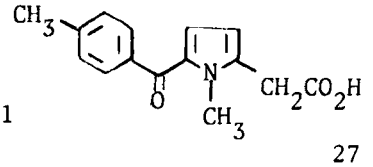
Reversible Protein Binding - The reversible binding of drugs to plasma proteins and to proteins in tissues may play an important role in the disposition and activity of many drugs. A comprehensive review of methods

for determining protein binding has been published.[57] The effect of protein binding on pharmacokinetics and therapeutic activity of antimicrobial drugs has been reviewed.[58] Differences in the binding of some drugs to plasma proteins of neonates and adult humans has been demonstrated.[59] Species differences have been observed for the binding of warfarin to plasma proteins.[60] A lack of correlation between in vitro activity (inhibition of PG-synthetase) and in vivo analgesic-antiinflammatory activity (phenylquinone-induced writhing in mice, and carrageenan-induced edema in rats) for indoprofen (25) and a series of its analogs (26) was attributed to differences in plasma protein binding.[61] Evidence of non-first-order behavior for plasma levels of tolmetin (27), a new nonsteroidal antiinflammatory drug, has been reported in man and attributed to reversible tissue binding.[62]



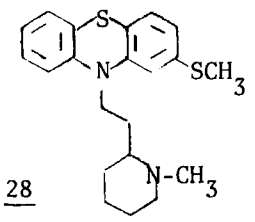
25 R = CH_3

26 R = H, ethyl, propyl or butyl



27

Pharmacokinetic-Pharmacodynamic Correlations - Pharmacokinetic evaluation of drugs and their metabolites can provide invaluable information regarding appropriate clinical dose regimens. Pharmacokinetic comparisons of analogs with similar potency in pharmacological models of human disease can assist in the selection of the compound to undergo clinical evaluation. Such studies are especially valuable when, for example, the time course of the pharmacological response can be related to that of the drug (and/or metabolites) in blood. A variety of methods are now available for obtaining serial blood microsamples from small laboratory animals and for the analysis of drug and/or metabolite concentrations in the samples.[63] Correlations of activity with blood, serum and plasma concentrations were reported for S-warfarin[64] in rats, and thioridazine (28)[65] and chlorpromazine (29)[66] in man. For chlorpromazine, an even better correlation was reported for one of its metabolites, 7-hydroxychlorpromazine (30).[67] Prolactin concentrations were found to correlate better than blood levels with clinical response following administration of thioridazine.[68] No correlation was observed between blood concentrations and antihypertensive activity of the cardioselective β-adrenergic blocking agent atenolol. (31)[69] The kinetics of the antiarrhythmic effect of procainamide showed a direct correlation with the kinetics of drug in saliva, but not in plasma.[70] This indicates that a good correlation between drug response and plasma concentrations of the drug is not always observed, but that by including metabolites in the analyses and by examining a variety of body fluids, such a correlation may be found. The effect of active drug metabolites on plasma level-response correlations has been reviewed,[71] and a pharmacokinetic model for simultaneous determination of drug levels in organs and tissues has been developed.[72]



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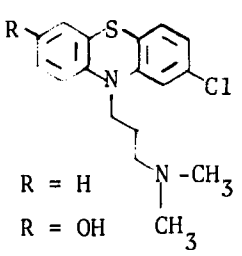
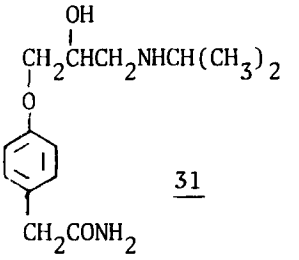


