INFORMATION TO USERS

This material was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.

2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again — beginning below the first row and continuing on until complete.

4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.

5. PLEASE NOTE: Some pages may have indistinct print. Filmed as received.

Xerox University Microfilms
300 North Zeeb Road
Ann Arbor, Michigan 48106

75-670

DOLBY, Thomas William, 1948-
MARIHUANA COMPONENTS AND CYCLIC NUCLEOTIDE ALTERATIONS IN THE CENTRAL NERVOUS SYSTEM.

The University of Michigan, Ph.D., 1974
Chemistry, biological

Xerox University Microfilms, Ann Arbor, Michigan 48106

THIS DISSERTATION HAS BEEN MICROFILMED EXACTLY AS RECEIVED.

MARIHUANA COMPONENTS AND CYCLIC NUCLEOTIDE ALTERATIONS IN THE CENTRAL NERVOUS SYSTEM

by

Thomas William Dolby

A dissertation submitted in partial fufillment
of the requirements for the degree of
Doctor of Philosophy
(Zoology)
in The University of Michigan
1974

Doctoral Committee:

Associate Professor Lewis J. Kleinsmith, Chairman
Professor John M. Allen
Assistant Professor George H. Jones
Assistant Professor K.M.J. Menon

All things have the life which we know,
but we do not see its root; they have
their goings forth, but we do not know
the door by which they depart. Men all
honor that which lies within the sphere
of their knowledge, but they do not know
their dependence on what lies without
that sphere-which would be their true
knowledge. May we not call their case
one of great perplexity? Ah! Ah!
there is no escaping from this dilemma.
So it is! So it is!

Chuang Tzu: Genius of the Absurd

ACKNOWLEDGEMENTS

While conducting my research during the last three years, I have had the opportunity to work with many fine people. Special mention goes to my thesis advisor, Dr. Lewis J. Kleinsmith, whose guidance, support, advice, and friendship has contributed greatly to my scientific pursuits. I would also like to thank Dr. George Jones, Dr. John Allen and Dr. K.M.J. Menon for their suggestions and advice while writing this manuscript.

Some very specialized equipment was required during certain phases of this thesis research and was kindly made available to me by permission of the following people of whom I wish to express my appreciation: Dr. Rees Midgley, Dr. Sinsheimer, Dr. K.M.J. Menon, Dr. Robert Beyer, Dr. Rowland Davis, Dr. George Jones, Dr. Gary Fowler.

My fellow researchers in the "Wonderlab" have also contributed greatly to my emotional and intellectual development. I have especially enjoyed the philosophical discussions with Mario (Oreo) Riolo and Dorothy Pumo. Dr. Linda Dokas, Dr. Ed Clemens, Dr. Robert Platz, Dr. Valeri Kish, Dan Ritschoff, Mitch Botany, Kathy Leonardson have also been instrumental in generating a stimulating and enjoyable atmosphere in the laboratory.

I would also like to thank a number of undergraduate students for their help in some of these investigations, Kim Pitts, Larry Loewe and Ariela Goldschmidt.

Finally, I would like to thank my wife, Evelyn, whose patience and understanding during my academic pursuits as well as typing

skills have contributed greatly to the culmination of this thesis program.

This thesis research was supported in part by Grant 5 R01 DA00116 (formerly MH 21003) from the National Institute of Mental Health.

PREFACE

To date, well over 4,000 articles have been published in scientific and medical journals around the world and numerous books and treatises compounded on the subject of marihuana. Unfortunately, no one reference has attempted to integrate all aspects of marihuana thoroughly making the processes of information gathering laborsome and time consuming for the researcher.

The purpose of this thesis is to provide a concise integration of the marihuana literature, stressing the conceptual and speculative aspects of cannabinoid action as it relates to many established molecular biological processes. Additionally, it provides a firm foundation of information upon which the significance of this marihuana research can be evaluated. The thesis is divided into three chapters. The aim of the first chapter of this thesis is to collate and correlate most of the pertinent literature on the subject perhaps to serve as a practical source book for researchers in this field. It deals with the botanical, historical, chemical, pharmacological, biochemical, physiological and behavioral investigations carried out on marihuana and its chemical components over the years. Since it would be presumptuous to assume that one book could cover all aspects of marihuana thoroughly, references were carefully selected in order that their bibliographies in turn would open new avenues of information. Much of the confirmed information on Cannabis and its actions is in the form of tables for easy reference and additions. The first chapter provides the necessary background information for the second two chapters which contain the methods, results and discussion of

this thesis work and how it correlates to others observations. Finally, a grand discussion speculates on the ramifications of this thesis research and presents some models for cannabinoid action. Eventually, this thesis will provide the basis for a comprehensive book.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS iii

PREFACE v

LIST OF APPENDICES xiii

LIST OF ABBREVIATIONS xv

CHAPTER I: LITERATURE REVIEW

THE BOTANY OF *CANNABIS SATIVA* L.

Origin of *Cannabis sativa* L. 2

Morphology from Seedling to Seed 2

Sex Expression 3

Cytology 6

HISTORICAL ASPECTS OF *CANNABIS SATIVA* L.

The History of the Uses of the Fiber of *Cannabis* . . 7

Medicinal Uses of *Cannabis* 9

Historical Aspects of Cannabis Use as an Intoxicant 13

THE CHEMISTRY OF CANNABIS SATIVA L. AND SOME SYNTHETIC DERIVATIVES

Botanical-Chemical Correlates 17

Historical Sketch of *Cannabis* Chemistry 19

Content of the Major Cannabinoids in *Cannabis sativa* L. 21

Biogenesis of the Cannabinoids 23

Isolation of the Cannabinoids from *Cannabis* 25

Qualitative and Quanitative Identification of the Cannabinoids 26

Chemical Synthesis of the Cannabinoids 27

Non-Cannabinoid Components of *Cannabis* 28

Structure-Activity Relationships 29

PHARMACOLOGY OF THE CANNABINOIDS

Classification of Marihuana 34

Routes of Administration and Cannabinoid Absorption Characteristics 35

Dose Response Relationships of Cannabinoid Action . 39

Minimum Effective Dosage 40

Interaction between Environment and Cannabinoid Action 42

Toxicology of the Cannabinoids 43

Physiological Concentration of the Cannabinoids in Plasma and Tissues 45

Blood Transport of Cannabinoids and their Metabolites 45

METABOLISM, DISPOSITION AND EXCRETION OF THE CANNABINOIDS

Metabolism of the Cannabinoids 48

Inhibitors and Cannabinoid Metabolism 50

Cannabinoids and Enzyme Induction 52

Plasma-Tissue Half-Lives of the Cannabinoids 54

Cannabinoid Disposition in the Central Nervous System 57

General Organ Disposition of the Cannabinoids . . . 59

Placental and Neonatal Distribution of the Cannabinoids 60

Subcellular Disposition of the Cannabinoids 60

Excretion of the Cannabinoids 61

PHYSIOLOGICAL AND BIOCHEMICAL ASPECTS OF CANNABINOID ACTION

Cannabinoids in the Peripheral and Central Nervous Systems 63

Cannabinoid Effects on Endogenous Levels of Brain Neurotransmitters and Related Compounds 63

Cannabinoids and Biogenic Amine Turnover 65

Cannabinoids and Other Neurotransmitters 67

Cannabinoids and the Electroencephalogram 70

Cannabinoids and Smooth and Striated Muscle Action . 71

Heart Muscle 73

Cannabinoids and General Human Physiology 73

Non-Human Physiological Actions of the Cannabinoids 74

Thermoregulation and Cannabinoid Action 76

Cannabinoid and Hormone Actions 76

Cannabinoids and Teratogenesis 79

Cannabinoid Actions at the Cellular and Subcellular Level 80

Cannabinoids and Nuclear Metabolism and Division . . 80

Cannabinoids and Vascular Membranes and Vesicles . . 83

Cannabinoids and Mitochondrial Processes 84

Cannabinoids and Nutrition 84

BEHAVIOR

Cannabinoids and Conditioned Behavior 86

Learning in Man 87

Other Behavioral Effects in Non-Humans 89

Biphasic Action of Cannabinoids on Overt Behavior . 90

Behavioral Tolerance to the Cannabinoids 92

Reverse Tolerance 94

CHAPTER II: EFFECT OF INJECTION OF THE CANNABINOIDS ON MAMMALIAN CYCLIC AMP SYSTEMS

RATIONALE 97

INTRODUCTION 98

MATERIALS 100

METHODS 101

Isolation of Cyclic AMP Dependent Protein Kinase from Beef Muscle 101

Procedure for Cyclic AMP Binding Protein Homogeneity Determination 103

Preparation of Kinase Inhibitor 103

Protein Binding Assay for Cyclic AMP 104

Assay of Adenyl Cyclase 105

Assay of Phosphodiesterase 106

Injection of Animals and Extraction of Cyclic AMP from Mouse Brain 107

Injection of Animals and Extraction of Cyclic AMP from Mouse Liver 108

Injection of Animals and Isolation of Adenyl Cyclase 109

Chronic Injection of Animals and Assay of Phosphodiesterase 109

Recovery of Injected THC in Mouse Brain 110

RESULTS

Isolation of the Cyclic AMP Binding Protein 111

Purity of the Cyclic AMP Binding Protein 111

Protein Binding Assay for Cyclic AMP 111

Fixation of Cyclic AMP Levels by Microwave Irradiation 112

Recovery of ^{3}H-THC in Brain after Intraperitoneal Injection 112

Effects of THC on Brain Levels of Cyclic AMP 113

Effect of Δ^{9}-THC on Liver Cyclic AMP Levels 114

Effects of the Injected Cannabinoids on the *In Vitro* Activity of Adenyl Cyclase from Mouse Brain 115

Phosphodiesterase Activity from Mice Chronically Treated with THC 115

DISCUSSION

In Vivo Effects of the Cannabinoids on Cyclic Nucleotide Synthesis and Steady State Levels in the Mouse Brain 116

CHAPTER III: THE IN VITRO EFFECT OF SEVERAL CANNABINOIDS ON THE ACTIVITY OF ADENYL CYCLASE AND PHOSPHODIESTERASE FROM MOUSE BRAIN

INTRODUCTION 122

MATERIALS 122

METHODS 122

Isolation of Adenyl Cyclase from the Mouse Brain . . 123

Preparation of Brain Slices and Incubation Conditions 123

In Vitro Assay of Phosphodiesterase in the Presence of Various Cannabinoids 124

RESULTS 125

In Vitro Effect of the Cannabinoids on the Activity of Adenyl Cyclase Isolated from the Mouse Brain . . 125

Interactions between the Biogenic Amines and the Cannabinoids on Adenyl Cyclase Activity from Mouse Brain Homogenates 126

Effect of the Cannabinoids on ^{14}C-Adenine Incorporation in ^{14}C-Cyclic AMP in Mouse Brain Slices 128

In Vitro Effect of the Cannabinoids on PDE Activity Activity from the Mouse Brain 129

DISCUSSION 130

GRAND DISCUSSION

Relationships Among Cyclic AMP, Nerve Function and Biogenic Amine Action 133

Cyclic AMP and Biogenic Amine Synthesis 142

Cyclic AMP and Behavior 144

Behavioral and Physiological Tolerance to the Cannabinoids 147

Cyclic AMP, Protein Kinases, RNA and Protein Synthesis and Memory 149

Cyclic AMP and Hormone Action 151

Cyclic AMP and Smooth Muscle Function 152

Cyclic AMP, DNA Synthesis, Cell Growth and Tumor Formation 152

Appendices 155

LIST OF APPENDICES

APPENDIX I: FIGURES

1 Anatomy of Cannabis sativa L. 155

2 Microanatomy of Cannabis sativa L. 157

3 Chemical Nomenclature 159

4 Farmilo's Proposed Cannabinoid Biogenesis 161

5 Mechoulam's Proposed Cannabinoid Biogenesis 161

6 Dolby's Proposed Cannabinoid Biogenesis 163

7 Model Cannabinoid 165

8 Metabolism of Δ^9-THC 167

9 Metabolism of Δ^8-THC 169

10 Metabolism of CBD and CBN 171

11 Absorbance - ^{3}H-Cyclic AMP Binding Profile 173

12 Standard Curve for Cyclic AMP Assay 175

13 Change in Levels of Cyclic AMP as a Function of Injected Δ^9-THC 177

14 Δ^9-THC and Adenyl Cyclase Activity 179

15 Effect of Various Compounds on Phosphodiesterase Activity 181

16 Effect of the Cannabinoids on PDE Activity 183

APPENDIX II: TABLES

I Naturally Occurring Cannabinoids 186

II Cannabinoid Content of Marihuana and Hashish 206

III Methods for the Qualitative Determination of the Cannabinoids 210

IV Methods for the Quantitative Determination of the Cannabinoids 218

V Synthetic Cannabinoids 223

VI Minimum Effect Dose of Cannabinoids 248

VII LD_{50} for Cannabinoids 250

VIII Δ^9-THC Metabolic Pathways in Various Animals 253

IX Δ^8-THC Metabolic Pathways in Various Animals 256

X CBN and CBD Metabolic Pathways in Various Animals . 258

XI Excretion of the Cannabinoids 260

XII Effects of Decapitation and Microwave Irradiation on the Brain Levels of Cyclic AMP, Adenyl Cyclase and PDE 262

XIII Effect of Δ^9-THC on Whole Mouse Brain Cyclic AMP Levels 264

XIV Effect of Δ^9-THC on Cyclic AMP Levels in Mouse Brain Regions 266

XV Effect of Injection of Δ^9-THC on Liver Cyclic AMP Levels 268

XVI Effect of Δ^9-THC and CBN when Injected on the In Vitro Activity of Adenyl Cyclase 270

XVII Effect of the Cannabinoids on the In Vitro Activity of Adenyl Cyclase 273

XVIII Interactions between the Cannabinoids and the Biogenic Amines on Adenyl Cyclase Activity 276

XIX Effect of the Cannabinoids on the Conversion of Labeled ATP to Cyclic AMP in Mouse Brain Slices in the Presence or Absence of Some Biogenic Amines . . 279

APPENDIX III

Procedure for Normalizing Data 282

APPENDIX IV

Procedure for t tests 284

APPENDIX V

Additional Bibliographical Listings 286

REFERENCES 287

LIST OF ABBREVIATIONS

ACTH	adrenocorticotrpoic hormone
CBD	cannabidiol
CBN	cannabinol
cyclic AMP	cyclic adenosine 3',5'- monophosphate
cyclic GMP	cyclic guanosine 3',5'- monophosphate
DNA	deoxyribonuclecic acid
5-HT	serotonin
i.c.	intracranial
i.g.	orally
i.p.	intraperitoneally
i.v.	intravenous
LH	leutinizing hormone
LRH	leutinizing releasing ractor
NE	norepinephrine
Δ^9-THC	Δ^9-tetrahydrocannabinol
Δ^8-THC	Δ^8-tetrahydrocannabinol
TRIS	tris(hydroxymethyl)aminomethane
TSH	thyroid stimulating hormone
RBC	red blood cell
WBC	white blood cell

CHAPTER I

THE BOTANY OF CANNABIS SATIVA L.

Origin of Cannabis sativa L.

In the 18th century, Carl von Linné (Linnaeus) established the genus, Cannabis, based on the ancient sanskrit vernacular used to describe the plant (cana = hemp or cane) (Bentowa, 1968). The name Cannabis sativa was used earlier by C. Bauhin (1623) but the binomial is generally attributed to Linnaeus as cited in his work, Species Plantarum. Formerly, the genus Cannabis was placed in the Urticaceae (nettle) or Moraceae (fig) family, however more recently taxonomists tend to classify it as a separate family, Cannabaceae (Schultes, 1970).

The origin of the Cannabis plant is under dispute, although it is thought that it most probably evolved around Central Asia or in the Himilayan mountains (Schultes, 1970). In earlier times, the genus Cannabis was believed to have over twenty distinct species, however botanists currently consider that Cannabis represents a single species which has not stablized to a common gene pool (Doorenbos et al., 1971; Camp, 1936) and displays numerous variations depending on the characteristics or morphological traits examined.

Morphology from Seedling to Seed

Cannabis is normally an annual plant of world wide distribution especially in tropical and temperate climates where it grows freely or is cultivated (U.S. Commission, 1972). Dewey (1913) noted that germination was most efficient if the seeds were planted 1.25 cm.

beneath the soil surface. Seed germination and viability of the offspring can be altered by the seed storage temperature, relative humidity, the application of disinfectants and the presoaking of the seeds in organic acids and metallic salts (Malinovskii, 1930; Stephan, 1932; Dorywalski, 1936; Robinson, 1943; Koehler, 1946). The seeds germinate within 3-7 days, generally in moist soils of low clay but high nitrogen content. The seedlings have tiny hairs distributed over the upper surface of their seed-leaves only. The first pair of true leaves are stalked from the stem and as they develop, they become multi-leaflet with serrate edges. The leaflets generally increase in number in an odd progression from 3-11 leaflets per leaf as one progresses from the bottom to the top of the plant. The leaves are arranged about the stem in an alternating fashion, each having small hairs (trichomes) distributed on both the upper and lower surfaces (Figure 1). Brazilian planters took advantage of apical dominance by clipping the terminal buds of the growing marihuana to produce branching, hence greater leaf content per plant.

The stem and branches are angular and sometimes hollow, especially as the plant grows older. They too are covered with minute hairs, curving downwards usually increasing in surface density with age (Dolby, 1973). Each hair is formed from one cell (Stearn, 1970) as can be seen from the anatomical drawings of the plant in Figure 2. Ballard (1915) made a histological comparison of American (Virginia) and foreign grown marihuana of unknown source. He describes the micromorphology of the two types of trichomes present in the epidermis of the leaf and stem. He relates that the trichomes often contain $CaCO_3$ crystals in the basal portion of their

cells and that the parenchyma cells contain large resin masses, chlorophyll granules and rosette crystals of calicum oxylate (Figure 2).

