

FC

# Cannabinoid Analysis in Physiological Fluids

Joe A. Vinson



ACS Symposium Series

98

# Cannabinoid Analysis in Physiological Fluids

**Joe A. Vinson, EDITOR**

*University of Scranton*

Based on a symposium  
sponsored by the Division  
of Analytical Chemistry at the  
173rd Meeting of the  
American Chemical Society,  
New Orleans, Louisiana,  
March 20–25, 1977.

A C S   S Y M P O S I U M   S E R I E S

**98**

AMERICAN CHEMICAL SOCIETY

WASHINGTON, D. C.      1979



Library of Congress CIP Data

Cannabinoid analysis in physiological fluids.  
(ACS symposium series; 98 ISSN 0097-6156)

Includes bibliographies and index.

1. Tetrahydrocannabinol—Analysis—Congresses. 2.  
Tetrahydrocannabinol—Metabolism—Congresses. 3.  
Chemistry, Pharmaceutical—Congresses.

I. Vinson, Joe A. II. American Chemical Society.  
Division of Analytical Chemistry. III. Series: American  
Chemical Society. ACS symposium series; 98.

RS431.T44C36 615'.323'962 79-10934  
ISBN 0-8412-0488-8 ASCMC 8 98 1-242 1979

Copyright © 1979

American Chemical Society

All Rights Reserved. The appearance of the code at the bottom of the first page of each article in this volume indicates the copyright owner's consent that reprographic copies of the article may be made for personal or internal use or for the personal or internal use of specific clients. This consent is given on the condition, however, that the copier pay the stated per copy fee through the Copyright Clearance Center, Inc. for copying beyond that permitted by Sections 107 or 108 of the U.S. Copyright Law. This consent does not extend to copying or transmission by any means—graphic or electronic—for any other purpose, such as for general distribution, for advertising or promotional purposes, for creating new collective works, for resale, or for information storage and retrieval systems.

The citation of trade names and/or names of manufacturers in this publication is not to be construed as an endorsement or as approval by ACS of the commercial products or services referenced herein; nor should the mere reference herein to any drawing, specification, chemical process, or other data be regarded as a license or as a conveyance of any right or permission, to the holder, reader, or any other person or corporation, to manufacture, reproduce, use, or sell any patented invention or copyrighted work that may in any way be related thereto.

PRINTED IN THE UNITED STATES OF AMERICA

## ACS Symposium Series

**Robert F. Gould, *Editor***

### *Advisory Board*

Kenneth B. Bischoff

Donald G. Crosby

Robert E. Feeney

Jeremiah P. Freeman

E. Desmond Goddard

Jack Halpern

Robert A. Hofstader

James D. Idol, Jr.

James P. Lodge

John L. Margrave

Leon Petrakis

F. Sherwood Rowland

Alan C. Sartorelli

Raymond B. Seymour

Aaron Wold

Gunter Zweig

## FOREWORD

The ACS SYMPOSIUM SERIES was founded in 1974 to provide a medium for publishing symposia quickly in book form. The format of the Series parallels that of the continuing ADVANCES IN CHEMISTRY SERIES except that in order to save time the papers are not typeset but are reproduced as they are submitted by the authors in camera-ready form. Papers are reviewed under the supervision of the Editors with the assistance of the Series Advisory Board and are selected to maintain the integrity of the symposia; however, verbatim reproductions of previously published papers are not accepted. Both reviews and reports of research are acceptable since symposia may embrace both types of presentation.

## PREFACE

The preparations of *Cannabis sativa* L., including marijuana and hashish, represent the most widely used group of illicit drugs in the world; they are consumed by an estimated 300 million people. A recent poll by the National Institute of Drug Abuse indicated that 53% of the population in the U.S. between the ages of 18 and 25 have tried marijuana and that the percentage is increasing.

In the last 15 years since Mechoulam isolated and identified the active ingredient in marijuana, tetrahydrocannabinol, scientific research has intensified, especially in the pharmacological area. The analytical chemistry of marijuana has progressed from the analysis of tetrahydrocannabinol and other cannabinoids in plant material to the much more difficult problem of quantitation of tetrahydrocannabinol and its metabolites in physiological fluids. Recent advances in physiological fluid analysis were discussed in a symposium at the 173rd ACS National Meeting in New Orleans. This book offers representative papers from the different analytical methods presented at that meeting from worldwide experts in the field. Following an introductory paper, analytical methodologies using gas chromatography, mass spectroscopy, radioimmunoassay, high-pressure liquid chromatography, and thin-layer chromatography are presented.