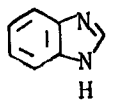
29 R = H

30 R = OH



31

Novel Biotransformation Pathways and Products - The understanding of how metabolites are formed is often as important as knowing the chemical structure of the metabolites. During the past few years there have been many reports of novel metabolites, the biogenesis of which apparently followed pathways not previously known. In most cases, the structures of these metabolites were established by spectroscopic methods. Novel biotransformation products were the subject of reviews,[73,74] and several other novel biotransformations have been reported separately, including the reductive cleavage of anthracycline glycosides by cytochrome c reductase.[75] The N-methylation of benzimidazole (32) by catechol-O-methyltransferase (COMT) is the first known example of N-methylation by COMT.[76] Metabolism of clocoumarol (33), a synthetic vitamin K antagonist, by rat liver microsomes produced the new compound 34.[77] Metabolites reported for the hypnotic triazolam (35) in dogs include 36, in which the methyl carbon group is lost, presumably by an oxidation-decarboxylation pathway.[78]



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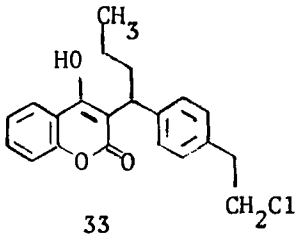

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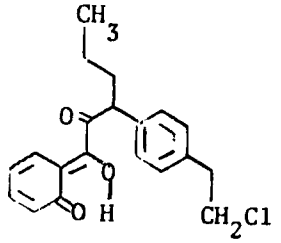
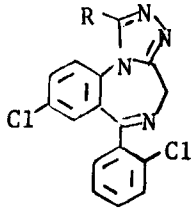


34 (cis-enol form)



35 R = CH_3

36 R = H

REFERENCES

1. V.I. Kantemir, G. Akder and O. Tulunay, Arzneim.-Forsch. 26, 261 (1976)
2. E. Shmunes, Annals of Allergy 39, 186 (1977)
3. D.M. Jerina, ed., "Drug Metabolism Concepts", American Chemical Society, Washington, D.C., 1977
4. J.W. Bridges and L.F. Chasseaud, "Progress in Drug Metabolism", vol. 2, John Wiley & Sons, London 1977
5. E.S. Vesell and M.S. Braude, eds., "Interactions of Drugs of Abuse" (Ann. N.Y. Acad. Sci.) 281, N.Y. Acad. Sci., New York 1976
6. D.G. Grahame-Smith, ed., "Drug Interactions", Univ. Park Press, Baltimore 1977
7. P.L. Morselli, ed., "Drug Interactions", Raven Press, New York 1974
8. J. Hirz, et al., "The Fate of Drugs in the Organism: A Bibliographic Survey", vol. 3, Marcel Dekker, Inc., New York and Basel 1976
9. E.R. Garret and J.L. Hirtz, ed., "Drug Fate and Metabolism: Methods and Techniques", vol. 1, Marcel Dekker, Inc., New York 1977
10. L.Z. Benet, ed., "The Effect of Disease States on Drug Pharmacokinetics", American Pharmaceutical Association, Wash., D.C. 1976
11. F.J. de Serres, J.R. Fouts, J.R. Bend and R.M. Philpot, eds., "In Vitro Metabolic Activation in Mutagenesis Testing", North Holland Publishers, Amsterdam 1976
12. A. Frigerio and E.L. Ghisaberti, eds., "Mass Spectrometry in Drug Metabolism", Plenum Press, New York 1977
13. R.B. Stewart, L.E. Cluff and J.R. Philip, "Drug Monitoring: A Requirement for Responsible Drug Use", William & Wilkins, Baltimore 1977
14. S.B. Matin, S.H. Wan and J.B. Knight, Biomed. Mass Spectrom. 4, 118 (1977)
15. D.L. Hachey, M.J. Kreek and D.H. Mattson, J. Pharm. Sci. 66, 1579 (1977)
16. M. Sano, Y. Yotsui, H. Abe and S. Sasaki, Biomed. Mass Spectrom. 3, 1 (1976)
17. M.E. Ward, I.R. Politzer, J.L. Laseter and S.Q. Alam, Biomed. Mass Spectrom. 3, 77 (1976)
18. C. Declume and P. Bernard, Toxicology 8, 95 (1977)
19. N.T. Wad and E.J. Hanifl, J. Chromatog. 143, 214 (1977)
20. P. Haefelfinger, J. Chromatog. 124, 351 (1976)
21. M.D. Faiman, E.D. Dodd, R.J. Nolan, L. Artman and R.E. Hanzlik, Res. Comm. Chem. Pathol. & Pharmacol. 17, 481 (1977)
22. W.R. Porter, R.W. Branchflower and W.F. Trager, Biochem. Pharmacol. 26, 549 (1977)
23. R.E. Billings, R.E. McMahon, J. Ashmore and S.R. Wangle, Drug Metab. Dispos. 5, 518 (1977)
24. J. Morland and H. Olsen, Drug Metab. Dispos. 5, 511 (1977)
25. T.D. Rogerson, C.F. Wilkinson and K. Hetarski, Biochem. Pharmacol. 26, 1039 (1977)
26. R.A. Wilson and F.E. Hart, Res. Comm. Chem. Pathol. Pharmacol. 17, 605 (1977)
27. K.W. Bock, U.C. von Clausbrunch, D. Josting and H.O. Henwalder, Biochem. Pharmacol. 26, 1097 (1977)
28. J. Kapitulnik, W. Levin, A.Y.H. Lu, R. Morecki, P.M. Dansette, D.M. Jerina, and A.H. Conney, Clin. Pharmacol. Ther. 21, 158 (1977)