Cannabis has a long tap root which divides and subdivides into branches depending on the plant density. The extent of root aborization is said to be from 1/3 - 1/5 the area of the plant superstructure. It has been reported that Cannabis in more advanced stages of growth may inhibit the germination of nearby competitors (allelopathy) by the production of several volitile terpenes and sesquiterpenes (Pratt and Youngken, 1951). Young marihuana produces little or no terpenes and is not effective in controlling weed competition.

The plant is usually dioecious, however occasionally a monoecious form occurs especially if the seed is sown in the winter and exposed to weak light (Schaffner, 1921; Doorenbos et al., 1971). It reaches from 2-30 feet in height at maturity after some 3-5 months of growth and may grow at a rate of 2-3 feet per week. The rate can be altered by photoperiod, hydration state of the soil and its nutrient content (Doorenbos et al., 1971; Black and Vessel, 1944; Krejci, 1970 and Wilsie et al., 1944). Optimal height is obtained by planting the seeds in April through July in the mid-latitudes with the light duration of 12 or more hours per day (Drake, 1971).

The flowers of the male plant consist of five whitish-green hairy sepals and five slender stamens in which the pollen is formed and contained prior to release (Figure 1). The pollen is spherical in shape with an average diameter of 25 microns and possesses

several germ pores. The male flowers are held on separate branches from the stem leaves (Stearn, 1970). The male dies about two weeks after shedding its pollen which is dispersed mainly by the wind.

The female flowers are borne in pairs, also on individual branches (Figure 1). Each flower has a bract or small structure surrounding the ovary, from which two stigmas project for conveying the pollen to the egg (Stearn, 1970). The bract has numerous resin secreting glands, which are present over much of the surface of the plant although not in as high of density. The resin secreted from these glands presumably protect the plant and its seeds from dehydration and herbaceous predators and contains many phenolic compounds (cannabinoids) which may serve to provide the plant with resistance to various bacterial diseases. One cannot distinguish the male from the female plant until flowering has occurred, although the male is usually thinner and less leafy than the female especially at the apical region (U.S. Commission, 1972).

After pollenation, the stigmas fall off and the egg grows to maturity in a seed containing two seed-leaves, a potential root, endosperm (food supply) and some 19-25% oils (Stearn, 1970; Drake, 1971) (See Figure 1). When pollenation is prevented, no seeds develop although the drug or resin content may be enhanced. The female generally survives until the first frost or dramatic change in climatic conditions. Birds and man are the major dispersing agents of the seeds (Haney and Bazzaz, 1970).

Sex Expression

The ratio of male to female plants in a particular locale is

believed to be influenced by the carbon monoxide concentration in the air (Heslop-Harrison, 1957), the age of the pollen and stigma (Wester, 1914; Bessey, 1928; Bose, 1930; Laskowska, 1961), the nitrogen content of the soil (Arnoux, 1966), the fertilizer type (Clark, 1945), the photoperiod (Schaffner, 1923; Limberk, 1959), the time of planting (Schaffner, 1931), the ambient temperature (Cheuvart, 1954), by clipping the floral branches (Pritchard, 1916), and by exposure of the seeds to ultraviolet light (Montemartini, 1928). For a general literature review of the subject of sexual expression in Cannabis with regard to carbohydrate and nitrogen ratios in the plant parts, see Talley (1934). For information about rejuvenation and sex reversal, consult Schaffner (1928).

Cytology

McPhee (1924) has rendered an excellent account of the meiotic cycle of the pollen of Cannabis sativa and identified 10 haploid chromosomes. He also noted that cell proliferation in the staminate buds was greatest between 7-11:30 a.m.

Other cytological karyotyping shows that Cannabis has 20 chromosomes (diploid) and is a functional allopolyploid (i.e. arises from a hybridization followed by polyploidization) (Schultes, 1970). Much of the Mendelian type genetics involved in sex determination of Cannabis has been studied by Hirata (1927).

HISTORICAL ASPECTS OF *CANNABIS SATIVA L.*

Mankind has discovered more uses for *Cannabis* than any other single plant in history. These uses can be historically divided into three catagories: the plant as a fiber source, the plant as a medicine and the plant as an intoxicant.

The History of the Uses of the Fiber of Cannabis

The stalk or stem of *Cannabis* contains approximately 70% cellulose and provides a suitable substitute for flax or any genus of the family, Linaceae, in which the fiber can be woven, braided or twisted.

The origins of *Cannabis* use are lost in antiquity, however it is generally believed that *Cannabis* evolved in Central Asia and was dispersed mainly by nomad migrations and later by equestrian mounted invaders from Central Asia to Egypt, India, Europe, Africa and perhaps China over a period of several centuries.

Neolithic peoples used hemp (*Cannabis*) fiber as textiles for clothes, ropes, fishlines and threads some 4,000-5,000 years ago in the Chikiang and Honan provinces of China (Merlin, 1972) and the remains of hemp found at an Egyptian archaeological site dating 3,000-4,000 years old (Schultes, 1970) indicate that *Cannabis* is one of our oldest non-food plants.

The use of hemp fiber continued in a domestic fashion until the invention of the horse-drawn chariot (1700 B.C.) and the use of bows and arrows in military endeavors initiated a wave of invasions exploiting their new found technique of war. With the constant inter-

mixing of cultures and peoples, the dispersion of Cannabis was greatly facilitated since it had innumerable uses, especially in warfare and was easy to transport the seeds and grow under a wide variety of climatic conditions.

By 500 A.D., Cannabis was being cultivated and utilized extensively for its fiber qualities throughout Central Asia, Europe, China, India and Egypt. About 5 centuries later, it was introduced into Spain but it is not known whether or not the Vikings or Columbus brought it to the New World. It is believed that the cultivation of Cannabis was introduced into North America by Louis Hébert, an employee of Champlain's Apothecary in 1606 in Nova Francia (Nova Scotia) (Canadian Commission, 1972). The first American crop of Cannabis was sown in 1611 near Jamestown, Virginia by early colonists and the pilgrims later planted it in New England for the production of clothing, cordage, sails, fine napkins, sheets, altar clothes, blankets and flags. By the 1630's, Cannabis had become a staple crop on the east coast and was also commercially cultivated in Kentucky, Virginia, Iowa, Wisconsin and Indiana (Bureau of Narcotics, 1933 (page 35); Rosevear, 1967; Grinspoon, 1971; Canadian Commission, 1972). The government of Virginia even awarded bounties for those who cultivated it and imposed penalties on those who did not (Boyce, 1900).

The invention of the cotton gin and the steam engine in the 1700's reduced the market for hemp sails and fiber for clothes and ropes except in the mid-west when, during the westward expansion, the pioneers used hempen fabric to clothe themselves and cover their wagons (Bloomquist, 1968). During the Civil War, hemp production

for fiber diminished and most of it thereafter was imported from the Philippines until the collapse of the stock market in the 1930's and finally the advent of World War II, when synthetics totally replaced the natural resource in most of the western cultures.

Currently, Cannabis is used for its fiber in relatively few regions of the world due to the international wholesale of cotton, wool and synthetics. It should be noted that not only is the fiber of the plant used but also the seeds. The seeds contain 20% oil which was formally used for lighting and soap-making and more recently in the manufacture of varnish and paints. The seed is also used for bird seed, as a food-stuff for cattle, bait for fish and as fertilizer (Bouquet, 1950).

Medicinal Uses of Cannabis

Cannabis and its preparations have been prescribed for the treatment of more disorders and diseases in former times than our modern day cure all, aspirin. In India, the first mention of Cannabis in terms of medicinal use was in a work known as the Sucruta (1000 B.C.) where it is called an antiphlegmatic (mild stimulant) (Walton, 1938). The legendary emperor, Shen Nung wrote a pharmacological treatise about 2700 B.C. in China in which he describes numerous remedies and potions for all sorts of aliments. The first detailed description of Cannabis occurs here and he prescribes it for beriberi, constipation, female weakness, gout, malaria, rheumatism and absentmindedness (Taylor, 1966). It should be noted that some believe that this book was actually written by scholars during the Han dynasty (300 B.C.) (Huard et al., 1968).

Cannabis remained an integral part of folk remedies for some 2,000 years as more of its medicinal applications were discovered. Just after the birth of Christ and for some four centuries thereafter, authorities on medicine and pharmacy from nearly every culture wrote treatises on drugs and medicinal herbs and most included at least a mention of Cannabis and some give descriptive accounts of its natural history which are not contrary to present day observations. The Greek physician, Dioscorides constructed accurate drawings of Cannabis but chose to place the male and female as separate species. The Roman historian, Pliny (100 A.D.) accurately described Cannabis and mentioned the curative properties of the plant for earache, edema and the use of stalks and stems for vegetables (Walton, 1938; Merlin, 1972). In the biography of the Chinese physician, Hoa-Tho (220 A.D.), it relates how the use of a Cannabis-wine mixture (Ma-Yo) was effective as an anesthetic to control pain during a surgical operation (Walton, 1938). For the next millennium, Cannabis was used extensively as a folk medicine in practically every culture to treat everything from diseases of the anus to madness.

In 1838, it was introduced into European medicine and crops were grown in Calcutta under British direction to supply it (Whineray, 1967). It was prescribed for mania, hysteria, as an antispasmodic, for sedation, in cough mixtures, for migraine headaches and sexual impotence in the form of tinctures (extracts of Cannabis in alcohol). The use of Cannabis in western medicine was reinforced by O'Shaughnessy (1843), who wrote about the theraputic and abusive uses of Cannabis. He relates that an oral administration alleviated pain, headaches, spasms, inflamed tonsils and was an effective anticonvul-

sant (Synder, 1971).

The Indian Hemp Drugs Commission (1893-94) reported that Cannabis was prescribed as an analgesic, restorer of energy, a hemostat, an ecbolic and an antidiuretic. It also proved to be useful in the treatment of hay fever, cholera, dysentary, gonorrhea, diabetes, impotence, urinary incontenance, swelling the testicles, open sores and chronic ulcers. In 1860, the Ohio State Medical Society's meeting on Cannabis indica reported that it was used successfully to treat neuralgic pain, dysmenorrhea, uterine hemorrhage, hysteria, delerium tremens, mania, palsy, whooping cough, infantile convulsions, asthma, gonorrhea, nervous rheumatism, chronic bronchitis, spasms, epilepsy, tetanus and appetite stimulation (McMeens, 1860).

In spite of the apparent theraputic value of the plant and its products to treat a variety of disorders, it was soon to be replaced by the opiates and its synthetic analogs due to the poor stability of the tincture preparations and the variation in potency. The use of Cannabis in medicine decreased until restrictive legislation was passed in 1937, which disinterested most potential clinical researchers.

Recently, Cannabis and its chemical components are returning to the position it once held as a theraputic drug. Frank and coworkers have acknowledged that the intraocular pressure can be reduced in a dose dependent fashion following the administration of marihuana which could be significant value for the treatment of persons with glaucoma (U.S. Commission, 1972). Alcohol extracts and paste preparations of marihuana have confirmed the old folk remedies that

marihuana is an effective antiseptic since these preparations have considerable antibacterial action on both gram negative and gram positive bacteria including those which are resistant to penicillin (Kabelikovi, 1952; Kabelik et al., 1960; Murphy, 1963; Veliky and Genest, 1970).

Sofia et al.(1973) has accumulated evidence that an active chemical component of marihuana (Δ^9-THC) is an orally effective anti-edemal and analgesic agent with 20 times the potency of aspirin and 2 times that of hydrocortisone. It was suggested that this may be effective in the treatment of adjuvant-induced arthritis. Additionally, the oral administration of Δ^9-THC into rats produces a marked antipyretic (fever reducing), analgesic and anti-inflammatory action subsequent to a pyrogenic inducer, a painful stimulus or an injected inflammatory agent, respectively (Kosersky et al., 1973). Again, confirming old anecdotes about Cannabis preparations.

It has been used with statistically significant effect for the treatment of grand mal epilepsy in children (David and Ramsey, 1949) and has had some success as an antidepressant (Stockings, 1947). It has recently been used for the relief of anxiety especially in ulcer patients and in the treatment of withdrawal from alcohol and drug addiction (Mikuriya, 1969; 1970; Thompson and Proctor, 1953). Marihuana and some of its active components may be useful in the treatment of certain types of asthma, since Taskin and others (1973) have found that both smoked marihuana and oral does of Δ^9-THC causes a definite dilation of the bronchial airways lasting from 60 minutes to 6 hours, respectively. Unofficial reports suggest that marihuana smoking may retard the rate of plaque formation around the teeth and

gums, presumably due to the antibacterial action of the chemicals in the smoke.

In summary then, marihuana is not presently employed as a general theraputic agent although precedents have been set by the aforementioned investigators which would indicate that perhaps it should reinstated to the Pharmacopeia.

Historical Aspects of Cannabis Use as an Intoxicant

The history of the plant use as an intoxicant dominates as extensive part of the Cannabis literature so only selected highlights are presented. For a more extensive history, see Gastinel, 1849; Grierson, 1892; Walton, 1938; Bouquet, 1950; 1951; Chopra and Chopra, 1957; Taylor, 1966; Solomon, 1966; Andrews and Vinkenoog, 1967; Rosevear, 1967; Bloomquist, 1968; Snyder, 1971; Drake, 1971; Grinspoon, 1971; Merlin, 1972 and all of the commissions and committees established to study marihuana as cited in the appendix.

Some of the oldest written literature in sanskrit contains numerous references to Cannabis and several words appear which can be translated as Cannabis or hemp; bhanga (f.) or bhang (m.), Indrashana and vijaya or jaya (Grierson, 1892). The Aryans or Indo-Europeans, who lived on the Iranian and Punjab plateaus, composed the Vedas which are a compendulum of hymns, prayers and genesis philosophies about natural phenomena dating from 3000-800 B.C. It is believed that these people brought the Cannabis seed with them into the Indus flood plain in northwest India around the 2nd millennium B.C. (Merlin, 1972) after invading Arabian nomads and Persians necessitated their migrations. The earliest known documented mention of bhang (Cannabis) occurs in

the Atharvaveda (circa 2000-1400 B.C.). Here the bhang plant is listed along with the Soma and some other herbs for the purpose of releasing the people from anxiety. Although the bhang plant is mentioned many times in the Vedas, its 'mode d'employe' is vague other than for bedding material or as an intoxicant to be used during Hindu religious ceremonies (Chopra et al., 1957).

Unlike the Chinese, who used hemp fiber for clothes and twine and condemned the use of Cannabis in favor of the opiates, the people of India, Egypt and Mesopotamia cultivated Cannabis for its resin production and its intoxicating properties and not solely for its fiber content. The Assyrians, a militant chariot driving group who lived around Mesopotamia, used Cannabis as an incense as well as a cure for the depression of the spirit. They called it, Azallu, Qunnapu or Quonoubou (Bouquet, 1950). The Scythians lived in the Altai mountains of Siberia and are believed to have cultivated Cannabis in the Volga river region since 1200 B.C. Herodotus rendered lucid accounts of the Scythians' use of heated Cannabis seed vapors as baths and their extensive use as an intoxicant (Merlin, 1972; Drake, 1971). The Getae, a Thracian tribe from Dacia had a mystic shaman cult, the Kapnobatai or "those who walk in smoke clouds", who presumably used Cannabis regularly for its intoxicating properties (Merlin, 1972). Thus we know from Aryan, Getae, Scythian and Assyrian evidences that Cannabis intoxication was popular by 500 B.C. in the Middle East, Central Asia and India.

Cannabis used continued and spread throughout Europe and other parts of Asia and around the Mediterranean for several centuries. Its use in Africa started about the 13th century A.D. where the

people of the tribes would throw Cannabis on a fire and stand around it and inhale the smoke. Then they became more sophisticated by breathing through hollow tubes or reeds placed near the embers of the burning marihuana. Later they built their fires on mounds or tree stumps in order that they might sit and smoke. The African bushman found that they could relieve the harshness of the smoke by holding a mouthful of water while smoking and some even fashioned waterpipes from antelope horns and bored out stones. As its use increased and spread thoughout Africa, waterpipes were constructed from pottery in the early 19th century (Bloomquist, 1968).

In Europe during the 1880's, many literary authors adopted the use of marihuana and hashish preparations and some even formed elite clubs centering around the use of a hashish containing confectionary, dawamesc. The members of this club used the drug for artistic and intellectual curiosity and many of the members relate these experiences in their works (Balzac, Hugo, Baudelaire, Gautier, Latouche, Musset, Dumas and Nerval) (Mikuriya, 1968).

In the New World about the 16th century, marihuana was used in Aztec religious ceremonies and is believed that Montezuma II partook of some kind of euphoric-soporific tobacco after lunch (Bloomquist, 1968) and that the practice of smoking the leaves is believed to have entered the United States with Mexican and Cuban migrant workers about 1900. Its use caught on in New Orleans about 1910 and enterprising sailors began smuggling it from Mexico and Cuba for sale at large profit margins and by 1930 nearly every large city had its share of marihuana smokers (Grinspoon, 1971). At this time, the public began to associate the use of Cannabis with the minority

groups and shortly thereafter the drug was placed under restriction by Marihuana Tax Act of 1937.

Today, marihuana and hashish are believed to be used by some estimated 300 million people (Mechoulam, 1970). Large populations of people in North Africa, the Middle East, Nepal, Europe, India, South America, North America, Southeast Asia, and Jamaica use marihuana on a daily basis in the form of drink or smoke.