It is a pleasure to express my gratitude to the participants in the symposium for their interest and enthusiasm as well as to their spirited discussions following presentation of the papers. To the authors of papers in this volume, a heartfelt thanks for their generous contribution of time and effort. The patience of my secretary, Debbie Camp, and the proofreading ability of my wife, Yvette, are also gratefully acknowledged.

University of Scranton  
Scranton, PA 18510  
January 2, 1979

JOE A. VINSON

# CONTENTS

<b>Preface</b> .....	<b>ix</b>
<b>1. A Survey of Metabolic Transformations of <math>\Delta^1</math>-Tetrahydrocannabinol</b> Sumner Burstein	<b>1</b>
<b>2. GLC and HPLC Analyses of Cannabinoids in Biological Fluids and Applications</b> .....	<b>13</b>
E. R. Garrett, A. J. Gouyette, and C. A. Hunt	
<b>3. Detection and Quantitation of Tetrahydrocannabinol in Physiological Fluids</b> .....	<b>39</b>
Monroe E. Wall, Dolores R. Brine, Joan T. Bursey, and David Rosenthal	
<b>4. Quantitation of <math>\Delta^9</math>-Tetrahydrocannabinol and 11-Nor-<math>\Delta^9</math>- tetrahydrocannabinol-9-carboxylic Acid in Body Fluids by GC/CI-MS</b> .....	<b>59</b>
R. L. Foltz, P. A. Clarke, B. J. Hidy, D. C. K. Lin, A. P. Graffeo, and B. A. Petersen	
<b>5. Improvement of the Mass Fragmentographic Technique for Quantification of Tetrahydrocannabinol in Human Blood Plasma</b> ..	<b>73</b>
Agneta Ohlsson, Stig Agurell, Jan-Erik Lindgren, and Kurt Leander	
<b>6. A General Approach to the Analysis of Cannabinoids from Physiological Sources</b> .....	<b>81</b>
J. Rosenfeld	
<b>7. Quantitation of <math>\Delta^9</math>-Tetrahydrocannabinol and Its Metabolites in Human Urine by Probability Based Matching GC/MS</b> .....	<b>93</b>
Donald E. Green, Fu-Chuan Chao, Kay O. Loeffler, and Saul L. Kanter	
<b>8. HPLC Analysis of <math>\Delta^9</math>-Tetrahydrocannabinol and Metabolites in Biological Fluids</b> .....	<b>115</b>
Seth R. Abbott, John R. Berg, Kay O. Loeffler, Saul Kanter, Leo E. Hollister, Joan Hawkins Abrams, Hugh L. Baras, and Reese T. Jones	
<b>9. Radioimmunoassay of Cannabinoid Compounds</b> .....	<b>137</b>
C. E. Cook	
<b>10. Antisera Raised Against Tetrahydrocannabinol and Their Use in the Radioimmunoassay of Cannabinoids</b> .....	<b>155</b>
J. D. Teale, Jacqueline M. Clough, P. L. Williams, A. C. Moffat, L. J. King, and V. Marks	

<b>11. HPLC Analyses of <math>\Delta^9</math>-Tetrahydrocannabinol and 11-Nor-<math>\Delta^9</math>-tetrahydrocannabinol-9-carboxylic Acid in Human Plasma . . . . .</b>	<b>175</b>
Jimmie L. Valentine, Owen H. M. Gan, H. C. Niu, and Everett D. Thompson	
<b>12. Detection and Quantitation of <math>\Delta^9</math>-Tetrahydrocannabinol in Plasma by Dansylation and Double Labeling . . . . .</b>	<b>207</b>
J. M. Scherrmann, H. Hoellinger, Nguyen-Hoang-Nam, R. Bourdon, and E. Fournier	
<b>Index . . . . .</b>	<b>225</b>



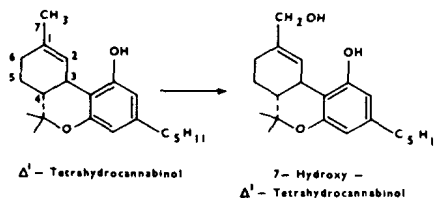
## A Survey of Metabolic Transformations of $\Delta^1$ -Tetrahydrocannabinol

SUMNER BURSTEIN

Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

Comprehensive reviews of the metabolism of  $\Delta^1$ -tetrahydrocannabinol ( $\Delta^1$ -THC) are available (1,2) so that this paper will be limited to an overview of the subject with a somewhat greater emphasis on recent developments.