29. J. Kapitulnik, P.J. Poppers, and A.H. Convey, Clin. Pharmacol. Ther. 21, 166 (1977)
30. G.E.R. Hook and J.R. Bend, Life Sciences 18, 279 (1976)
31. H. Hoensch, C.H. Woo, and S.B. Raffin, Gastroenterology 70, 1063 (1976)
32. H.Y. Ando, N.F.H. Ho, and W.I. Higuchi, J. Pharm. Sci. 66, 1525 (1977)
33. W.A. Mahon, T. Inaba, and R.M. Stone, Clin. Pharmacol. Ther. 22, 228 (1977)
34. L.M. Ball, A.G. Renwick, and R.T. Williams, Xenobiotica 7, 101 (1977)
35. P.A.F. Dixon, J. Caldwell, and R.L. Smith, Xenobiotica 7, 695 (1977)
36. ibid 7, 707 (1977)
37. L. Teng, R.B. Bruce, and L.K. Dunning, J. Pharm. Sci. 66, 1615 (1977)
38. D.R. Feller and L. Malapeis, Drug Metab. Dispos. 5, 37 (1977)
39. L.R. Pøhl, W.R. Porter, W.F. Trager, M.J. Fasco, and J.W. Fenton, II, Biochem. Pharmacol. 36, 109 (1977)
40. N. Gerber, R.M. Leger, P. Gordon, R.G. Smith, J. Bauer, and R.K. Lynn, J. Pharmacol. Exp. Ther. 200, 487 (1977)
41. S.B. Matin, P.S. Callery, J.S. Zweig, A. O'Brien, R. Rappoport, and N. Castignoli, Jr., J. Med. Chem. 17, 877 (1974)
42. J. Gal, J. Pharm. Sci., 66, 169 (1977)
43. N.P. McGraw, P.S. Callery, and N. Castagnoli, Jr., J. Med. Chem. 20, 185 (1977)
44. A.L. Misra and R.B. Pontani, Drug Metab. Dispos. 5, 556 (1977)
45. A.J. Atkinson, Jr., W.-K. Lee, M. Quinn, W. Kushner, M.J. Nevin, and J.M. Strong, Clin. Pharmacol. Ther. 21, 575 (1977)
46. D.E. Duggan, K.F. Hooke, E.A. Risley, T.Y. Shen, and C.G. Van Arman, J. Pharmacol. Exp. Ther. 201, 8 (1977)
47. D.E. Duggan, L.E. Hare, C.A. Ditzler, B.W. Lei, and K.C. Kwan, Clin. Pharmacol. Ther. 21, 326 (1977)
48. F. Schaffner, Vet. Pathol. 12, 145 (1975)
49. S.D. Nelson, M.R. Boyd, and J.R. Mitchell, in Drug Metabolism Concepts, D.M. Jerina, ed., ACS, Washington, D.C. 1977, pp. 155-185
50. Y. Woo, J.C. Arcos, and M.F. Argus, Biochem. Pharmacol. 26, 1535 (1977)
51. R.B. Harbison, B. Mantilla-Platt, and D.J. Lubin, J. Pharmacol. Exp. Ther. 202, 455 (1977)
52. J. DiGiovanni, T.J. Slaga, D.L. Berry, and M.R. Juchau, Drug Metab. Dispos. 5, 295 (1977)
53. M. Davis, N.G. Harrison, G. Ideo, B. Portmann, D. Labadarios, and R. Williams, Xenobiotica 6, 249 (1976)
54. M. Davis, D. Labadarios, and R.S. Williams, J. Int. Med. Res. 4, 40 (1976)
55. P.L. Madan, J. Clin. Pharmacol. 555 (1977)
56. G.J. Merrit, and P.U. Joyner, Drug Intell. Clin. Pharm. 11, 458 (1977)
57. H. Kurz, H. Trunk, and B. Weitz, Arzneim.-Forsch. 27, Nr. 8 (1977)
58. W.A. Craig and Peter Willing, Clin. Pharmacokinetics 2, 252 (1977)
59. H. Kurz, H. Michels, and H.H. Stickel, J. Clin. Pharmacol. 11, 469 (1977)
60. E.M. Sellers, M.L. Lang-Sellers, and J. Koch-Wesser, Biochem. Pharmacol. 26, 2445 (1977)
61. R. Ceserani, M. Ferrari, G. Goldaniga, E. Moro, and A. Buttinoni, Life Sciences 21, 223 (1977)