THE CHEMISTRY OF CANNABIS SATIVA L. AND SOME SYNTHETIC DERIVATIVES

Botanical-Chemical Correlates

Marihuana contains a group of closely related terpenophenolic compounds which are almost unique to that species, the cannabinoids. There are some 40 different naturally occurring cannabinoids discovered to date, however the most important in terms of pharmacological activity are 1-(-)-Δ^9-6a,10a-trans-tetrahydrocannabinol (Δ^9-THC), its isomer Δ^8-THC, cannabidiol and some other carboxylic acid derivatives (See Routes of Administration). As stated previously, the quality of the soil greatly effects the growth and height of the plant. Crowding and treatment with gibberellins (Atal et al., 1961) seems to increase the fiber content in the stem of the plant but none of these factors seem to alter the cannabinoid content of a particular strain which was grown under minimal growth conditions (Doorenbos et al., 1971). This indicates that the biosynthetic pathway for the cannabinoids is not linked to the phenotypic expression of those characteristics normally associated with the general health of the plant. In regard to this Krejci (1970) showed that Czechoslovakian Cannabis sativa displayed marked differences in growth rate and height (10-20%) depending on the soil nutrient but the antibiotic activity (presumably cannabidiol and cannabidiolic acid) remained the same or rose to even higher levels in marihuana grown in poorer soils. For more information concerning the indifference of strain type, age, fertilizer treatment, region of cultivation and sex on the intensity of the Beam test, a colorimetric index of cannabidiol, cannabigerol and cannabidiolic acid (See Qualitative Tests) has been published by

Matchett et al., 1940; Robinson and Matchett, 1940; Kechatov, 1959). Krejci also presents evidence that cannabidolic acid appears very early in the plant development (8 weeks after germination) and it is believed that this compound is the precursor for most of the remaining cannabinoids present in the plant at later stages of growth (See Biogenesis of the Cannabinoids).

The drug content (active components) of the male and the female of the same variant are equivalent at comparable stages of growth except for the bracts of the female which tend to contain a higher percentage by weight of the active compounds due to the high density of the resin secreting glandular hair cells (Fetterman et al., 1970; Ohlsson et al., 1971). The drug content of the plant parts (male and female) is variable but it usually follows this order of decreasing cannabinoid percent by weight: bracts (female only)/flowers/leaves/stems/seeds/roots (Doorenbos et al., 1971).

The variants of Cannabis were formerly classed into the fiber and drug phenotype depending on the type and proportions of cannabinoids present during analysis. Generally, the drug type is higher in Δ^9-THC and low in cannabidiol while the fiber type shows the inverse distribution. More recently, Small and Beckstead (1973) have presented data which suggests that there are three phenotypes that can be characterized from analysis of various strains of marihuana. Along with the previously described types, there exists an intermediate type which possesses comparable cannabidiol and Δ^9-THC content. These phenotypes appear to be genetically determined, since for at least three generations the cannabinoid content remains the same regardless of the locale in which the plant was grown (Sabalit-

schka, 1925; Waller, 1971; Doorenbos et al., 1971). These phenotypes and the proportions of the cannabinoids contained in them probably reflect multiple isoenzyme pathways under independent regulation or the absence of negative feedback inhibition by an end product(s) which results in the accumulation of certain intermediates (See Biogenesis and Figure 6).

Historical Sketch of Cannabis Chemistry

For information concerning the literature on marihuana and its chemistry before 1850, the following references should be consulted: Blatt, 1938; Todd, 1946; Adams, 1942 and Bibliography, 1951. Although several preliminary investigations were carried out previously, interest in the chemical aspects of Cannabis and its euphoria producing components began in the late 1850's, when the Smith Brothers (1857) and other contemporary researchers demonstrated that an active component of marihuana could be obtained from an alkali-insoluble, high-boiling preparation of an alcoholic extract of marihuana. Some 40 years later, Wood, Sprivey and Easterfield (1897) isolated a compound they called cannabinol after a lengthy distillation and acetylation process. They believed this to be the active compound present in marihuana but were unable to establish its structure due to some unfortunate chain of events. Cahn (1933) worked with various diazotinized derivatives of cannabinol in an attempt to purify it. He correctly established the general chemical structure of cannabinol but not the steric arrangement of the moieties or the position of the double bonds. In the 1940's, Adams and co-workers (1940a; 1940b; 1940c) piloted an extensive investigation into the chemistry

of Cannabis, formulated new extraction schemes and synthesises, established the final conformation of cannabinol and isolated a new compound, cannabidiol. Both of these compounds appeared pharmacologically inactive with the Loewe dog ataxia test and the Gayer rabbit cornea areflexia test (Adams, 1942). This initiated a series of experiments synthesizing and testing numerous analogs and homologs of the cannabinoid type for psychoactivity, shedding a wealth of information on the structural-activity requirements of the complex terpenoids (Adams et al., 1942a; 1942b). Contemporary researchers, Ghosh and co-workers, established synthesis procedures for cannabinol and other dibenzopyran derivatives (Ghosh et al, 1940; 1940a).[1] Little elucidation of the structures of the other cannabinoids present in marihuana was accomplished until 1964, when the major psychomimetic principle was isolated from the plant material and its structure confirmed as 1-(-)-$\Delta^9$6a,10a-trans-tetrahydrocannabinol (Gaoni and Mechoulam, 1964). Minor amounts of an isomer of nearly equivalent potency and greater stability, Δ^8-THC, was first synthesized by Adams and Cain et al., 1941 and was subsequently isolated from marihuana preparations by Hively et al., 1966. Later, other investigators synthesized the isomer, among them were Taylor et al., 1960; 1966; Petrzilka and Sikemeier, 1967 and Jen et al., 1967.

---

[1] Although the isolation of the active component of marihuana is generally accredited to Gaoni and Mechoulam, it is very likely that it was first isolated in 1940. Haagen-Smit et al (1940) isolated a crystaline substance from Minnesota Cannabis which had a melting point of 128-129°C. Although Δ^9-THC is an oil and not normally crystallizable, this substance when administered orally to a dog at 0.38 mg/kg produced many of the symptoms characteristic of the active THC's. They called this material, Cannin. Unfortunately, their procedures are too vague to duplicate.

Since 1964, many other naturally occurring cannabinoids have been isolated from marihuana, their structures established and their synthesises accomplished. Table I gives all of the cannabinoids discovered to date with their structure, common name and abbreviation currently used, relative pharmacological potency and pertinent chemical properties. The structures and stereochemistry of the cannabinoids have been illucidated by gas-liquid chromatography combined with mass spectroscopy, nuclear magnetic resonance spectroscopy, infrared and ultraviolet spectroscopy and chemical synthesis correlations. For specific information concerning these aspects of cannabinoid chemistry, see Table I with accompanying citations.

Content of the Major Cannabinoids in Cannabis sativa L.

The spectrum of the major cannabinoids and their percent by dry weight in the plant material as determined by gas-liquid chromatography analysis by various researchers are given in Table II. As seen from the table, the THC content of marihuana and hashish is variable. This has been shown to be dependent on the genotype (Doorenbos et al., 1971) and the time of analysis after harvesting, since pure Δ^9-THC is dehydrogenated by atmospheric oxygen to cannabinol at a rate of approximately 0.4-0.5% per day at 25°C (Pars and Razdan, 1971) and studies with the plant material stored at various temperatures indicate that about 6.92% of the Δ^9-THC is lost per year at 22°C (Turner et al., 1973). Other cannabinoids will undergo cyclization and decarboxylation upon exposure to natural irradiation and heat (See Biogenesis of the Cannabinoids). The active components are believed to decompose more rapidly in hashish than

marihuana, presumably due to the presence of a natural anti-oxidant in the leaf material or perhaps due to the fact that the resin protected from aerial oxidation by the encapsulation of considerable quanitities of the secretions in the glandular hairs. Razdan et al. (1972) reported tha anti-oxidants like BHA, BHT, ascorbic acid and nordihydroguariaretic acid had no effect on the stability of pure Δ^9-THC on filter paper at 80°C. This suggests that other mechanisms are responsible for the reported stabilization effect. The white-grey powdery material occasionally found on the outer surfaces of hashish is probably cannabinol and phenolic polymers.

Table II shows that in the preparations analysed, the THC content of marihuana ranges from trace to 5.8% by weight (higher levels have been found) and that of hashish from trace to 14.3%. While hashish is not only more potent due to the higher average content of the tetrahydrocannabinols and their respective acids and cannabidiol which are converted to the active form upon smoking or heating (Mikes and Waser, 1971), it also contains different proportions of the cannabinoids than does marihuana. Lerner and Zeffert (1968) and recently, Small and Beckstead (1973) have confirmed that the ratio of Δ^9-THC/Δ^8THC in fresh marihuana with an average total THC content of 1.2% as 99.9/0.1 to 98.8/1.2, which would indicate that the Δ^8-THC component would have a negligible pharmacological effect with so low an endogeneous level. Very recently, data suggests that 90% of the Δ^9-THC present in the fresh plant may be in the form of carboxylic acids (Straight et al., 1973).

These two compounds (Δ^9-THC and Δ^8-THC), in light of the present evidence and testing techniques appear to be the only

psychomimetically active components in the plant, however some data exists to suggest that other cannabinoids may act synergistically or competitively with these active terpenoids (Lemberger, 1972; Paton and Pertwee, 1971; Karniol and Carlini, 1972). (See Biochemistry, Routes of Administration and Metabolism).

Biogenesis of the Cannabinoids

As early as 1942, the idea that the cannabinoids were formed in the plant by a condensation of a terpene derivative, methatriene with olivetol to produce cannabidiol (CBD) was proposed by Sinonsen and Todd. As more information about the cannabinoids became available, other alternative pathways were devised. Farmilo et al. (1962) proposed that cannabidiolic acid was formed from the condensation of olivetolic acid and menthadiene which in turn provided the precursor for all of the remaining cannabinoids. This pathway had its limitations since it postulated for the synthesis of Δ^8-THC and not the more abundant isomer, Δ^9-THC (See Figure 4).

More recently, Mechoulam (1970; Mechoulam and Gaoni, 1967) has proposed that the biosynthesis of Δ^9-THC and its acids proceeds from cannabigerol and cannabigerolic acid through cannabidiol and cannabiolic acid. Cannabigerol and cannabigerolic acid have their origins with mevalonate, the carotenoid and phytol pathway precursors analogous to the steriod pathway in animals (See Figure 5).

Various homologs of the cannabinoids have been recently isolated from marihuana and hashish, especially of Asian origin in which the 3-position is substituted with methyl, propyl as well as the common pentyl group. These homologs manifest themselves as Δ^9-THC-C_1,

Δ^9-THC-C_3 and Δ^9-THC-C_5; CBD-C_1, CBD-C_3 and CBD-C_5; CBN-C_1, CBN-C_3 and CBN-C_5 (Vree et al., 1971; deZeeuw et al., 1972). This would indicate that perhaps the side chain at the 3-position would be synthesized by an acetate fragment addition process since the alkyl side chain increases in increments of two.

By analysis of the more recent data concerning the newly discovered cannabinoids, I have generated a more complete pathway for the biogenesis of the cannabinoids than that presented by Mechoulam as shown in Figure 6. One must bear in mind that this pathway is speculative but based on established phytochemical pathways involved in the synthesis of phenolic compounds.

These synthesis pathways have recently been questioned due to the analysis of a South African variant of Cannabis sativa which contained no CBD but relatively large quanitities of Δ^9-THC-C_5, Δ^9THC-C_3, CBG-C_5, CBC-C_5, CBL-C_5, CBG-MEt-C_5 and CBN-C_5 (Turner and Hadley, 1973a). If CBD is one of the pathway intermediates during the synthesis of the cannabinoids, then according to Turner and others, the plant should not totally lack it. An alternative explanation for this effect could be that the enzymes involved in this pathway in the variant have a lower K_m or disassociation constants driving the reaction to completion and leaving little or no CBD for GLC detection. Other investigators have disputed Turner's findings on the basis that some chromatographic supports cannot effectively separate CBC from CBD. Small and Beckstead (1973) have devised a support which will resolve CBD from CBC and have found no plants in their samples which lack CBD.

Many of the products found in marihuana are believed to be

decomposition products of the synthesized cannabinoids since the presence of numerous double bonds and phenolic groups makes them labile to acids (mainly H_2CO_3 and natural organic acids), ultra-violet irradiation and oxidation by atomospheric oxygen as well as polymerization reactions. The CBDA or CBD and other high energy intermediates formed from the enzymatic synthesis could be converted to THC by natural irradiation and heat (Mikes and Waser, 1971), although some investigators have not found this to be true (Shoyama et al., 1969). The Δ^9-THC in turn is dehydrogenated to CBN by atmospheric oxygen and other oxidizing agents during the heating process (Mikes and Waser, 1971; Shoyama et al., 1969). All of the carboxylic acid derivatives of the cannabinoids are thermodynamically unstable and given enough time would decarboxylate. This process can be enhanced by heating (Schultz and Hafner, 1958; Shoyama et al., 1968 and Agurell and Leander, 1971) or ultra-violet and other high energy irradiation. Cannabichromenic acid has been shown to be converted to cannabicyclolic acid in the presence of light (Shoyama et al., 1972). Some recent data suggests that hexahydrocannabinol may be a decomposition product of one of the cannabinoids (Turner et al., 1973). A few of the cannabinoids are reported to be heat stable during smoking CBG, Δ^8-THC and CBN (Shoyama et al., 1969).

Isolation of the Cannabinoids from Cannabis

Column chromatography has been the major technique used in the bulk isolation of the cannabinoids from marihuana. A powdered or loosely ground preparation of the plant material or hashish is subjected to repeated extractions with an organic solvent such as

petroleum ether, n-hexane, chloroform, methanol, ethanol, ethyl ether, ethyl acetate or combinations of these in order to dissolve the active cannabinoids. The remaining compounds can be extracted with benzene yielding phenolic polymers and cannabielsoic acids. The cannabinoids are then separated into basic and acidic fractions by pH alterations to be chromatographed separately over florosil, silica gel, calicum phosphate-silver nitrate or aluminum oxide matrixes and eluted in increasing polarity with an organic solvent (Mechoulam, 1970; 1973; Mechoulam and Gaoni, 1967; Miras et al., 1964; Turk et al., 1971; Davis et al., 1970 and Fairbairn and Liebmann, 1973). Besides column chromatrography, partition chromatography (De Ropp, 1960; Turk et al., 1971), absorption chromatography (De Ropp, 1960), accelerated microparticulate gel chromatography (Ribi et al., 1973), preparative thin layer chromatography (Hively et al., 1966) and counter current distribution (Korte and Sieper, 1960) techniques have been successfully used to isolate the cannabinoids. For a comparison of some of the extraction schemes utilized, see Fairbairn and Liebmann (1973).

Qualitative and Quanitative Identification of the Cannabinoids

Numerous colorimetric tests have been used to identify Cannabis due to the presence of its unique cannabinoids. Table III gives some of the tests used for the identification and qualitative determination of the cannabinoids in marihuana. A few of these techniques are mainly for the field identification of the plant but for the most part, thin layer chromatography is the preferred method due to its specificity and resolution. The most common dye used to visualize the cannabinoids following TLC is Fast Blue B salt in dilute NaOH.

Many techniques have also been established for the determination of pico and nanogram levels of the cannabinoids from plant extracts and biological fluids. The most sensitive being gas-liquid chromatography (GLC) perhaps combined with mass spectroscopy (Vree et al., 1972) or flame photometric detectors (McCallum, 1973), electron capture systems detecting heptafluorobutyrate and pentafluorobenzoate derivatives of the cannabinoids (Garrett et al., 1973; Exley, 1968) capillary gas chromatography (Novotny et al., 1973) and the antibody fluoresence quenching technique for Δ^9-THC (Grant et al., 1972). See Mechoulam (1973) for additional sources.

Gas-liquid chromatography is the preferred method for the analysis of hashish and marihuana material for identification and quanitative evaluation of the cannabinoids present. Numerous chromatographic stationary phase supports have been utilized for the separation of the cannabinoids but there seems to be a preference for 1-5% medium polarity types like OV-17 (Lerner and Zeffert, 1968; Mechoulam, 1970; Vree, Breimer and others, 1971; Fetterman et al., 1971; Verwey and Witte, 1972) and SE-30 (Farmilo and Davis, 1961; Betts and Holloway, 1967; Shoyama et al., 1970; Aramaki et al., 1968; deZeeuw, Malingré and Merkus, 1972). deZeeuw, Wijsbeek and Malingré (1973) give data comparing the commonly used stationary supports for the separation of the cannabinoids by GLC. For a more complete listing of the available quanitative techniques, see Table IV.

Chemical Synthesis of the Cannabinoids

The chemical synthesis of most all of the cannabinoids, including isotopically labeled (^{14}C and ^{3}H) cannabinoids, have been

accomplished by numerous researchers using a variety of reactions. For information on these aspects of cannabinoid chemistry, see Mechoulam and Gaoni, 1967; Fahrenholtz et al., 1966; 1967; Nilsson et al., 1969; Burstein and Mechoulam, 1968; Agurell et al., 1973; Neumeyer and Shagoury, 1971; Idanpaan-Heikkila et al., 1969; Gill and Jones, 1971; Ben-Zvi et al., 1971; Taylor et al., 1966; Razdan and Handrick, 1970; Mechoulam et al., 1968; Petrzilka et al., 1969; Agurell et al., 1968; Pitt and Wall, 1969; Ghosh et al., 1940; Jen et al., 1967; Timmons et al., 1969 and the citations in Table V. For an excellent extensive review of synthesis pathways for most of the cannabinoids and data on cannabinoid physical constants, consult Mechoulam and Gaoni, 1967; Mechoulam, 1973.

Non-Cannabinoid Components of Cannabis

Other than the typical proteins, nuclecic acids, fatty acids, glycerides and carbohydrates occurring in all plants, many other non-cannabinoid compounds have been found in Cannabis but are not unique to it.

Among these include waxes, oils, terpenes, muscarine, choline, trigonelline (Gill et al., 1970; Bercht et al., 1971), several alkaloids, Cannabamine A, B, C, D with no apparent toxicity but some suppression on activity in mice (Klein et al., 1971), low molecular weight piperidines, and the following triterpenes: campesterol, β-sitosterol, stigmasterol, freidelin, epifriedelanol, N-(p-hydroxy-β-phenylethyl) and p-hydroxy-trans-cinnamanide which are found in high concentrations in the roots of Cannabis (Doorenbos et al., 1971; Slatkin et al., 1971; Mole & Turner, 1974). Obata and Ishikawa

(1960) have extracted eugenol and guaiacol and a piperidine associated with amino acids from marihuana preparations.