It is less than eight years since the first reports on this subject have appeared. At that time, four groups almost simultaneously published findings on both  $\Delta^1$  and  $\Delta^6$ -THC, Nilsson *et al.* (3), Wall *et al.* (4), Foltz *et al.* (5) and Burstein *et al.* (6). All four laboratories independently showed that the 7 position was hydroxylated in both  $\Delta^1$  and  $\Delta^6$ -THC (Figure 1). This has since been shown to be the major initial point of metabolism in virtually every system tested thus far.



Annals of the New York  
Academy of Sciences

Figure 1. Oxidation of  $\Delta^1$ -THC (7)

The characteristics of this reaction were studied by Burstein and Kupfer (7) who showed that it followed the pattern of a typical mixed-function oxidase system. They showed that all the metabolizing activity occurred in the microsomal fraction and that oxygen and NADPH were required. Other agents such as SKF-525A ( $\beta$ -diethylaminoethyldiphenylpropyl acetate), Burstein and Kupfer (8), and DDT, Kupfer *et al.* (9), were reported to inhibit this hydroxylation process.

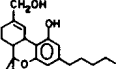
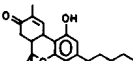
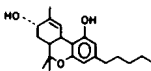
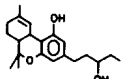
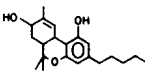
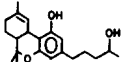
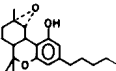
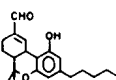
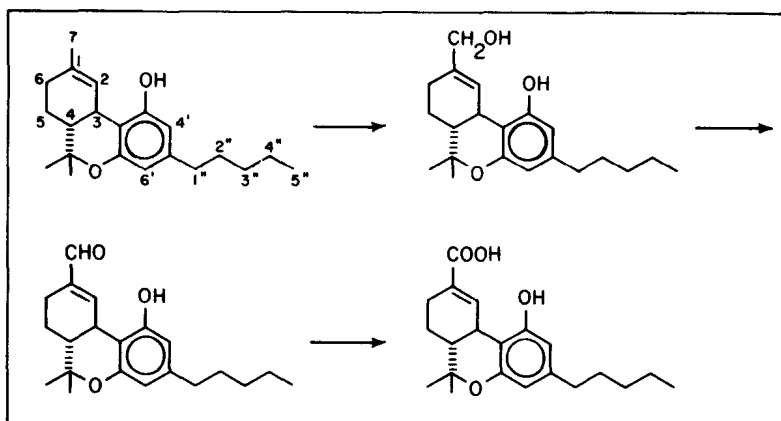
Metabolite	Metabolizing system	Metabolites	Metabolizing system	Authors
	rabbit liver rat liver rat liver man		monkey liver	Gunn et al. (1972)
	mouse rabbit liver man		dog lung dog liver	Widman et al. (1975) Widman et al. (1975)
	rabbit liver		dog lung dog liver	Widman et al. (1975) Widman et al. (1975)
	monkey liver rabbit liver			
	rat liver			

Figure 2. Oxygenated metabolites of  $\Delta^1$ -THC

In the subsequent years, a number of other mono-hydroxy- $\Delta^1$ -THC derivatives have been isolated from various metabolizing systems (Figure 2). These have been of two types: hydroxyls allylic to the  $\Delta^1$ -double bond and sidechain hydroxyls. No evidence for aromatic hydroxylation has thus far been reported, although these positions are chemically reactive and there is biochemical evidence for C-C glucuronide formation at the 4'-position of  $\Delta^6$ -THC. Possibly such catechol type metabolites of  $\Delta^1$ -THC are unstable and may be lost during the extraction and isolation procedures.

Another expected monohydroxy- $\Delta^1$ -THC which has thus far remained undiscovered is 3-OH- $\Delta^1$ -THC. This position is both allylic and benzylic and would therefore be expected to be highly susceptible to attack. The possibility exists that this product is, in fact, formed but may readily dissociate leading to a  $\Delta^{1,3}$ -diene which could serve as a precursor to other more stable metabolites such as cannabinol (CBN). This point will be discussed further in connection with certain observed transformations in a later section of this paper.