62. J.W. Ayres, D.J. Weidler, E. Sakmar, and J. Wagner, Res. Comm. Chem. Pathol. Pharmacol. 17, 583 (1977)
63. B.H. Migdalof, Drug Metab. Rev. 5, 295 (1976)
64. A. Yacobi and G. Levy, J. Pharm. Sci. 66, 1275 (1977)
65. R.G. Muusze and F.A.J. Vanderheeren, Eur. J. Clin. Pharmacol. 11, 141 (1977)
66. L. Rivera-Calimlim, H. Nasrallah, J. Strauss, and L. Lasagna, Am. J. Psychiatry 133, 64 (1976)
67. O.T. Phillipson, J.M. McKeown, J. Baker, and A.F. Healey, Brit. J. Psychiat. 131, 172 (1977)
68. G. Nikitopoulou, M. Thorner, J. Crammer, and M. Lader, Clin. Pharmacol. Ther. 21, 422 (1977)
69. A. Amery, J.-F. De Plaen, P. Lijnen, J. McAinsh, and T. Reybrouck, Clin. Pharmacol. Ther. 21, 691 (1977)
70. R.L. Galeazzi, L.Z. Benet, and L. Sheiner, Clin. Pharmacol. Ther. 20, 278 (1976)
71. A.J. Atkinson, Jr., and J.M. Strong, J. Pharmacokinetics Biopharm. 5, 95 (1977)
72. C.N. Chen and J.D. Andrade, J. Pharm. Sci. 65, 717 (1976)
73. Z.H. Disraili, P.G. Dayton, and J.R. Kiechel, Drug Metab. Dispos. 5, 411 (1977)
74. P. Jenner and B. Testa, Xenobiotica 8, 1 (1978)
75. T. Oki, T. Komiyama, H. Tone, T. Inui, T. Takeuchi, and H. Umexawa, J. Antibiotics 30, 613 (1977)
76. C.D. Arnett, P.S. Callery, and N. Zenker, Biochem. Pharmacol. 26, 377 (1977)
77. N. Thonnart, J. Med. Chem. 20, 604 (1977)
78. F.S. Eberts, Jr., Drug Metab. Dispos. 5, 547 (1977)