Nigam et al. (1965) and Hood et al. (1973) have identified numerous compounds present in the volitle headspace of Cannabis leaves from stem distillates of Mexican marihuana among them are: α-pinene, camphene, β-pinene, 2-methyl-2-heptene-6-one, Δ^3-carene, myrcene, α-terpinene, limonene, β-phellandrene, cis and trans-ocimene, γ-terpine, terpmolene, p-cymene, linalool, fenchyl alcohol, borneal, α-bergamotene, sabinene hydrate, caryophyllene, β-farnesene, α-terpinenol, β-humulene, α-selinene, β-bisabolene, curcumene and caryophyllene oxide.

Adams, Pease and Clark (1940b) isolated from a steam distillate of marihuana, quebrachitol (1-inositol monomethyl ether). n-Hexacosane, n-pentascosane, n-octacosane, as well as other high molecular weight alkanes have recently been confirmed as natural products by deZeeuw et al. (1973) and Adams et al. (1973).

Structure-Activity Relationships

The discovery that the synthetic $\Delta^{6a,10a}$-tetrahydrocannabinols had pharmacological activity led to the synthesis by Adams, Todd and others of numerous derivatives of this series. These were tested for pharmacological potency by using basic autonomic reflexes as indices of action. The Loewe dog ataxia and the Gayer corneal areflexia test were used extensively by these investigators in order to establish relative potency factors of the $\Delta^{6a,10a}$ series (Loewe, 1945; 1946; 1947; Gayer, 1928). More recently, Loev et al. (1973), Edery et al. (1971), Pars and Razdan (1971) and some others have synthesized and compiled a large number of synthetic derivatives of

the cannabinoids and have tested them for activity by physiological and behavioral criteria in various mammals.

Many of the structure-activity relationships as described by Loev et al. (1973) differ markedly from those previously reported by Adams and Loewe in the dog and Todd and co-workers in the rabbit. This points out one of the limitations of these bioassay techniques in that the response to a given dose of drug on a weight basis varies not only within one animal species but also between animal species. (See Route of Administration and Biochemistry.) The second point of caution when interpreting the structure-activity relationships is the differences in solubilities, metabolism, deposition, excretion and permeabilities caused by the subtle alteration of the moieties and overall stereochemistry. For example, although a particular compound may be five times as potent as Δ^9-THC, this may not reflect an increased affinity for a particular receptor but an enhancement of the transport rate, solubility or various other factors. Finally, many of the earlier structural-activity studies involved the use of racemic and isomeric mixtures so the reported results are due to the multiple effects of any of the administered stereomorphs.

The structure, nomenclature, relative pharmacological activity and citations as to the structure and properties of some synthetic cannabinoids are collated in Table V. By careful comparisons of the stereochemistry of the compounds in this list, several conclusions can be drawn concerning the structure-activity relationships of the cannabinoids in some mammalian systems.

Figure 7 shows a model cannabinoid with certain rings labeled with letters for reference to the following conclusions.

In the $\Delta^{6a,10a}$ and Δ^9-THC series, with an increase in non-branched alkyl-side chain length in the A ring in the 3-position, there is an increased activity until the n-hexyl (Adams et al., 1942a) or the n-nonyl (Loev et al., 1973) homolog is reached. Further extension beyond this point results in a decreased activity depending on the animal tested. Secondary groups added to the alkyl-side chain produces compounds with higher activity, especially if side groups are located close to the benzene ring (Adams et al., 1942a; Loev et al., 1973). In the $\Delta^{6a,10a}$-THC series, the α-dimethylheptyl side chain produces maximum activity (500-1000 times more potent than Δ^9-THC) with the α,β-dimethylheptyl derivative closely behind. The introduction of a double bond in certain positions of the side chain are without effect in pharmacological action (Loev et al., 1973). Alkyl ethers of the 3-position were reported by Morrison et al. (1943) to be relatively inactive, however Loev and co-workers (1973) present conflicting data.

Esterification of the 1-hydroxyl position of the A ring of either Δ^8 or Δ^9-THC molecules renders it slightly less potent, while etherification generally abolishes activity (See Table V.). The liver esterases rapidly convert the THC esters to THC, although plasmalogens have an ether linkage, it is not known if etherases can use the cannabinoids as substrates. The removal of the 1-hydroxyl group altogether renders the compound totally inactive (Alles et al., 1942).

The substitution of a methyl, carboxyl, acetyl or carboxymethyl group at the 2 or 4 position of the A ring renders the molecule generally inactive, while an alkyl group at the two position still retains some activity (See Table V.).

In the B ring, the removal of the 6-dimethyl groups and the addition of a carbonyl function abolishes activity in the CNS while a diethyl substitution decreases potency by 10-250 times depending on the animal tested (Adams et al., 1941a; Loev et al., 1973).

In the C ring, the reduction of any of the double bonds (Δ^8, Δ^9, $\Delta^{6a,10a}$) results, in a decrease in activity (Hexahydrocannabinol) (See Table V.). Ethylation at C-9 position and methylation at C-7 or C-8 and dimethylation of C-9 yields a less active molecule (Loev et al., 1973). The 9-demethylated form which still retains some activity, indicates that the hydroxylation of the 11 carbon is not absolutely necessary for activity (like 11-OH-Δ^9-THC). The trans configuration must be about the $\sigma^{6a,10a}$ bond in order to retain any activity (cis form-totally inactive; see Table V).

α-Hydroxylation of the Δ^8-THC at the 9 or 10 position in ring C, yields an equally active compound, while the β isomer has reduced activity in the monkey, indicating that the substitutients at that position must be equatorial for activity. A metabolite of Δ^9 and Δ^8-THC, 11-OH-Δ^9(Δ^8)-THC is nearly equipotent in man and mouse (Lemberger et al., 1970; Christensen et al., 1971). For more information about the activity of the polyhydroxylated derivatives formed as metabolites, see section on Metabolism and Tables VIII, IX and X.

Dextrorotatory THC's are inactive while the levorotatory forms are the most active (Mechoulam, 1970; Edery et al., 1971). Most of the heterocyclic nitrogen analogs of the THC's still retain some pharmacological activity (Dewey et al., 1970).

Although Δ^8 and Δ^9-THC are frequently referred to as having equivalent activities, there are some noteworthy exceptions. Scheckel

et al. (1968) noted that racemic Δ^8-THC lacked a depressant effect on behavior and did not produce vomiting in squirrel monkeys while Δ^9-THC did. (See Behavior - Chapter I.) Jarbe et al. (1973) and Sjoden et al. (1973) found that Δ^8-THC produces a greater reduction in normal weight gain than Δ^9-THC in female rats. (See Cannabinoids and Nutrition.) Ho et al. (1972a) observed that Δ^8 and Δ^9-THC have different effects on monkey brain norepinephrine and serotonin levels. (See Biogenic Amines.) Finally, Ho et al. (1973) found that Δ^8-THC is metabolized slower than Δ^9-THC. Therefore, although Δ^8 and Δ^9-THC appear to have similar effects on immediate overt behavior and certain pharmacological tests, the biochemical interactions and metabolism of these two isomers are very different. (See Metabolism.) For additional structural-activity relationships and a more extensive lising of synthetic cannabinoids, see Mechoulam, 1973.

PHARMACOLOGY OF THE CANNABINOIDS

Classification of Marihuana and the Cannabinoids

The pharmacological classification of marihuana and its "active" and "inactive" cannabinoids has been rendered extremely difficult due to their multiple pharmacological effects. These effects appear to be dependent on the type of cannabinoid, the experimental animal and its physiological state, the dosage, the route of administration, the suspension vehicle, the environmental setting and the antagonists used. Marihuana has been pharmacologically classified as a stimulant, analgesic, sedative, hyponotic, narcotic, depressant-tranquillizer, hallucinogen or psychedelic, which covers most of the possible catagories.

This indicates the complexity of the action of marihuana which may reflect the varying proportions of the 40 known naturally occurring cannabinoids, their potential metabolites (estimated between 60 to 250) formed in vivo and numerous other low molecular weight compounds (See the non-cannabinoid components of marihuana under Chemistry.). All or some of these compounds may exert some degree of physiological alteration which results in the summation of all of the synergistic and competitive interactions of these compounds to produce some grand overall change in the animals physiological and/or behavioral state.

Interestingly enough, the thorough examination of hundreds of papers present in the pertinent literature on marihuana indicates that in most of the experimental animals studied, regardless of the parameters tested, the index of variance (range, standard error of

the mean or standard deviation) of the groups treated with marihuana components is 10 to 40% higher than those controls run simultaneously. This suggests that the administration of the cannabinoids gives rise to a "dynamic state" which allows for a greater latitude of behavioral or physiological response than the "normal state". The following literature review will bear on this variability phenomena and will express the extreme importance of the route of administration, the suspension vehicle, the environmental conditions, the age, sex, weight and nutritional state of the animal, dosage and the type of cannabinoid administered on the degree or level of psychotomimetic activity produced in the experimental animal.

Routes of Administration and Cannabinoid Aborsption Characteristics

Numerous methods and suspension vehicles have been employed to administer the cannabinoids to experimental subjects. The absorbtion kinetics and percent incorporation of these cannabinoids has been shown to be dependent on the route of administration, the suspension vehicle and in some cases the type of cannabinoid (Ho et al., 1971; 1973; Leighty, 1973; Perez-Reyes et al., 1971; Agurell and Leander, 1971; Sofia, 1972; Dewey et al., 1972; Shannon and Fried, 1972; Fried and Nieman, 1973). For a comparison of the common suspension vehicles, routes of administration and stability of the cannabinoids in these solutions, see Rosenkrantz et al. (1972) and Borgen and Davis (1973).

The most efficient method for the administration of these drugs must be determined for each experimental situation since each of the various modes of administration has its particular advantages and disadvantages. Smoking and intravenous administration require the

smallest dose per unit weight for the production of a definite physiological or behavioral effect; thus about 2-5 times more effective absorbtion of the cannabinoids is obtained as compared with the oral, intraperitoneal, intramuscular or subcutaneous modes (Isbell et al., 1967; U.S. Commission, 1972; Hollister, 1971; Lemberger et al., 1971a; Mechoulam and Gaoni, 1967). The smoking method has its drawbacks since it is very difficult to establish the precise delivered dosage without the use of label cannabinoid tracers.

Additionally, substantial evidence has accumulated indicating that some of the Δ^9 and Δ^8-tetrahydrocannabinolic acids, cannabidiol and cannabidiolic acid present in marihuana may be converted to the Δ^9 and Δ^8-tetrahydrocannabinols upon smoking and the cannabinol levels in the smoke mainstream may be elevated slightly with this heating process (Mikes and Waser, 1971; Claussen and Korte, 1968; Fehr and Kalant, 1972). Thus, this smoking process would increase the effective dose of the "active" cannabinoids delivered which would not normally manifest itself by other routes of administration.

Truitt (1971), Manno et al. (1970), Mikes and Waser (1971), and Agurell and others (1971) have presented data implying that between 14-60% of the Δ^9-THC present in a "joint" (marihuana cigarette) is actually delivered to the smoker while on the order of 50% is delivered with a pipe. The smoke or air flow rate whether intermittant or constant seems to be directly related to the recovery of the Δ^9-THC present in a "joint" (Fehr and Kalant, 1972), which could possibly explain the large variations observed in dose delivery reported by these investigators. Recently, a smoking machine has been devised to quanitiative the mainstream dose delivered to rodents

(Fried and Nieman, 1973).

It has been previously stated that the action of the cannabinoids may be affected by the route of administration. For example, an intravenous injection may allow considerable systemic dispersion of the drug before it passes through the liver and lungs which would metabilize it. (See Freudenthal et al., 1972 for example.) Alternatively, an intraperitoneal injection would most readily be abosorbed via the mesenteric and gastro-intestinal vessels which would ultimately coalesce into the hepatic portal system, thereby passing most of the absorbed drug through the liver without any significant deposition except at the injection site and the area immediately around it. In regard to this, Christensen and co-workers (1971) and Ho et al. (1971) have noted that about 10% of an intraperitoneal dose remains at the injection site and is not absorbed, although the suspension vehicle and the time of analysis can have a dramatic effect on the process of absorbtion. Perez-Reyes and others (1972) reported that the intravenous injection in man of Δ^9-THC in quanitities greater than 0.5 mg (dissolved in ethanol) causes vessel irritation, hemolysis and plasma protein precipitation. The use of human serum albumin as a suspension vehicle alleviated this undesirable effect.

Additional support for the importance of the route of administration on the subsequent pharmacological action of the cannabinoids have been presented by Christensen and co-workers (1971), who observed that when cannabidiol and cannabinol are injected by an <u>intracerebral</u> (i.c.) route into mice, the effects noted are very similar to those of Δ^9 and Δ^8-THC. Conversely, when CBD and CBN are injected <u>intravenously</u> (i.v.) into mice, these compounds are functionally

inactive. Moreover, Karniol and Carlini (1973) have recently demonstrated that CBD has a significant effect on locomotor activity and produces catatonia in mice when injected intraperitoneally (i.p.). Finally, Gallager and others (1970) found that intraperitoneal injections of Δ^9-THC did not effect the levels of certain biogenic amines in the rat brain while intracerebral injections did. These studies also indicate that the common belief that the only active cannabinoids are the tetrahydrocannabinols may be a gross oversimplification.

The oral (i.g.) route of administration requires the minimum amount of animal handling as well as accurate dose delivery. However the rate and degree of absorption of the cannabinoids is not consistent and is dependent on the suspension vehicle and the gastrointestinal contents during ingestion (Perez-Reyes and Lipton, 1971).

Using isotopically labeled Δ^9-THC, Lemberger and co-workers (1971a; 1972a) examined the absorption rate and the onset of "symptoms" indicative of intoxication for various modes of administration in man. They found that the intravenous route produced label in the blood immediately, smoking gave rise to the label shortly after inhalation (6-15 minutes) and the oral route resulted in a delay of blood appearance form 15-30 minutes depending on the subject. Concomitantly, subjective effects ("high") were noted at the onset of the appearance of the labeled cannabinoids in the blood, especially during the first several hours (Perez-Reyes et al., 1971; Lemberger et al., 1971b; Galanter et al., 1972; Heath, 1973; Lemberger, 1973a).

The intramuscular and subcutaneous routes are probably the most variable in terms of absorbtion, although there are no specific

studies indicating so. The rate and degree of absorbtion by these routes would be expected to be dependent on the local blood flow through the tissue at the injection site. (See Transport of Cannabinoids.). Regional blood flow has been shown to be affected by the local oxygen and carbon dioxide tension, pH, nervous innervation, electrolyle concentration, humoral factors and metabolic products of the particular area (Rothe, 1966; Friedman and Selkurt, 1966).

Dose-Response Relationships of Cannabinoid Action

It has been fairly well established that there is a direct relationship between the quanitity of dose delivered to the subject (man and other animals) and the degree of physiological and behavioral alterations produced by both marihuana, Δ^9-THC, Δ^8-THC and some of the active metabolites (Waskow et al., 1970; Dornbush and Freidman, 1971; Weil et al., 1968; Meyer et al., 1971; Clark et al., 1970; Tinklenberg et al., 1970; Kiplinger et al., 1971; Dewey et al., 1972; Kubena and Barry, 1972; Meleges et al., 1970; Cappell et al., 1972; Lemberger et al., 1971a; 1972; Volavka et al., 1971; Perez-Reyes et al., 1973).

Isbell et al. (1967a), Kiplinger and Manno (1971), Perez-Reyes et al. (1973) Galanter et al. (1972), Chappell et al. (1972) and Renault et al. (1971) have found that the heart rate is a direct physiological index of the degree of subjective "highness" produced in man and that this parameter does change (adapt or habituate) with continued usage. It should be noted that Weil and others (1968) did not observe a dose dependent relationship between the heart rate and the two doses tested by the inhalation technique in both naive and

chronic smokers.

Although the blood pressure, conjunctivitis (pinkeye), hypothermia, glycogen stores, glucose levels, static equilibrium (wobbliness), mobility, respiration rate, pupillary dilation, ptosis (squint eye), ataxia, sleepiness and numerous decrements on various perceptual, psychological (man) and conditioned learning paradigms have been reported to follow a dose dependent relationship (not necessarily linear) in many animals, much conflicting data also exists (Bromberg, 1939; Tayleur-Stockings, 1947; Allentuck and Bowman, 1942; Chopra and Chopra, 1957; Isbell et al., 1967; Hollister, Richards et al., 1968; Johnson and Domino, 1971; Dewey et al., 1970a; Domino, 1971; Weiss et al., 1972; Shiffrin, 1971; Casswell and Marks, 1973; Weil and Zinberg, 1969; Kiplinger and Manno, 1971; Waskow et al., 1970; Mills and Brawley, 1972; Sprague et al., 1973; Ho et al., 1971a; Meleges et al., 1970; Forney and Kiplinger, 1971; Bowman and Pihl, 1973). The significance of some of these experiments will be discussed under the Physiology and Biochemistry of Cannabinoid Action.

Minimum Effective Dosage

The minimum effective dose required to produce definite observable physiological and/or behavioral alterations in various animals is extremely variable and not consistent even when administered on a proportionate weight basis. This suggests that the absorbtion, transport, metabolism, and mode of action is different for different animals. There has been much data to support this concept (See Metabolism, Disposition and Excretion of the Cannabinoids.).

Although the minimal effective dose varies with the animal, its

physiological state, the conditions during administration and the type of cannabinoid, Table VI gives some of the approximate values obtained for a number of animals for the purpose of comparison. It is interesting to note that the greater the mass of the animal, the less the minimum effective dose. Perhaps this correlates with the weight specific metabolic rate which has also been shown to be an inverse function of the animals mass in homeotherms and some reptiles (Morrison, 1948; Pearson, 1948 and Bartholomew et al., 1964).