Research Communications in  
Chemical Pathology and Pharmacology

Figure 3. Metabolism of  $\Delta^1$ -THC at the 7-position (10)

Several of the monohydroxy-THCs are further oxidized to the corresponding aldehydes or ketones. Ben-Zvi and Burstein (10) have identified 7-oxo- $\Delta^1$ -THC (lower left of Figure 2) as a product of  $\Delta^1$ -THC when incubated with rat liver microsomes. Although it has not been demonstrated, it seems certain that 7-OH- $\Delta^1$ -THC is the precursor for this transformation (Figure 3). The levels of this aldehyde in human tissues are unknown at this time, however, it is of interest to speculate on the possible toxicological effects of such a substance particularly in a chronic exposure situation. The  $\alpha,\beta$ -unsaturated aldehydes such as crotonaldehyde are highly toxic and have been implicated in carcinogenesis. While 7-oxo- $\Delta^1$ -THC shows no acute effects, long term exposure to this unsaturated aldehyde may produce undesirable reactions.

Gurny et al. (11) have isolated 6-keto- $\Delta^1$ -THC from the incubation of monkey liver with  $\Delta^1$ -THC. As with the aldehyde above, it seems likely that the corresponding hydroxy-THC is the precursor of this metabolite.

The compound  $6\alpha$ -OH- $\Delta^1$ -THC has been found by Wall (12) in human plasma so that it would not be surprising if there are detectable levels of the ketone as well.

A monooxygenated metabolite which arises by a different process is the  $1\alpha,2\alpha$ -epoxide (Figure 2). This substance was first reported by Gurny et al. (11) as a monkey liver product from  $\Delta^1$ -THC; it has since been isolated by Ben-Zvi and Burstein (12) who used rabbit liver microsomes (Table 1).

Table 1

Metabolism of  $\Delta^1$ -THC by rabbit liver microsomes (12)

T.l.c. zone	Rf	Assignment*	Retention time (min)	Principal ionst (M/e)
1	0.67	$\Delta^1$ -THC acetate		
2	0.40	1,2 $\alpha$ -Epoxyhexahydrocannabinol acetate	5.7	372(25), 357(25), 330(50), 315(75), 312(45) 298(100), 280(55), 274(75), 231(88)
		6 $\alpha$ -Hydroxy- $\Delta^1$ -THC diacetate	7.0	372(34), 354(100), 339(21), 312(82), 297(65), 295(18)
3	0.30	7-Hydroxy- $\Delta^1$ -THC diacetate	7.5	372(38), 354(43), 312(100), 297(28), 259(31)
4	0.13	6 $\alpha,7$ -Dihydroxy- $\Delta^1$ -THC triacetate	11.3	412(34), 397(5), 370(9), 355(12), 337(47) 310(46), 295(100)

\* All materials were acetylated prior to t.l.c. with a mixture of acetic anhydride and pyridine. T.l.c. system: Silica gel G, hexane ether (7:3)

† The spectra were obtained on a Finnegan 1015 at 70 eV. The column conditions were: 2 ft., OV-1; 180-240 (8°/min); carrier gas, He; injector temp., 255°. Numbers in parentheses refer to relative intensities.

Biochemical Pharmacology

This metabolite like the 7-oxo product mentioned above may also have toxicological consequences. Although chemically quite stable, it may react *in vivo* with cellular components such as DNA which could lead to profound alterations in cellular processes.

The further oxidation of the 7-position, to a carboxylic acid group (Figure 3) leads to a major group of metabolic end products. The first substances identified in this series were side-chain hydroxylations. The 1"-and 2"-hydroxy derivatives which were reported by Burstein et al. (13) and shown in Figure 4. These were found in rabbit urine and appeared to be present as base-sensitive conjugates.