Shortly after the pure active compounds became available to the researcher, a plethora of pharmacological experiments were reported in the literature. During this time, the doses employed by various investigators were on the order of 10-500 mg/kg. These doses were believed to be very reasonable since the investigators were trying to illicit a definite observable behavioral response to correlate with their quantitative data. Elsomore and Fletcher (1972) published data which suggested that Δ^9-THC produced "adverse" effects in the rat when administered at doses greater than 0.5 mg/kg (oral or i.p.). This hypothesis arose from experiments which paired a novel tasting drink (saccharine solution) and increasing doses of Δ^9-THC to thirsty rats. Presumably, if the dosage paired was unpleasant for the rat then it would not return to drink the saccharine solution. A similar effect was noted by Corcoran (1973) although he used hashish resin rather than pure cannabinoids. Since doses from 10-1000 times the physiological levels encountered during normal use were employed (See Tissue Plasma Concentrations and Table VI.), the data gathered from these experiments may only represent responses to toxicological states and are not likely to be confronted in society. Therefore it

appears that most of the biochemical and pharmacological studies prior to early 1972 and some subsequent to this must be repeated employing more reasonable doses of the cannabinoids.

Interaction between Environment and Cannabinoid Action

It has become apparent that the environment has a great deal of influence over the effect of the cannabinoids in animals. Waskow et al. (1970), Jones and Stone (1970) and Jones (1971) contend that the environmental setting has the greatest influence over the type of "high" produced in man and not necessarily the potency of the marihuana smoked. Jones relates that those subjects in isolation upon smoking marihuana appeared to have less euphoria and more of a sedative action than in those in a group situation (euphoria, activity) and that some of the subjects mistaked the placebo for the marihuana after smoking. This suggests that there may be some degree of learning involved in the process of getting "high" and that the social atmosphere is an important consideration.

Other non-human studies by Barry and Kubena (1969) lend support to the effect of environment on cannabinoid action. The behavioral response to a given dose of Δ^9-THC was enhanced in non-acclimated rats while those acclimated to the laboratory environment for 5-7 days responded much less. Other studies have emerged which indicate that the action of Δ^9-THC can be altered by the application of a stressful situation to the experimental animal. Ng and others (1973) correlated a decrease in serum dopamine β-hydroxylase activity with continued subcutaneous administration of Δ^9-THC at very high doses (400 mg/kg) only when the rats were in the unrestrained state. Upon

restraint (inducing stress) THC was ineffective in blocking the stress induced elevation of serum dopamine β-hydroxylase. A similar phenomena was observed by Lamprecht et al. (1973) with the adrenal medulla catecholamine forming enzymes. In non-immobilized rats, Δ^9-THC did not affect their activities while upon immobilization and administration of the cannabinoid their activities were dramatically enhanced.

Clearly then, animal manipulations during the course of the experimental investigation can drastically alter the results. Perhaps this is an additional factor contributing to the enormous variations in response to the cannabinoids.

Toxicology of the Cannabinoids

Marihuana and its preparations are surprisingly non-toxic, based on the quanitities required to produce death in 50% of the experimental animals tested (LD_{50}). As far as can be determined, there are no documented reports of marihuana or hashish overdose in man which have resulted in death (Brill et al., 1970; Smith, 1968). Lethal doses have been administered to most laboratory rodents (rabbit (?)), although most other animals (dogs, cats and monkeys) are relatively tolerant to the cannabinoids. The LD_{50}'s for various experimental animals have been tabulated in Table VII. Several generalities can be concluded from this data. The ability of a particular dose to produce death is related to the mode of administration (i.v.>i.p.> i.g.) and studies using the same animal and route of administration differ significantly in their LD_{50}'s, again indicating that the suspension vehicle, animal strain, sex, region of injection and other factors may be decisive in drug absorbtion and delivered or effective

dose. In regard to the animal strain and concomitant cannabinoid action, Park and Tilton (1970) observed that rats of different strains performed differently on behavioral avoidance tasks to an equivalent dose of marihuana smoke. Additionally, Cohn et al. (1972) saw differences in the behavioral responses between male and female rats (females become more "intoxicated"; effects last longer) of the same strain to marihuana extracts administered orally.[2] Additional data, more directly related to the toxicology and sex differences has been presented by Thompson and co-workers (1973). They found that male and female rats of the same strain have very different LD_{50}'s (See Table VII.).

Some of the available data suggests that in addition to the route of administration and the suspension vehicle, the mass of the animal may alter the effective lethal or minimal effective dose (Dewey et al., 1972; De Ropp et al., 1972; Thompson et al., 1973). If one of the metabolites formed in vivo is responsible for these toxic symptoms, this would account for death in the smaller animals which would metabolize the active cannabinoids to this metabolite at a faster rate than the blood and body clearance or dilution could counteract when compared to larger mammals (vis. weight-specific metabolic rate).

---

[2] Other male-female specific characteristics have been observed. Burstein et al. (1971) published evidence which implied that Δ^9-THC is metabolized two times faster in the male than in the female rat to 11-OH-Δ^9-THC. Perhaps this could be related to Cohn's findings. Borgen et al. (1973a) observed more pronounced hypothermia in female rats (0.5-1°C) than male rats to given dose of Δ^9-THC.

Physiological Concentration of the Cannabinoids in Plasma and Tissues

A few studies have been carried out in which the blood and tissue levels of Δ^9-THC and its metabolites have been correlated with observable behavioral alterations. Studies from many laboratories indicate that in most mammalian organisms, the blood-tissue concentrations produced by a minimum effective dose and moderately high doses (7-10 mg/kg) are between 10^{-8} and 10^{-4} M (Dolby and Kleinsmith, 1974; Ho et al., 1970; Galanter et al., 1972; Nilsson et al., 1973; Ryrfeldt et al., 1973; Layman and Milton, 1971; Ho et al., 1973; Klausner and Dingell, 1971a). This indicates that the blood levels of the cannabinoids can potentially vary 10,000 fold, depending on the dosage administered to the experimental animal. It would be of interest to know the range of the blood levels of the cannabinoids encountered during social marihuana use in humans.

Blood Transport of the Cannabinoids and their Metabolites

Most the cannabinoids display a marked insolubility in aqueous media as do the steroids. Similarly, many of the cannabinoids and their metabolites have been found to bind and be transported by the plasma proteins and some are excreted as sulfate and glucuronide conjugates (See Excretion of the Cannabinoids.). The examination of space filling models of the cannabinoids and several steroids suggests very similar general stereochemistry (planarity and ring structure).

Agurell (1970), Widman et al. (1971) and Walquvist et al. (1970) have demonstrated that there is some 30-60% of labeled Δ^9-THC bound to the albumin fraction and some 20-30% associated with the transferrin

band by using disc electrophoresis and zonal floatation centrifugation techniques on whole blood previously incubated with 0.08-0.3uM labeled Δ^9-THC. Additional evidence of high albumin affinity of Δ^9 and Δ^8-THC has been accumulated in our laboratory. By using P-30 Biogel and A5M agarose gel filtration chromatography it appears that that the labeled THC's have a considerable affinity for bovine serum albumin and hemoglobin among other proteins in vitro (Riolo and Kleinsmith, 1973). Interestingly, Agarose gel electrophoresis profiles differ significantly from the polyacrylamide gel electrophoresis patterns in that the label seems to be predominantly associated with the α, β and γ globulins (90%) (Walquvist et al., 1970). Walquvist accounts for the discrepancy between these data in that the lipoproteins are impermeable to the disc gel matrix and remain at the origin.

There are some indications that the cannabinoids may be transported by the corpuscles (RBC, WBC and platlets) in the plasma. A few investigators have reported a stabilization of red blood cells to hypoisosmotic hemolysis when incubated with Δ^9-THC and CBD at concentrations greater than 10^{-6} M (Chari-Bitron, 1971; Raz et al., 1972). This suggests that the cannabinoids bind to the RBC membranes and alter the surface tension or structural organization of the plasma membrane preventing disruption. This membrane binding phenomena was confirmed by Seeman et al. (1972) in red blood cells and synaptosome preparations. They also present evidence that tetrahydrocannabinol may have two binding sites with different affinities, although they failed to account for the cannabinoids high affinity for glass which contributes greatly to the bound fraction (Dolby, 1973; Riolo and

Kleinsmith, 1973). Oddly, McMillan et al. (1973) did not find any ^{3}H-THC associated with pigeon RBC's after an intramuscular injection, although his RBC preparation procedure prior to scintillation counting is somewhat questionable.

Although the cannabinoids are predominantly bound to the plasma lipoproteins, their affinity for these proteins must be considerably less than that of the tissues since very rapid redistribution occurs after an intravenous injection (See Plasma-Tissue Half-Lives.). Interestingly, this redistribution seems to be non-specific in nature since the partitioning of labeled Δ^{9}-THC between the plasma and tissue phases (in vitro) is unaffected by heating of the tissue homogenate phase to 90^{o}C for five minutes (Dingell et al., 1973). Admittedly, any specific binding to cellular receptors could also be masked by the general non-specific absorbtion to cellular hydrophobic material. Indeed, this has been the major stumbling block encountered during subcellular receptor localization experiments with the cannabinoids. The cannabinoids are probably dispersed throughout the cells of the tissues by transport within the endoplasmic reticulum and other particulate structures, although this has not been experimentally established.

These investigations suggest that the rate of redistribution of the cannabinoids throughout the organism following their administration, should be directly related to various hemodynamic parameters and the extent of cellular vascular reticulation in the tissues. Due to cannabinoid insolubility (5-10 nmoles/ml; Dolby, 1973), the aqueous diffusion components of the small percent of unbound cannabinoids can be effectively ignored. It is very probable that the cannabinoids

can form micelles or chylomicron like structures of considerable "aggregate" molecular weight (>5 x 10^6), since labeled cannabinoid experiments with molecular sieving chromatography (A5M) show that a small but consistent portion of the label is eluted in the void volume (Riolo and Kleinsmith, 1973; Dolby, 1973).

In summary, it can be concluded that most of the more hydrophobic cannabinoids and metabolites are bound to and are transported by the plasma lipoproteins and small amounts are also associated with other hemoproteins and corpuscles, a dynamic equilibrium of the cannabinoids exists between the plasma and tissues.

METABOLISM, DISPOSITION AND EXCRETION OF THE CANNABINOIDS

Metabolism of the Cannabinoids

Many metabolic studies have been carried out on the fate of the major cannabinoids present in marihuana (Δ^9-THC, Δ^8-THC, CBD and CBN) and two of their *in vivo* metabolites (11-OH-Δ^9 and 11-OH-Δ^8-tetrahydrocannabinols) in various animals. The general metabolic pathways involving these and other related compounds are given in Figures 8 to 10 and the references to studies of the species specific metabolism are given in Tables VIII to X.

Generally, the cannabinoids are extensively hydroxylated during their metabolism (*in vivo* and *in vitro*) as are many other drugs by the endoplasmic reticulum localized NADPH-cytochrome P_{450} oxidoreductase system (Burstein and Kupfer, 1971; Bailey and Toft, 1973; Kupfer et al., 1972; Cohen et al., 1971). Even though the microsomal hydroxylase system shows a general lack of substrate specificity, most of

its potential substrates have one characteristic in common, high lipid solubility. Perhaps membrane permeability controls the availability of substrate to the microsomal electron transport system. Indeed, Dingell et al. (1973) present evidence that there is a competitive sequestering of the cannabinoids between the nuclear and microsomal membranes (See Subcellular Distribution.). This could be of considerable importance in regard to cannabinoid action in those cell types which lack extensive endoplasmic reticulum development but still have a nuclear membrane with similar surface area or volume as those cells with an elaborate endoplasmic reticulum.

The polyhydroxylated metabolites are formed mainly in the liver, lung and spleen (Christensen et al., 1971; Nakawaza and Costa, 1971; Ho et al., 1973) and not to any significant extent in the brain, kidney or small intestine of most mammals (rodents) (Christensen et al., 1971; Ho et al., 1973; Dolby, 1973). Nakazawa and Costa (1971) present data which implies that the lung may utilize a different type of hydroxylation system than the liver cytochrome P_{450} oxidoreductase complex. They find that methylcholanthrene (a potent microsomal enzyme inducer) specifically induced the lung cannabinoid metabolizing enzymes and not that of liver, except for those enzymes responsible for the formation of the 11-OH-Δ^9-THC and a very similar product with a comparable R_f value. The lung also produced at least two different but specific metabolites not seen in the liver preparations.

The binding affinity of the terminal oxidase of the liver NADPH-cytochrome P_{450} oxidoreductase system for the major cannabinoids present in marihuana decreases in the following order: CBD-C_5>CBN-C_5> Δ^8-THC-C_1>Δ^8-THC-C_3>Δ^8 and Δ^9-THC-C_5 (Cohen et al., 1971; Bailey and

Toft, 1973; Kupfer et al., 1972; Fernandes et al., 1973). This data supports other evidence that the naturally occurring cannabinoids and their metabolites may exert competitive and/or synergistic effects on their own and one anothers metabolism and action (Jones and Pertwee, 1972; Cohen et al., 1971; Karniol and Carlini, 1972; 1973; Krantz et al., 1971).

Interestingly, the 11-OH-Δ^8-THC metabolite of Δ^8-THC does not seem to act competitively with any of the tested cannabinoids for the cytochrome P_{450} terminal oxidase (Kupfer et al., 1972), indicating that the further hydroxylations are either coupled prior to this step in the system or involve another system altogether.

Metabolic Competitive Inhibitors and Cannabinoid Metabolism

The metabolic inhibitor SKF-525A (2-diethylaminoethyl, 2,2-diphenylvalerate HCl) has been shown to inhibit the conversion of THC→ 11-OH-THC (Burstein and Kupfer, 1971). SKF-525A when administered to rats at doses of 10mg/kg prior to Δ^9-THC, causes some 3-5 minutes delay in the onset of the "classical" syndrome of marihuana intoxication, indicating that perhaps some of the metabolites are very active and account for a considerable proportion of the psychotomimetic activity (Peterson et al., 1971). Ho et al. (1973) relate that SKF-525A injection prior to ^{3}H-11-OH-Δ^8-THC administration, yields a 30% increase in incorporation of label in the brain but not liver. Paradoxically, it was shown that SKF-525A inhibits the hydroxylation of 11-OH-Δ^8-THC to the more polar forms and shunts the 11-OH-Δ^8-THC to a SKF-525A insensitive system of 1-methylation (See Figure 9). In the discussion following Lemberger (1973), Truitt also supports the

contention that SKF-525A is a much more potent inhibitor of the 11-OH-THC conversion to more polar dihydroxy-THC's than the THC to 11-OH-THC reaction.

Competitive experiments with confirmed microsomal hydroxylase substrates have only compounded the confusion of the cannabinoid-microsomal interactions. Cohen et al. (1971) found that Δ^9-THC inhibited the demethylation of ethylmorphine and Dingell et al. (1971) noted a decreased formation of hydroxy-hexabarbital from hexabarbital in the presence of Δ^9-THC (competitor) implying that these drugs compete for a common coupler or intermediate of the liver cytochrome P_{450} oxidoreductase system. On the other hand, Burstein and Kupfer (1971) did not see an inhibition of Δ^9-THC hydroxylation by rat liver microsomes in the presence of hexabarbital (competitor). However the competitive inhibitor concentration (hexabarbital) used in this study was approximately ten fold less than the apparent K_m for the terminal oxidase (Rubin et al., 1964) which may possibly explain the unexpected results. Alternatively, the cannabinoids may alter the conformation or integrity of the microsomal membranes such that other (non-cannabinoid) substrates cannot reach or interact with the couplers of this electron transport system, while the cannabinoids remain equally permeable and have access to active sites.

Clearly then, a more thorough investigation of the oxidative pathway of cannabinoid metabolism and the elucidation of any interactions of the cannabinoids and other potential substrates are in order. Research of this type has special clinical significance since the use of multiple drugs at the same time has become prevalent in some parts of the world.

Cannabinoids and Enzyme Induction

The continued use of marihuana is believed to induce the formation of more "detoxification enzyme" or perhaps to increase the activity of the enzyme systems already present in the lung and liver, although this is a highly disputed point (Dolby, 1973; Marcotte and Witschi, 1972; Witschi et al., 1972; Ho et al., 1973; Okamoto et al., 1972). Many compounds displaying a Type I difference spectrum with the terminal oxidase of cytochrome P_{450} system have been shown to induce their own metabolism. Δ^9-THC has been claimed to show a Type I difference spectrum (shift of blue light absorbtion from 420 to 390 nm upon substrate binding to the oxidized cytochrome P_{450}) (Bailey and Toft, 1973; Cohen et al., 1971), although White and Hine (1972) did not observe a Type I or II spectrum during their investigations with the cannabinoids.

Ho and others (1973) found with the oral administration of Δ^9-THC to the rat at 1 mg/kg for 3 weeks, that the lung metabolism was not altered but that the liver metabolism was elevated by 50%. This latter figure is probably a conservative estimate since with repeated administration there is a gradual increase in the pool size of the cannabinoids due to their long retention times and slow excretion rate (See Body Half-Lives.). These slow clearing cannabinoids may compete with the labeled substrate during the enzyme assays, thus producing an underestimate of induction effect (Dolby, 1973).

In opposition, Kupfer et al. (1973) found no induction of the (male) rat liver microsomal hydroxylase or demethylase by the repeated administration of crude marihuana extract or Δ^9-THC by the oral or intraperitoneal routes for 3 or 9 days. They did find a significant

induction and a two fold increase in metabolism of Δ^9-THC when DDT was the inducing agent (i.p. for three days).

Finally, Witschi and Saint-Francois (1972) reported that upon administering Cannabis resin extract (500 mg/kg) orally to rats, that between 1-2 days later there was a dose dependent induction of both liver (5-6 fold) and lung (30-40 fold) benzpyrene hydroxylating enzymes which showed no apparent saturation. Interestingly, when pure cannabinoids were administered, Δ^9-THC induced the lung metabolism while Δ^8-THC enhanced the liver metabolism of benzpyrene to 8-OH-benzpyrene in a dose dependent fashion. This induction phenomena was observed only when incredibly high doses were employed.

In regard to this self-induction phenomena, Lemberger et al. (1971b) shows that in man the plasma half-lives of Δ^9-THC after equilibrium, is 27 hours for chronic marihuana smokers and 56 hours for naive users, suggesting a definite increase in the metabolic rate (induction) and/or excretion in the chronic users (See Plasma-Tissue Half-Lives of the Cannabinoids). This supports the concept of self-induction but by no means confirms it.