The parent  $\Delta^1$ -THC-7-oic acid (lower left of Figure 4) has since been found in the monkey by Wall and Brine (14) and in the mouse, Harvey and Paton (15). The latter authors have also reported the 3"-hydroxy derivative and the 2",6-dihydroxy derivative as metabolites in the mouse (16). A series of acids in which the carboxyl group is in the side-chain have been isolated by Martin et al. (17). The structures were established by mass spectral analysis and by conversion to the

corresponding alcohols which had been synthesized. The metabolites were shown to be 5"-nor- $\Delta^1$ -THC-4"-oic acid, 4",5"-bisor- $\Delta^1$ -THC-3"-oic acid, and 3",4",5"-trisor- $\Delta^1$ -THC-2"-oic acid; the second substance was a major product in the guinea pig. A fourth metabolite in which the side-chain was reduced to a single carboxyl group was tentatively identified; this substance is analogous to the CBN derivative which was tentatively reported by us (18,19). Nordquist *et al.* (20) identified a bisnor-dicarboxylic acid metabolite from the rabbit in which the side-chain is reduced to three carbon atoms (Figure 4). All of these acids and hydroxyacids probably represent detoxification products of  $\Delta^1$ -THC since they are a major fraction of the excreted metabolites.

Polyhydroxy derivatives of  $\Delta^1$ -THC have also been isolated by several groups (Figure 4). The positions involved are the same as those found for the monohydroxy-THCs and for the hydroxy-acids suggesting that these all arise by similar pathways.

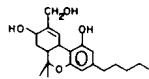
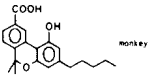
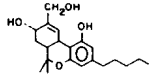
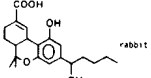
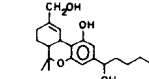
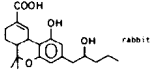
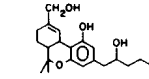
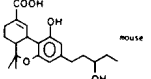
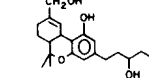
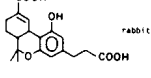
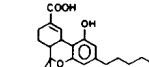
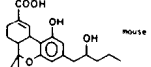
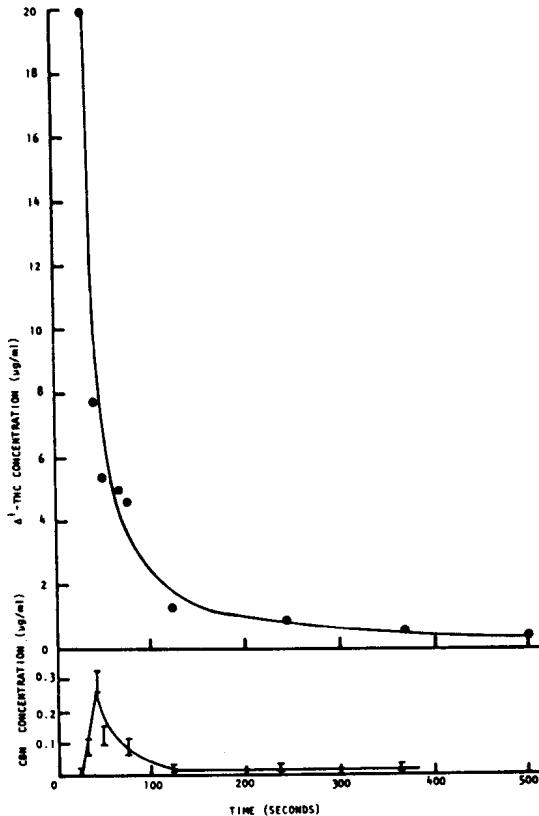
Metabolite	Metabolizing system	Authors	Metabolite	Metabolizing system	Authors
	rat liver	Wall <i>et al.</i> (1970)		monkey	Ben-Zvi <i>et al.</i> (1974)
	rat liver	Wall <i>et al.</i> (1970)		rabbit	Burstein <i>et al.</i> (1972)
	monkey liver	Wall & Brine (1976)		rabbit	Burstein <i>et al.</i> (1972)
	monkey liver mouse	Wall & Brine (1976) Harvey & Paton (1976)		mouse	Harvey & Paton (1976)
	monkey liver mouse	Wall & Brine (1976) Harvey & Paton (1976)		rabbit	Nordquist <i>et al.</i> (1974)
	monkey liver mouse	Wall & Brine (1976) Harvey & Paton (1976)		mouse	Harvey & Paton (1976)

Figure 4. Polyoxygenated metabolites of  $\Delta^1$ -THC

For some time it has been suspected that  $\Delta^1$ -THC may be transformed into CBN *in vivo*. Widman *et al.* (21) found a small amount of CBN in rat bile which

could not be explained as a contaminant of the  $\Delta^1$ -THC which was administered. McCallum *et al.* (22) have given more convincing evidence by monitoring rat blood at short time intervals post injection (Figure 5). An interesting feature of their findings was the very transient nature of the CBN in blood. It seems that the pharmacokinetics of CBN may be somewhat different than that of  $\Delta^1$ -THC.