Therefore, it has not been unequivocally established that the cannabinoids can induce their own metabolism, although several other microsomal hydroxylase substrates (hexabarbital, methylcholanthrene, DDT, synthetic opiates, etc.) upon pretreatment can definitely enhance the turnover of these cannabinoids.

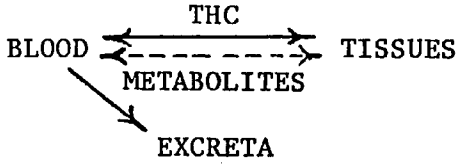
These polyhydroxylated metabolites are still for the most part very hydrophobic (except for their acid forms and perhaps some conjugates) and probably compete with each other for sites on the blood lipoproteins. This competiition, along with the metabolites' par-

tition coefficient which is a function of degree of hydroxylation, are probably the most important factors in determining their disposition and elimination.

As a final note, the metabolism of the synthetic cannabinoid, $\Delta^{6a,10a}$-dimethylheptyl-tetrahydrocannabinol, has recently been examined in vitro and in vivo in various animals, and is believed to follow a similar pattern as that of the naturally occurring THC's (Lemberger et al., 1973).

Plasma-Tissue Half-Lives of the Cannabinoids

It has been observed in most animals studied to date that the absorption and subsequent elimination of tetrahydrocannabinol and its metabolites from the blood follows a biphasic pattern. After an injection of labeled Δ^9-THC, the initial phase (α) involves a very rapid uptake and redistribution of the cannabinoid to the tissues. After a brief period of equilibrium, the second phase (β) ensues which is very slow (approximately linear with time) and probably reflects the secondary redistribution of the newly formed polyhydroxylated metabolites to the tissues and their gradual renal filtration, tubular secretion and bile elimination to the feces (Agurell et al., 1969; Turk et al., 1973; Harbison et al., 1972; Lemberger et al., 1971; Lemberger, 1973). Thus we have the following dynamic steady state occurring over time:



The plasma half-lives during the initial (α) rapid phase of redistribution after the administration of labeled THC varies from 7-16 minutes in the rabbit following an intravenous dose (Agurell et al., 1970), to about 60 minutes in the mouse (i.v.) (Ryrfeldt et al., 1973), and 10 minutes (chronic smoker, i.v.) and 15 minutes (naive smoker, i.v.) in man (Lemberger et al., 1971b). This phase presumably represents the initial absorbtion and subsequent metabolism of the cannabinoids. The secondary phase (β) of plasma half-lives is a composite of the elimination pattern of both the administered Δ^9-THC and the metabolites formed in vivo. But Δ^9-THC is eliminated to such a small extent in the unmetabolized form (Agurell et al., 1969) the secondary phase more accurately reflects the dispersion and elimination of the polyhydroxylated metabolites and the redistribution of the Δ^9-THC as displaced by the newly formed metabolites. In the rabbit this secondary phase is approximately 2 hours (Agurell et al., 1970), 20-25 hours for the mouse (Ryrfeldt et al., 1973), 21-24 hours in the rat (Klausner and Dingell, 1971) and in man, 27 hours for chronic users to 56 hours in naive users (Lemberger et al., 1971a; 1971b).

It is interesting to note the marked difference in the plasma clearance rate betweeen chronic and naive users of marihuana in both the α and β phases. A rationale for the faster redistribution of the cannabinoids with continued use (α phase) is the hypothesis that the capacity of the blood lipoproteins for the cannabinoids is enhanced or perhaps the rate of cellular incorporation is increased. Lemberger demonstrated that this enhanced initial redistribution phenomena in chronic users over naive users is not related to differences in

disposition from volume distribution studies. The process of enzyme induction could possibly explain the enhancement of the β phase in that an increased hydroxylation may render the cannabinoids more prone to elimination.

An additional factor, which may prolong the half-lives of the cannabinoids in mammalian systems is enterohepatic recirculation. Those cannabinoids sequestered by the gall bladder or choleductus and secreted into the small intestine during the digestive cycle are believed to be reabsorbed (by the lymph and blood) and thus recirculated throughout the system repeatedly (Klausner and Dingell, 1971; Miras and Couselinis, 1970).

As would be expected from the linear secondary phase of cannabinoid redistribution and elimination, the cumulative appearance of the labeled cannabinoids in both the feces and urine display a hyperbolic form over time (Lemberger et al., 1971b; Agurell et al., 1970; Klausner and Dingell, 1971).

Table XI gives data as to the various species specific body half-lives of the cannabinoids. It is of interest to note that the total rentention of the cannabinoids exceeds 1 week in many species (See Citations, Table VI), although the pharmacological effect is observed only for a short time after administration. This implies that most of the lingering cannabinoids are inactive, or their concentrations are below the threshold levels required to produce intoxication or that they are sequestered in certain specific tissues (adiapose) and are slowly released over time. The following discussion will shed more light on this selective sequestering of the cannabinoids.

Cannabinoid Disposition in the Central Nervous System

Current dogma presumes that the psychotomimetic action of the cannabinoids lies in the central nervous system (CNS), and in lieu of this, numerous investigators have tried to correlate selective brain region accumulation of the cannabinoids and concomitant behavioral and physiological alterations generally associated with these regions.

Within 15 minutes after an intravenous injection of ^{3}H-Δ^{9}-THC, McIsaac et al. (1971) found the monkeys' frontal cortex, visual cortex and cerebellular cortex show similar levels of label to that of the plasma while other gross brain structures showed less incorporation. This rapid selective cortex accumulation was also observed in the rat (Shannon et al., 1972). Conversely, Milton and Layman (1971) with the mouse and later, Freudenthal et al. (1972) with the rat observed a uniform distribution of label about all of the gross structures of these rodent brains, even for some 30 days following an intraperitoneal injection of labeled Δ^{9}-THC.

Therefore at this time, it is not known whether or not there is a selective accumulation of the cannabinoids in certain brain areas which is species dependent or whether the route of administration, suspension vehicle and aformentioned factors affect the disposition of the cannabinoids in these tissues. In regard to this possibility, inhalation techniques produce a two fold greater accumulation of labeled Δ^{9}-THC in the rat brain after 5 minutes than the intravenous mode (Leighty, 1973).

It has been confirmed that there is a selective deposition of labeled Δ^{9}-THC after an i.v. administration to the mouse, monkey and

rat in the grey matter over the white matter (Kennedy and Waddell, 1972; McIsaac et al., 1971; Shannon and Fried, 1972). This is unexpected since the partition coefficients would predict the accumulation of label in the more lipid-rich white matter (axons and sheath cells) than the grey matter (cell bodies and astrocyte cells), although the composition of mylein in the white matter is rather unique to that of general membrane composition.

Data has been accumulated suggesting that the cannabinoids may have different permeabilities to the blood-brain barrier as well as different retention times in the brain. Ho et al., (1973) found upon comparison of labeled Δ^9-THC, Δ^8-THC, 11-OH-Δ^8-THC and CBN, that after 15 minutes following the intravenous injection into rats, the cannabinoids reached similar levels of concentration in the whole brain except CBN which was about 20% less. After 30 minutes post-injection, 11-OH-Δ^8-THC and CBN had dropped to similar levels, while Δ^8 and Δ^9-THC remained relatively stable. Finally, after 60 minutes, Δ^8 and Δ^9-THC levels began to fall about 30% while CBN and 11-OH-Δ^8-THC had been eliminated to about 60% their original level. This data implies that both Δ^8 and Δ^9-THC are retained fairly well by the brain and are not redistributed until competitively displaced by other influxing metabolites since Christensen et al. (1971), Dolby (1973), and Ho et al. (1973) demonstrated the brains' poor capacity for metabolism of the cannabinoids.

The problem of differential permeability to the blood-brain barrier is rendered even more complex by other potential cannabinoid interactions. Jones and Pertwee (1972) found that by preinjection with CBD, followed by ^{3}H-Δ^9THC, that between 20 to 30 minutes later

the distribution of the labeled THC was 1.4 to 2.0 times greater in the brain than the controls which received no CBD. CBD could either inhibit the metabolism of Δ^9-THC (See Metabolism) and/or enhance THC's permeability to the blood-brain barrier and thereby account for THC's increased disposition.

General Organ Disposition of the Cannabinoids

The disposition of the cannabinoids in other body tissues is no more consistent in pattern than that of the brain. An intravenous injection of labeled Δ^9-THC into rats yields the following order of disposition after 30 minutes: (lung; liver)>fat>intestine>kidney>heart>muscle>(spleen; brain)(Klausner and Dingell, 1971). Similar results have been obtained by Shannon et al. (1972), Freudenthal et al. (1972) and Kennedy and Wadell (1972) except the lungs ranked lower and the spleen and adrenals ranked higher when the mode of administration was intraperitoneal or subcutaneous. Understandably, the intestine ranked higher when the THC was given orally.

These generalities are subject to great variation depending on the animal, its nutritional state (fat or lean), time of analysis after administration and the particular metabolite examined, the route and the suspension vehicle employed (Agurell et al., 1970; Ryrfeldt et al., 1973; Harbison et al., 1972; Christensen et al., 1971; Leighty, 1973; Kreuz and Axelrod, 1973; Ho et al., 1970). For example, the acute subcutaneous injection of ^{14}C-Δ^9-THC in rats produces an accumulation of a ten fold excess of label in fat tissues over brain, lung or liver, while the repeated administration results in only a 5-8 fold greater deposition in the adipose tissue after 26 days. Even with chronic

injections, the brain displayed the lowest incorporation. The 11-OH-Δ^9-THC and 8,11-di-OH-Δ^9-THC metabolites were also found to accumulate with time in the fat tissues but oddly the more polar di-OH-THC metabolite(s) accumulated more than the others (Kreuz and Axelrod, 1973). This indicates that the brain and the fat tissues behavor oppositely in their retention of the hydroxylated metabolites.

Placental and Neonatal Distribution of the Cannabinoids

Idaanpaan-Heikkila and others (1969) studied the permeability of Δ^9-THC to the placenta and its incorporation into fetal hamsters. Either by the intraperitoneal or subcutaneous routes of administration, the labeled THC incorporation was 2-3 times higher in the placenta than the fetus but the fetus was only slightly more concentrated with label than the maternal plasma. Unmetabolized THC accounted for 55% of the isotope in the placenta but only 25% in the fetus after 30 minutes. Similar placental transfer was seen by Harbison et al. (1972) in the mouse with Δ^9-THC.

The neonatal distribution of labeled Δ^9-THC, when the mode of administration was via the maternal lactation to the offspring, was very similar to the adult. Most of the label was associated with bone formation areas, liver, lung, spleen, heart and the lowest incorporation in the brain. (See Teratogenesis Under Physiology.)

Subcellular Disposition of the Cannabinoids

Dingell and others (1973) found the subcellular distribution of labeled THC to be mainly associated with the nuclei and endoplasmic reticulum. When THC was incubated with liver homogenates (in vitro)

about 30-40% of the label was associated with the nuclei and crude pellet, 10-30% with the mitochondria, 25-30% with microsomes and about 7-10% was soluble depending on the THC concentration and the conditions.

In our laboratory extensive subcellular investigations have been carried out with much higher resolution than that achieved by Dingell and his co-workers. Riolo and Kleinsmith (1973) found a tissue specific subcellular radioisotopic distribution of Δ^9-THC and its metabolites, 30 minutes postinjection of labeled THC intravenously into rats. In the rat brain, most of the label was associated with the plasma and nuclear membranes while the remainder was distributed similarly through the mitochondrial, lysosomal, microsomal and soluble fractions. The liver and kidney had a 2 to 3 fold greater incorporation of counts in the microsomal fraction and 6-8 times more soluble counts than the brain (perhaps acid metabolites and conjugates of glucuronic acid and sulphate). The kidney showed a major portion of the incorporated counts as polar metabolites while the brain displayed most of the label in the form of unmetabolized THC. This subcellular data correlates well with other investigators observations previously presented (Ho et al., 1973, for example).

Excretion of the Cannabinoids

As previously stated, the cannabinoids are transported by the plasma proteins like the steroids. Similarly, the excretion of the cannabinoids is predominantly in the form of sulfates and glucuronide conjugates. The pattern of excretion of the cannabinoids is species specific and is dependent on the route of administration, the suspension vehicle, the type of cannabinoid and numerous other yet uniden-

tified parameters. Much of the specific information on cannabinoid excretion in various experimental animals is given in Table XI.

Generally, Δ^9 and Δ^8-THC are not recovered to any significant extent in the unmetabolized form from the urine or feces of most animals studied (Agurell et al., 1969; Hollister et al., 1972) and presumably the excretion of the cannabinoids is enhanced by hydroxylation.

Humans seem to be intermediate between the rat which excretes most of its metabolites of THC in the feces (Agurell et al., 1969; Klausner and Dingell, 1971) and the rabbit, which eliminates most of its metabolites in the urine (Agurell et al., 1970). Lemberger et al. (1971b; 1972) noted that with continued use of marihuana in man (chronic users), there appears to be a shift from feces elimination to urine elimination (5-10%) of the polyhydroxylated metabolites, and 11-OH-Δ^9-THC may account for about 20% of the total label in the feces. Whether this is related to a faster metabolism in the chronic smokers than the naive smokers remains to be established.

Forrest and co-workers (1972) reported that a very slight amount (0.3-1.2%) of an administered dose of Δ^9-THC was eliminated in the body hair or fur of the monkey over a period of 14 weeks. Although, the form of the label is unknown, perhaps the cannabinoids are metabolized to some common intermediary metabolite which is then incorporated into the keratin protein or perhaps secretions from the sebaceous glands at the base of the hair follicles.

PHYSIOLOGICAL AND BIOCHEMICAL ASPECTS OF CANNABINOID ACTION

Since most of this thesis work involves the action of the cannabinoids on the central nervous system (CNS), the physiological and biochemical aspects of cannabinoid action in the CNS will be covered exhaustively.

Cannabinoids and the Peripheral and Central Nervous Systems

With the partial elucidation of the processes involved in synaptic transmission, intensive biochemical studies have been carried out on the effects of various drugs and inhibitors on the levels of the neurotransmitters and related compounds in the mammalian brain. The use of drugs and inhibitors to gain insights into the molecular biological processes involved in "normal" cellular action seems at first contradictory. It becomes apparent that one cannot normally measure or establish relationships among cellular processes (or any natural or physical phenomena) without perturbing the system in some way ("Heisenberg uncertainty principle"). Thus when examining the effect of drugs and enzyme inhibitors on biochemical processes, the information gained not only provides data on drug action but also the functioning in the "normal" state.

As with most of the literature concerning marihuana and its components, the variability of its action(s) on the levels of various neurotransmitters and related compounds in the brain is overwhelming.

Cannabinoid Effects on Endogenous Levels of Brain Neurotransmitters and Related Compounds

Several investigators have observed a significant elevation in whole brain or specific brain regions (hypothalamus, midbrain and cerebellum) of serotonin (5-HT), norepinephrine (NE), dopamine (DA), 2-phenylethylamine (PEA) and 5-hydroxyindoleacetic acid (5-HIAA) levels in rats, mice and rabbits following the administration (i.p., i.v., i.c.) of Δ^9-THC, Δ^8-THC, CBN or marihuana and hashish extracts at doses of 5.5-10 mg/kg or greater (Sofia et al., 1971a, Bose et al., 1964; Holtzman et al., 1969; Schildkruat and Efron, 1971; Welch et al., 1971; Ho et al., 1972a; Gallager et al., 1970; Waters et al., 1973; Constantinidis et al., 1971; Sabelli et al., 1974).

On the other hand, Leonard (1971) injected Δ^9-THC (i.p.) into rats and saw no change in the whole brain (minus cerebellum) 5-HT, NE,DA,5-HIAA or normetaephrine during the 10 days postinjection. Similarly, Gallager and others (1972) observed no effect of Δ^9-THC on the endogenous levels of 5-HT or 5-HIAA in the forebrain or brain-stem when administered intravenously or intraperitoneally. The chronic administration of Δ^9-THC at low doses for 1 week did not alter the brain or adrenal concentration of NE or DA (Mazurkiewicz-Kwilecki et al., 1973). Comparable data of Welch et al. (1971) show that CBN and Δ^9-THC do not effect the mouse brain levels of NE or DA following acute doses.

Paradoxically, some researchers have also observed that certain biogenic amines levels are reduced following the administration of Δ^9-THC in various animals. Schildkraut and Efron (1971) saw a slight depression of NE in rat brain and Ho et al. (1971b) found with the repeated inhalation of Δ^9-THC impregnated cigarettes, rats show a significant depression of 5-HIAA and normetaephrine by 20-40% at the

end of 2-3 weeks. In the monkey (Ho et al., 1972a) showed data which indicated a depression of both NE and 5-HT after a small intramuscular injection of both Δ^8 and Δ^9-THC (Discussion follows Cannabinoids and Biogenic Amines Turnover.).

Cannabinoids and Biogenic Amine Turnover

The effect of the cannabinoids on the enzymes responsible for the synthesis and degradation of the biogenic amines is as variable as their actions on the endogenous levels of these neurotransmitters.

Ho et al. (1973) studied the effect of repeated intravenous administration of 10 mg/kg Δ^9-THC, twice a day for a total of 5 weeks, on the *in vitro* activity of tyrosine hydroxylase, dopamine decarboxylase and tryptophan hydroxylase, the enzymes involved in NE and 5-HT synthesis respectively. Pretreatment with Δ^9-THC in rats, enhanced tyrosine hydroxylase (50%) and dopamine decarboxylase (15%) but had no effect on tryptophan hydroxylase and thus had no effect on 5-HT synthesis. It was concluded that NE synthesis is enhanced by chronic Δ^9-THC treatment (See Grand Discussion.). Similar results were noted by Maitre (1971) and Mazurklewicz-Kwilecki et al. (1973) in the whole rat brain in that Δ^9-THC increased the formation of DA and NE from the ^{3}H-tyrosine pool.

Leonard (1971) reported that Δ^8-THC injected at 100 mg/kg i.p. into rats did not alter the synthesis of DA or NE but did inhibit the uptake of both tyrosine and γ-amino butyric acid in the brain (not tryptophan). This inhibition of tyrosine uptake by the rat brain was also seen by Ho et al. (1971b). Along these lines, De Ropp et al. (1972) found no alteration in mouse brain dopamine decarboxylase,

glutamate decarboxylase and monoamine oxidase after exposure to marihuana smoke or residue. Sofia et al. (1971a), Schildkraut et al. (1971) and Holtzman et al. (1969) also present data which suggest that THC does not affect monoamine oxidase activity. Finally, Sofia et al. (1971a) observed a 50% reduction in the synthesis of 5-HT upon injection of 20 mg/kg Δ^9-THC into rats.

It is very difficult to draw conclusions from such conflicting data. Although it has been proposed that THC alters the uptake and release of various biogenic amines from synaptic vesicles and nerve terminals (Sofia et al., 1971a; Schildkraut et al., 1971; Truitt and Anderson, 1971) few studies have been carried out demonstrating this directly. Sofia and co-workers (1971b) found that Δ^9-THC at 10^{-7} M or greater inhibited the <u>in vitro</u> uptake of labeled 5-HT into rat brain homogenates, suggesting that THC alters the transport of 5-HT into nerve endings and vesicles. Apparently, the route of administration, suspension vehicle, dosage, age and sex of the experimental animal, time of assay after cannabinoid administration and a host of other factors play an important role in the neurochemical action of the cannabinoids.

An additional difficulty in dealing with the neurochemical and other biological parameters is the diurnal or circadian rhythmns of the endogenous neurotransmitters in the mammalian brain. Unfortunately, these aspects have not been investigated thoroughly. Scheving et al. (1968), Fernstrom et al. (1974) and Reis et al. (1968) have presented convincing evidence of circadian rhythmns of 5-HT in the brain of some mammals and some preliminary data from our laboratory implies that cyclic AMP levels in the mouse brain may be cyclically

affected. Circadian rhythmns seem to be a reoccurring phenomena in biological systems in that cyclical changes in blood sugar, mitosis in gonadal tissue, locomotor activity, body temperature, oxygen consumption and numerous other parameters have been demonstrated (Aschoff, 1960).

Although the data concerning the effect of the cannabinoids on the brain levels of certain biogenic amines in various animals is quite contradictory, some studies indicate a biphasic process may be underlying the action of the cannabinoids. Low doses of Δ^9-THC cause a general hyperexcitability behavior and an elevation of brain NE and a depression of 5-HT while high doses produce a depression and catatonia and an increase in brain 5-HT and a depression of NE (See Biphasic Effects of the Cannabinoids and Grand Discussion.). It is tempting to speculate that the biphasic effects of the cannabinoids on behavior, body temperature, biogenic amines and cyclic AMP levels may be related to the alteration of the cyclical or circadian rhythmns, such that the oscillations are increased or decreased in frequency over time. The ramifications of these biphasic effects comprise the bult of this thesis research and will be covered more thoroughly under the discussion in Chapter II and Chapter III.

Cannabinoids and Other Neurotransmitters

In studies with small mammals, Domino (1971) gives evidence of a slight increase in whole brain acetylcholine (ACH), although moderately high doses were required for this effect. The release of ACH into the suprasylvian gyrus was generally reduced in cats by pretreatment with low doses of Δ^9-THC. This effect is antagonized

by the amphetamines.

Leonard et al. (1973) found that Δ^8-THC decreased rat whole brain (minus cerebellum) γ-amino butyric acid (GABA) levels. Interestingly, GABA was shown to be an inhibitory neurotransmitter in the CNS (Krnjevic and Schwartz, 1966) and the decrease in GABA may be involved in the hyperexcitability behavior seen during certain phases of cannabinoid intoxication.

An important factor of cannabinoid action in the CNS may be the differential interaction of the cannabinoids on bilaterally symmetrical structures. Waters et al. (1973), using the water soluble ester of Δ^9-THC found that after an i.p. injection, the brain striati (bilateral) exhibited a dose-dependent difference in DA ratios but no change in the mean level of the two. Perhaps THC interacts with the nigrostriatal system in a random fashion which results in an excitation and concomitant depression of the bilateral counterpart by some feedback process. Eventually this differential action and biphasic effects of the cannabinoids on various biochemical processes may emerge as a reoccurring theme of cannabinoid action (See Grand Discussion.).

It is interesting that although marihuana has been classified as a hallucinogen, very few studies have been carried out examining this aspect. Berger and Hockman (1973) found a differential effect of Δ^9-THC on the activity of the neurons in the lateral geniculate nuclei of the rat brain. The "on" neurons (discharge upon light stimulus to the retina) are inhibited by THC while the "off" neurons (fire in the dark, inhibited by light) show no effect or an increased firing rate. This phenomena is correlated with hallucinations which would manifest themselves at the visual cortex level.

The actions of the cannabinoids in the peripherial nervous system are poorly understood. Byck and Ritchie (1973) found that 0.5 uM to 3 mM Δ^9-THC applied to in vitro isolated unmylenated vagus nerve from the rabbit caused a 3-15% decrease in the compound action potential in a dose dependent fashion and that recovery did not occur for 2 hours after wash out of the cannabinoids. A slight slowing in the conduction velocity (3%) was noted over the treatment period although it is probably due to nerve degeneration over time. No significant effect on the electrical threshold required to elicit an action potential or any impairment in the Na^+-K^+ pump was noted by the application of the cannabinoids. The observed decrease in the compound action potential may reflect the action of THC on the Na^+ and K^+ conductances during the course of the action potential as it is generated sequentially along the axon. 11-OH-Δ^9-THC showed a similar effect to THC while CBN displayed none of these effects. Cavero et al. (1972), Layman and Milton (1971) and Dewey et al. (1970) observed no impairment of the stellate ganglion (ACH) transmission in the parasympathetic nervous system which implies some specificity as to the sites of action of the cannabinoids, in terms of the biogenic amines.

In regard to the possibility that the cannabinoids alter cation transport or distribution in the CNS, Izquierdo and others (1973) reported that CBD, CBN, Δ^9-THC and Δ^8-THC (in that order), decreased the susceptability of rat brain hippocampus to experimently induced seizure-like discharges when applied topically or via i.p. injection. Since high concentrations of extracellular K^+ can elicit seizure-like discharges from various brain structures (by elevating the resting potential closer to threshold), it was hypothesized that the canna-

binoids may alter the release or transport of K^+ from the hippocampal neurons such that a stabilization of the membrane occurs. Worthy of mention, is that CBN and CBD caused more pronounced supression of seizures than the "active" cannabinoids. Finally, Boggan et al. (1973) observed a similar decreased susceptability of mice pretreated with Δ^9-THC to audiogenic induced seizures.

Cannabinoids and the Electroencephalogram

Electroencephalography (EEG) has been used as a diagnostic tool to ascertain the gross electrochemical activity of the brain in normal and pathological states. Although much of the data gained from EEG investigations of various animals is reproducable, many of the cause and effect interpretations boarderline the metaphysical. With this in mind, the deterministic relationships between the cannabinoids and the EEG profiles outlined should be considered tenative at this time.

Marihuana extracts and the tetrahydrocannabinols given orally, intravenously or intraperitoneally to rats, rabbits, monkeys, dogs and cats give rise to a general reduction in EEG potentials and the appearance of synchronized periodic bursts from the recording leads monitoring various regions of the mammalian brain and a host of other species specific alterations of the α, β, δ and θ wave forms (Lipparini et al., 1969; Bose et al., 1964; Boyd et al., 1971; Domino, 1971; Martinez et al., 1972; Masur and Kazhan, 1970; Pirch et al., 1972; Hockman et al., 1971; Fujimori et al., 1973; Lewis, 1972).

In man a decrease in alpha wave activity is generally noted after the assimilation of certain active cannabinoids (Williams et al.,

1946; Ames, 1958; Jones and Stone, 1970) although conflicting accounts exist (Hollister et al., 1970; Gibb, 1970; Rodin et al., 1970; Volavka et al., 1971). The variability of the actions of the cannabinoids on the electroencephalogram profile probably reflect a species and state specific response which varies according to the recording method and aforementioned absorbtion variables. In regard to this, Heath (1973) demonstrated that the exposure of Cannabis smoke to monkeys caused a fast wave activity with periodic bursts. The type of wave formation observed may reflect the condition of the individual animal and whether or not deep or superficial recordings of brain electrical activity were performed. Heath (1973) further correlates the changes seen in the wave form from the septal area of the brain by THC with heightened awareness and pleasure normally associated with this region.

Cannabinoids and Smooth and Striated Muscle Action

Several investigators have reported that the cannabinoids have the capacity to reduce the contraction force of smooth muscle preparations. Gascon and Peres (1973) studied the effect of Δ^9-THC on the contraction of in vitro isolated guinea pig vas deferens smooth muscle. THC at 10^{-8} M produced a biphasic response on muscle contraction induced by ACH. Initially, a potentiation of contraction occurred, followed by a depression. Incubation concentrations of THC greater than 10^{-7} M were inhibitory to the development of contraction force. Interestingly, Δ^9-THC was more effective in inhibiting muscle contraction induced by ACH than Δ^8-THC. Similarly, Layman and Milton (1971a) found the Δ^9-THC but not CBD reduced the twitch response of the ileum intestinal smooth muscle by 50% at 10^{-7} M and

100% at 10^{-6} M when stimulated transmurally by electrodes or induced to contract in the presence of acetylcholine (ACH). Both Δ^9-THC and CBD were effective in inhibiting the release of ACH at the neuromuscular junction in the ileum preparations. Therefore it may be concluded from these studies that some cannabinoids may interfere with the release of ACH from the presynaptic neuromuscular endplates or perhaps alter its uptake into the presynaptic axon. Additionally, the cannabinoids may impair the excitation-contraction coupling process directly, since transmurally stimulated smooth muscle preparations are also affected by the cannabinoids.

Interestingly, Gill et al. (1970) and later, Dewey and co-workers (1972) found no effect of Cannabis extracts or Δ^8 and Δ^9-THC on chemically induced contractions of ileum or vas deferens in in vitro preparations when the cannabinoid concentrations were less than 10^{-5} M.

It has been consistently observed that Δ^9-THC at 10^{-7} to 10^{-5} M does not alter the contraction or neuromuscular transmission of ACH of striated muscle preparations in the rat, frog or cat (Layman and Milton, 1971a; Gascon and Peres, 1973).

Interestingly, Brown (1971) cited some evidence which links the action of Δ^8-THC with anticholinergic activity. Those drugs which inhibit cholinergic activity in mammals (atropine and related alkaloids) also prevent behavioral habituation and extinction of learned responses. Δ^8-THC was also found to prevent habituation. (See Behavior.)

Heart Muscle

Cavero et al. (1972) also presented evidence which implied that Δ^9-THC inhibits the release of ACH from the vagus nerve, thus accounting for the tachycardia observed upon the administration of the cannabinoids in man. Additionally, when propranolol (β blocker) was administered orally subsequent to the smoking of marihuana in man, there was a significant antagonism of the tachycardia induced by marihuana. This suggests that a β-cholinergic mechanism may underlie the elevation in heart rate during marihuana intoxication (Martz et al., 1971).

Cannabinoids and General Human Physiology

The effect of marihuana and/or THC on various human physiological parameters are listed below. The blood pressure was found to decrease after administration of the cannabinoids (Hollister et al., 1968; Isbell et al., 1967; Waskow et al., 1970), to increase (Johnson and Domino, 1971; Domino, 1970; Weiss et al., 1972) or to fluxuate over time (Perez-Reyes et al., 1971; U.S. Commission, 1972). The following parameters remained unaffected by the cannabinoids: body temperature (Hollister et al., 1968; Isbell et al., 1967), respiratory rate (Domino, 1970; Isbell et al., 1967; Weil et al., 1968), red blood cell count, platlet count, blood electrolyte concentration, serum proteins, hemoglobin levels, blood cholesterol, blood alkaline phosphatase, lactate dehydrogenase, glutamate oxaloacetic transamylase activities, no proteinuria, glycosauria, ketouria, hematuria (U.S. Commission, 1972), blood sugar (Dornbush and Freidman, 1971; Hollister and Gillespie, 1970; Hollister, 1971; Hollister et al., 1968; Isbell

et al., 1967; Manno et al., 1970; Weil et al., 1968) blood fatty acids and cortisol (Hollister, 1971), although a few investigators have reported conflicting accounts (Manno et al., 1970; Miras, 1965; U.S. Commission, 1972). Urinary excretion of epinephrine was reported to be elevated by the use of marihuana (Chopra, 1969; Weiss et al., 1972) while Hollister et al. (1970) and Messiha and Suskin (1973) found no alteration in excretion of 5-HT, E, NE, 5-HIAA, 3-methoxy-tyramine or metanephrine but a slight depression of elimination of norepinephrine, dihydroxyphenylmandelic acid, vanillylmandelic acid, homovanillic acid and dihydroxyphenylacetic acid after 2 hours post-marihuana administration.

Although, the enhancement of visual acuity, somatic sensations, vibratory and touch thresholds and hearing have been reported (Caldwell et al., 1970; Ames, 1958; Bouquet, 1951; Clark and Nakashima, 1968), it has not been consistently observed by other investigators and probably reflects individual responses.

Campbell et al. (1971) reported an increase in the size of both the lateral and third ventricles of the human brain, implying brain tissue atrophy with marihuana smoking. The incidence was higher in marihuana smokers than controls, however the marihuana smokers were very atypical in case histories, in that many other drugs had been used, periodic syncope and some signs of epilepsy were prevelant among the 10 people studied. The inferences made by these investigators are highly tenative.

Non-Human Physiological Actions of the Cannabinoids

Data regarding the actions of the cannabinoids on general

physiological processes in non-human animals is not as well documented as that of man.

Sprague et al. (1973) found that the acute or chronic administration of Δ^9-THC (i.v., s.c. or inhalation) to the rat, rabbit or monkey caused a significant depression by 20-75% of the liver glycogen stores. Since corticosterone levels are elevated by Δ^9-THC (See Cannabinoids and Hormone Action) and the glucocorticoids have been implicated with sugar transport and metabolism, perhaps there is some correlation between the corticosteroids and the Cori cycle. Similar results were obtained by El-Sourogy and others (1966) in the rabbit upon the intramuscular administration of CBN.

Finally, Ho et al. (1971) reported no impairment of liver or kidney function or change in organ weight after 6 weeks or daily exposures of rats to marihuana smoke.

Cavero et al (1972a) found that Δ^9-THC produced a marked hypotension in dogs and Birmingham (1973) revealed that female rats with experimently induced hypertension would display a reduction of systemic blood pressure upon the intraperitoneal injection of Δ^9-THC. The administration of Cannabis extracts or Δ^9 and Δ^8-THC to various animals (rat, monkey, dog and cat) generally results in bradycardia (Kubena et al., 1971a; Dewey et al., 1972a) which is opposite to the general effect observed in man (See Dose-Response Relationship - Chapter I). Respiratory rate is generally reduced in rats and dogs upon the administration of marihuana components (Kubena et al. 1971a; Oskoui, 1972) perhaps by reduction in phrenic nerve discharge rate.

Thermoregulation and Cannabinoid Action

It has been established in mice and rats that the administration of Δ^8 or Δ^9-THC causes a general dose dependent reduction in body temperature (0.5-6^{o}C) which may last for several hours to 24 hours (Miras, 1965; Leonard, 1971; Rating et al., 1972; Garattini, 1965; Gill, Paton and Pertwee, 1970; Lomax, 1971; Holtzman et al., 1969; Borgen et al., 1973a). It is interesting that Welch (1968) noted that hypothermia causes an accumulation of brain catecholamines in mice suggesting that the two may be related by temperature dependent catecholamine transport and synthesis. Finally, Sofia (1972) reported a biphasic effect of Δ^9-THC on the rectal body temperature of the rat. A hyperthermic response was observed after the administration of low doses of Δ^9-THC while a profound hypothermia occurred following high doses.

Cannabinoid and Hormone Actions

Several investigators have reported interactions between the cannabinoids and certain hormone systems. Burstein et al. (1972a; 1973) found that 10^{-5} to 10^{-4} M Δ^9-THC inhibited the *in vitro* synthesis of PGE_2 in a dose dependent fashion and that CBN, CBDA, Δ^8-THC, CBD, CBC, Δ^9-THC and finally CBL inhibited, in decreasing order of effectiveness, the conversion of a PGE_1 precursor to PGE_1. The active site of the cannabinoid molecule was indicated to be the olivetol moiety since olivetol showed a high degree of inhibition also.

Ling et al. (1973) observed no effect of Δ^9-THC chronically administered to rats on gonad weights or ^{3}H-testosterone uptake in

liver, adrenals or sex organs. Δ^9-THC did cause involution of the thymus gland but no increase in the in vitro production of corticosteroid was observed after i.p. administration of Δ^9-THC at 4 or 16 mg/kg. The elevation of blood corticosteroid, preceded by the increased synthesis is usually associated with the involution of the thymus gland shortly after birth, indicating that perhaps THC is mimicking the corticosteroid action or enhancing its release from the adrenals since its synthesis was not effected. In regard to this possibility, Kubena et al. (1971) found a dose dependent elevation of rat blood corticosterone levels by Δ^9-THC administration at 1-4 mg/kg i.p. After hypophysectomy, even 16 mg/kg was incapable of altering the blood corticosterne levels suggesting that THC is working at a higher level of organization than the anterior hypophysis, possibly enhancing the release of corticostropin releasing factor from the median emminance or higher brain centers controlling the release.