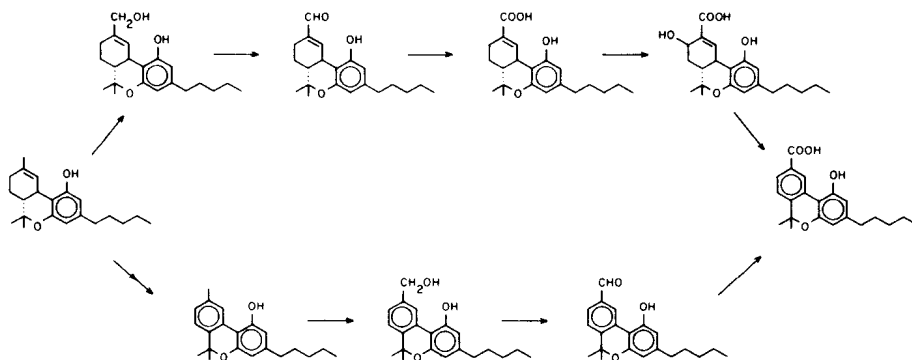


Raven Press

Figure 5. Cannabinoid blood concentrations after *iv* administration of 1 mg of  $\Delta^1$ -THC to the rat (22)

If CBN is formed *in vivo*, the appearance of oxygenated CBN metabolites as excretion products would be expected and this has proven to be the case. Quite independently, Ben-Zvi *et al.* (23) has identified CBN-7-oic as a sizable fraction of the urinary monkey metabolites of  $\Delta^1$ -THC. It is, of course, possible that this metabolite could arise by a route not involving

CBN itself. As shown below in Figure 6, the aromatization process could occur after the oxidation of the methyl group by a sequence such as outlined in the upper part of the figure. All of the intermediates in this proposed sequence have been shown to be metabolites of  $\Delta^1$ -THC lending support to this pathway.



Raven Press

Figure 6. Possible routes for the production of CBN-7-oic acid from  $\Delta^1$ -THC (19)

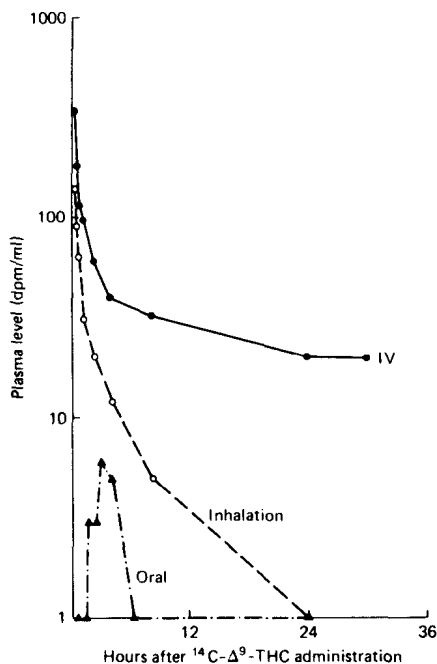
The alternate route via CBN shown in the lower part of Figure 6 is also supported by experimental findings. CBN has been reported to be converted to 7-OH-CBN by Widman *et al.* (24) and the further transformation to the acid was demonstrated by Burstein and Varanelli (18).

The question of how  $\Delta^1$ -THC could be transformed to CBN was touched upon earlier in this paper. A plausible explanation is that one of the three allylic monohydroxy metabolites of  $\Delta^1$ -THC undergoes a loss of water to give the corresponding diene; this in turn could be readily oxidized to the aromatic system (25).

In view of the nature of this symposium, it is appropriate to mention several other aspects of THC metabolism as a background for subsequent papers. As in the above discussion on transformation products, the subjects of route of administration, species variation, excretion patterns and plasma levels are covered in depth in several monographs by Mechoulam (25), Braude and Szara (26) and Nahas *et al.* (27). Therefore, only a limited number of reports will be cited as examples of the type of data which has appeared.

As can be readily seen from Figure 7, Lemberger (28) has found the route of administration has a major

effect on plasma levels of  $\Delta^1$ -THC. The intravenous route gave the highest values, inhalation was lower and the oral route gave the lowest. This seems reasonable since the drug in the first instance is injected directly into the compartment where it is subsequently being measured. The plasma levels of metabolites are somewhat higher than those of THC as is shown in Figure 8; also there are the expected variations in subjects.



Springer-Verlag

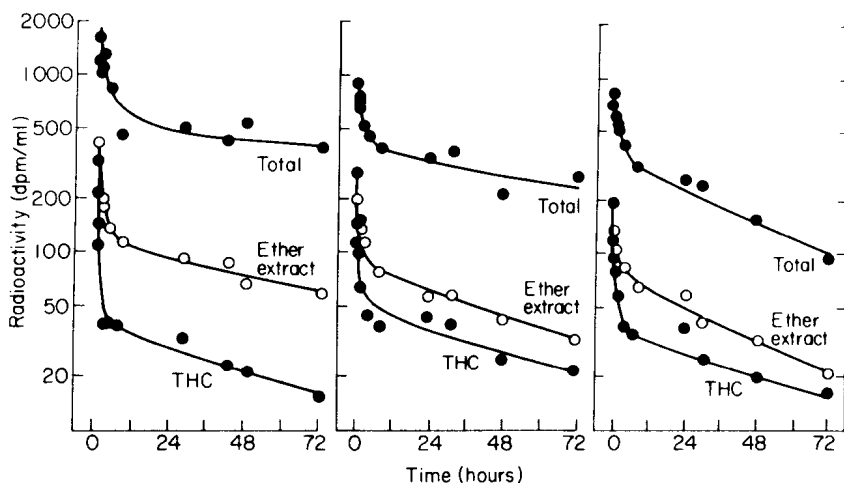
Figure 7. Plasma levels of unchanged  $^{14}\text{C}-\Delta^9\text{-THC}$  after oral or iv administration and inhalation of  $^{14}\text{C}-\Delta^9\text{-THC}$ . Each curve represents a typical subject (28)

Agurell (30) has observed species differences in excretion patterns as is illustrated in Figure 9. The rat resembles man in having a low proportion of metabolites in the urine; the rabbit, on the other hand, disposes of most of the metabolites via the kidney. These differences are also reflected in the rate of excretion; the rabbit being the most rapid.

The composition of the metabolite mixtures shows a quantitative dependence on species. Table 2 compares the transformation of  $\Delta^1$ -THC by rat and mouse liver microsomes under similar conditions. While the principal products were the same in both species, there

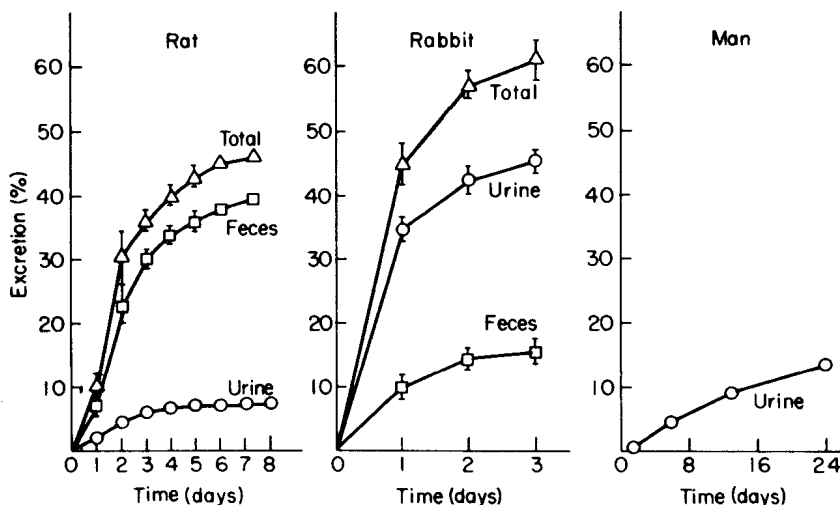


were substantial differences in the proportions of each. Moreover, the mouse appeared to be more effective in metabolizing  $\Delta^1$ -THC overall.



Science

Figure 8. Plasma levels of  $\Delta^1$ -THC, ether-extractable radioactivity, and total radioactivity after iv injection of  $^{14}\text{C}$ - $\Delta^1$ -THC (5.6–7.9  $\mu\text{g/kg}$ ) in three human subjects (29).



Churchill Livingstone

Figure 9. Cumulative excretion of label after administration of  $^3\text{H}$ - $\Delta^1$ -THC in rat, rabbit, and human urine and feces (30)