Additional evidence for competitive interactions between the cannabinoids and the steroids comes from the work of Drew and Slagel (1973). They found that the intraperitoneal injection of 9 mg/kg of Δ^9-THC into adrenalectomized rats selectively impaired labeled corticosterone uptake by the hippocampus and septum of the brain. Perhaps the hippocampus (or related structures) is the regulatory center for blood corticosterone levels and THC effectively blocks the receptors to prevent the inhibition of this control system by circulating blood corticosterone. Further evidence of neural-hormonal disruption by cannabinoids comes from the finding that Δ^9-THC is capable of abolishing the serum surge of pituitary leutinizing hormone and thus the subsequent ovulation during the menstrual cycle

of the rat (10 mg/kg i.p.) (Nir et al., 1973). It could be hypothesized that THC may directly effect the ovary as well as inhibit the release of leutinizing releasing hormone (LRH) and/or leutinizing hormone (LH) from the pituitary and associated brain regions. Also the systemic injection of Δ^9-THC causes the depression of thyroid I^{131} release implying an inhibition of thyroid stimulating hormone (TSH) release from the hypothalamus or the impairment of the second messenger coupling at the thyroid level (Lomax, 1971). Although not related, support for this concept can be seen from experiments of Kelly and others (1973). In human lung fibroblasts in culture, Δ^9-THC inhibited the elevation of cyclic AMP induced by the application of either PGE_1 or epinephrine in a dose dependent fashion (from 0.32-32 uM). This effect was reversed by the washing out of the cannabbinoid. This certainly does imply an impairment of the allosteric interaction of these compounds with the cyclic AMP generating system within the membranes of these lung cells (See Chapter III.). Alternatively, THC could enhance the activity of phosphodiesterase although this does not seem to be the case with mouse brain phosphodiesterse preparations (See Chapter II and III.).

Finally, Welch et al. (1971) found that Δ^9-THC but not CBN depleted adrenal epinephrine by 30% within 10 minutes after an intraperitoneal injection of 10 mg/kg in mice. However, this could be related to stress induced during the experimental procedure as other investigators have pointed out (See Effect of Environment of Cannabinoid Action.).

Cannabinoids and Teratogenesis

The effects of the cannabinoids on gestation and offspring development are quite controversial. Pregnant mice injected with Cannabis resin (4.2 mg/kg) on days 1-6 of gestation, showed an increased incidence of stunted and malformed offspring, while injections after day 6 caused complete fetal reabsorbtion (Persaud and Ellington, 1967). Other investigators have demonstrated that the injection of 20-500 mg/kg of the cannabinoids into pregnant rats, hamsters and rabbits prior to the 6th day of gestation also produced malformed offspring and in some cases were actually embryocidal (Gerber, 1969; Gerber and Schramm, 1969). It should be noted that Cannabis resin probably contains different compounds than those obtained by more conventional preparation procedures and that the doses employed by the latter investigators were approaching the LD_{50} for the maternal parent.

Paradoxically, Miras (1965) orally administered 0.2% marihuana extract to rats for several months and found a marked reduction of fertility but the offspring were normal for 90 days postparturition. Perhaps this decrease in fertility is related to the supression of leutinizing hormone release. Continuing along these lines, pregnant rats injected with Δ^9-THC at 10 mg/kg (s.c.) on days 10-12 of gestation did not produce any defects in offspring or stillbirths, although some degree of latency in learning parameters, tooth eruption and growth rate were seen in the in utero treated animals, after delivery (Borgen et al., 1971; 1973b). Similarly, Pace and co-workers (1971) found that Δ^8-THC, Δ^9-THC or marihuana extracts administered (s.c., i.v., i.p.) to pregnant rats, rabbits and hamsters generally

produced smaller litter size and stunted offspring (at high doses) but little or no abnormalities or death.

Therefore it seems that very high doses of marihuana preparations and some pure cannabinoids can produce considerable teratogenic responses in various rodents depending on the time of administration during gestation and a host of other as yet unidentified parameters. On the other hand, more physiological doses of the cannabinoids seem to have little effect on the normal development of the offspring postparturition, except possibly on the growth rate.

Cannabinoid Actions at the Cellular and Subcellular Level

In spite of the advances in Cannabis chemistry, cannabinoid metabolism, disposition and excretion and numerous other elegant experiments with the cannabinoids and mammalian life processes, little attention has been turned toward the cannabinoids and their effect on biochemical processes at the cellular level.

Cannabinoids and Nuclear Metabolism and Division

The currently available information on the effects of marihuana components on cell physiology and nuclear metabolism of macromolecules is quite limited. Leuchtenberger et al. (1973) studied the effects of marihuana smoke and standard Kentucky cigarette smoke on human lung cell explants. They found an increase in the size of the cytoplasm, nuclei, nucleoli and a concomitant increase in the DNA content of both the cigarette and marihuana smoke exposed cultures over control cultures. Mitotic abnormalities were noted, which included chromosome lag and breakage during metaphse and anaphase as well as tripolar meta-

phases and larger spindle formations. The frequency of these abnormalities over controls was 10-25%, depending on the age of the cultures. In a similar experiment conducted by Leuchtenberger et al. (1971) with mouse lung epithelial cells in culture, it was found that the cigarette smoke was cytotoxic to these cells while mixtures of tobacco and marihuana smoke had little cytotoxicity. The nuclei and nucleoli were larger than controls and contact inhibition was depressed along with an increased mitotix index and nuclear DNA content in these mixture exposed cells. Also, lagging chromosomes were noted during metaphase and an increase incorporation of tritiated thymidine into DNA of the marihuana-tobacco mixture was observed. Neu and co-workers (1970) and Martin (1969) reported a decreased mitotic index but no chromosomal abberations of cultured human leukocytes which were incubated with Δ^9 and Δ^8-tetrahydrocannabinol at concentrations exceeding 0.1 mM.

Many other researchers have found no relationship between the use of marihuana and chromosomal abberations in the human white blood cells of chronic or casual users, first generation offspring of rodents whose parents were treated with Δ^9-THC or chronically treated hamsters (Gilmore et al., 1971; Dorrance et al., 1970; U.S. Commission, 1972; Pace et al., 1971; Nicholson et al., 1973). Nahas et al.(1974) found upon comparison of marihuana smokers and non-users lymphocytes, the former had a reduced capacity (40%) for induced division and the users lymphocytes also incorporated less ^{3}H-thymidine than the non-user.

Furthermore, experiments with cultured protozoa, Tetrahymena which were synchronizied by a series of heat shocks at 34^{o}C, showed

a dose dependent reduction in growth rate during log phase and a dose dependent lengthening of the mean division time upon exposure to Δ^9-tetrahydrocannabinol (Zimmerman and McClean, 1973). These studies indicate the necessity for further investigations into the effects of the cannabinoids on impairments of the cell cycle or as potential prophylactics to disease and abnormal states induced by other factors.

Biochemical information on the effects of the cannabinoids on transcription and translation processes are wanting. Some of the available data suggests that either in vitro incubations of rat brain cortex slices with 0.1 mM Δ^9-THC or isolated rat brain chromatin from rats, previously injected with 10 mg/kg of Δ^9-THC (i.p.), display a marked inhibition of RNA synthesizing capacity and a decreased rate of incorporation of ^{14}C-leucine into proteins (Jakubovic et al., 1972; Hodgson et al., 1973). CBD and CBG were also shown to possess some inhibitory activity on transcription and translation (Jakubovic et al., 1972). Additionally, Zimmerman and McClean (1973) utilizing synchronous Tetrahymena cultures noted significant reduction of the incorporation of ^{3}H-uridine into acid-insoluble fractions and only a slight decrease in ^{3}H-thymidine incorporation into DNA and ^{14}C-phenylalanine into protein. The investigators also present data which suggest that Δ^9-tetrahydrocannabinol inhibits the transcription of mRNA or its assembly into polyribosomes in these protozoa.

On the other hand, rats treated with 20 or 40 mg/kg of Δ^9-THC or crude extracts (75 mg/kg) and 90 minutes later various brain slices were assayed for the incorporation of ^{14}C-uridine, showed no effect on its incorporation into RNA (Roberge et al., 1972).

An explanation for these inconsistent results may be found in the

time of assay after the administration of the cannabinoids or in the particular protocols used. Although the doses used in most of these studies exceed those encountered under normal physiological conditions during use, they do open avenues for potentially fruitful areas of research.

If RNA and protein synthesis are generally impaired in the presence of the cannabinoids, then the observation made by Hattori et al. (1973) on the percentage of nuclear attached ribosomes in the infant rat brain confirms this hypothesis nicely. The administration of the cannabinoids at 10 mg/kg (s.c.) to infant rats decreased the percentage of nuclear membrane attached ribosomes after 3 hours in the following order of decreasing potency Δ^9-THC, Δ^8-THC, 11-OH-Δ^9-THC>CBG> CBN and CBD. Therefore if either rRNA synthesis or ribosomal protein synthesis is inhibited by the cannabinoids, then the RNP particle production of the cell should also be inhibited.

Unexpected data has emerged from our laboratory in that doses between 0.5-5 mg/kg (i.v.) of both Δ^9-THC and CBN stimulated both Mg^{++} and Mn^{++} dependent nucleolar and heterogenous rat brain and liver DNA dependent RNA polymerase activity (in vitro) in a dose dependent fashion (Botany and Kleinsmith, 1974).

Cannabinoids and Vascular Membranes and Vesicles

Recently, it has been shown that very high concentrations of Δ^9-THC and CBD (5×10^{-4} M) cause the release of acid phosphatase from in vitro liver lysosomal preparations (Raz et al., 1973). Since high concentrations are required for this action it is doubtful that this effect has any relevance during normal marihuana consumption in society.

For information concerning the cannabinoids and interactions with the endoplasmic reticulum, see Metabolism, Disposition and Transport of the Cannabinoids.

Cannabinoids and Mitochondrial Processes

Δ^8 and Δ^9-THC at relatively high incubation concentrations (0.1 mM) enhances rat liver mitochondria ATPase activity (Chari-Bitron and Bino, 1971; Bino et al., 1972) and produces a biphasic effect on oxygen consumption by these mitochondria. Low doses stimulate oxygen uptake while high concentrations produce an inhibition (Bino et al., 1972). Perhaps this is related to the disruption or lysis of the mitochondria when incubated with high concentrations of the cannabinoids (Bino et al., 1972; Mahoney and Harris, 1972) and the uncoupling of oxidative phosphorylation (Mahoney and Harris, 1972).

Interestingly, the Na^+-K^+ pump of red blood cells which also involves ATPase activity is also altered by Cannabis resin. Porter et al. (1970) demonstrated that the RBC Na^+-K^+ transport as monitored by the uptake of $^{86}Rb^{++}$, is inhibited only by very high concentrations of cannabinoid extracts.

Cannabinoids and Nutrition

The administration of the "active" cannabinoids (Δ^8 and Δ^9-THC) to rodents for varying lengths of time (days) causes a consistent reduction in food intake (Manning et al., 1971; Borgen et al., 1971; Rating et al., 1972; Nahas et al., 1973a) and either a decreased water intake (Jarbe et al., 1973; Sjoden et al., 1973; Borgen et al., 1971) or no change in water consumption (Manning et al., 1971) and

a general decrease in the rate of weight gain as compared to controls or a weight loss (Sjoden et al., 1973; Manning et al., 1971; Borgen et al., 1971; Jarbe et al., 1973; Nahas et al., 1973a). Interestingly, Δ^8-THC is more effective in producing a weight loss than Δ^9-THC (Sjoden et al., 1973; Jarbe et al., 1973). Perhaps there is some correlation with the cannabinoid induced depletion of glycogen stores (Sprague et al., 1973) and the weight loss observed by these researchers, since glycogen depletion would lead to the metabolism of fat and muscle proteins to serve as alternate food stuffs.

Rating and others (1972) observed an initial decrease in food consumption and a concomitant weight loss in rats injected with Δ^8-THC, however a physiological tolerance developed within 7 days such that the weight returned to control levels. The initial decrease in food intake and subsequent weight loss was attributed to the cataleptic state induced by the cannabinoids, such that the animal was incapable of eating.

BEHAVIOR

Cannabinoids and Conditioned Behavior

The effect of the naturally occurring cannabinoids and their metabolites on various behaviorally learned paradigms in rodents is quite perplexing. Several researchers have reported an enhancement of learning, no effect or a depression in the acquisition of certain behavioral tasks. If learning may be loosely defined as the change in performance on a behavioral task that occurs as a result of experience, then insights as to the effects of the cannabinoids on the acquisition of particular behavioral tasks can be gained directly form classical conditioning experiments.

When rats and monkeys are under the influence of the cannabinoids and their metabolites, Jaffe and Baum (1971), Pirch et al. (1972), Scheckel et al. (1968) and Barry et al. (1971) demonstrated an enhanced acquisition of certain behavioral tasks when shock avoidance was employed as negative reinforcement. With positive reinforcement methods, Carlini and Kramer (1965) saw an improved maze learning ability in some rats following the administration of the active tetrahydrocannabinols. Additionally, Δ^9-THC when injected into rats at 2.5 or 10 mg/kg (i.p.) produces "winning" behavior on competitive tasks between previously "loser" rats paired with uninjected winners (Masur et al., 1971a; Masur et al., 1972).

On the other hand, several investigators have reported a reduction in the acquisition of reinforced learning upon the administration of Δ^9-THC, Δ^8-THC, marihuana smoke or extracts in rats, pigeons, monkey and two month old rats which were exposed to marihuana extracts

in utero (Pradhan et al., 1972; Carlini et al., 1970; Henriksson and Jarbe, 1971; Grunfeld and Edery, 1969; Ferraro and Billings, 1973b; McMillian et al., 1970; Gianutsos et al., 1972).

Marihuana and its components upon administration to many animals which has previously established some conditioned behavior causes a disruption of response for food and/or water (Black et al., 1970; McMillian et al., 1970; Ford et al., 1971; Frankenheim et al., 1971; Carlini, 1968), rope climbing for food (Carlini, 1968), maze running for food reward and shuttle box performance (Orsinger et al., 1970).

These diametrically opposed results may be attributed to whether or not the animal is learning new behavior or repeating old conditioning and the specific type of learning paradigm tested. Robichaud and others (1973) reported that the acute administration of Δ^9-THC impaired performance of mice to conditioning while the chronic administration for 5 days resulted in an enhanced acquisition of learning. Perhaps a state dependent learning phenomena is responsible for some of these variable effects observed during conditioned behavior experiments (See Tolerance.). Herring (1972) saw a similar phenomena with rats under the influence of Δ^9-THC.

Many investigators have pointed out an "all or none" phenomena on behavioral paradigms produced by the cannabinoids. Some of the treated animals show no impairment of behavioral acquisition while others will show a high degree of alteration (Carlini et al., 1965; Orsinger et al., 1970; Ribichaud et al., 1973).

Learning in Man

Studies of state dependent learning in man subsequent to the

smoking of marihuana show only certain cognitive psychological and perceptive tasks are affected by marihuana, especially those processes involved in short term sequential memory (Hill et al., 1973; Pearl et al., 1973; Manno et al., 1971).

It is commonly believed that the human memory process can be grossly divided into a short term memory (limited capacity register) and a long term memory which represents the information pool transferred from the short term register (Atkinson and Shiffrin, 1971). Some evidence has accumulated which suggests that marihuana smoking may affect the process of recall of information from the short term register but not the long term memory in both humans and monkeys (Darley et al., 1973; Abel, 1971; Casswell and Marks; 1973; Weil et al., 1969; Tinklenberg et al., 1970; Zimmerberg et al., 1971).

In man, the cognitive functions are generally impaired, although there is much variation among subjects, especially between long term or chronic users and naive subjects. Decrements in serial addition and subtraction, reading comprehension, digit memory (forward and reverse) and elapsed time estimations have been reported rather consistently (Weil et al., 1968; Clark and Nakashima, 1968; Meleges et al., 1970; Domino, 1971). Presumably, these impairments were due to interference with the decoding and recall processes associated with the short time memory (Meleges et al., 1970).

It should be noted that Bowman and Pihl (1973) conducted an extensive investigation on chronic Jamaican marihuana smokers and nonsmokers from Jamaica. They found absolutely no impairment on any of the psychological, physiological or sensory tests between the two groups. This study was unique in that the experimental subjects were

tested while in their own environment and using their own marihuana, which was sampled by the investigators for GLC analysis.

Other Behavioral Effects in Non-Humans

Several reports have appeared which support the concept of reduced aggression shortly after the administration of the "active" cannabinoids (McDonough et al., 1972; Carlini and Masur, 1969; Carlini et al., 1972; Kilbey et al., 1972) to rodents, although the aggressiveness may be dramitically affected by the animals nutritional state (Carlini and Masur, 1969). Carlini and others found that starved animals are even more aggressive while under the influence of the cannabinoids than those well satiated. They pinpointed the increased aggressiveness with stress produced by hungar or perhaps hypothermia and not hypoglycemia due to the fact that the intraperitoneal injections of glucose did not alter the aggressiveness in the starved rats (Carlini et al., 1972).

Other evidence suggests that Δ^9-THC, Δ^8-THC or crude extracts administered to rats induce vocalization upon tactile stimulus (Carlini and Kramer, 1965; Hendriksson and Jarbe, 1971a), a decreased sexual performance in male rats (Merari et al., 1973), definite alteration in gross behavioral activity in an open field area (Masur et al., 1971; Drew et al., 1972), an elevation in pain threshold (Parker and Dubas, 1973) and a supression of the maternal pup retriving behavior at doses greater than 20 mg/kg (Abel, 1972).

The continued use of marihuana has been reported to give rise to an "amotivational syndrome" in man (Kolansky et al., 1972; National Institute of Mental Health, 1971), although this phenomena is highly

disputed. In regard to this, Masur (1973) gives data which suggests that a decreased motivation does not occur in rats but that they are remotivated toward different behavior upon exposure to THC. He conducted positive reinforced experiments with rats paired as worker and manager relationships to a water reward. Seven of the twelve pairs tested showed that upon injection of 5 mg/kg of Δ^9-THC (i.p.) into the worker rats caused a reversal or inversion of the heirarchy in that the manager became the worker and the worker would adopt the manager role of reward for work performed by the worker. This phenomena was reversible upon the ceasation of THC.

Biphasic Action of the Cannabinoids on Overt Behavior

Many investigators have reported a biphasic action of the cannabinoids on overt behavior. Small doses of Δ^9-THC or Δ^8-THC causes hyperexcitability, increased motor activity or behavioral response rate, while high doses of the tetrahydrocannabinols causes an initial hyperexcitable state followed by a depression, decreased response rate and catalepsy (Leonard, 1971; Ling et al., 1971; Schildkraut and Efron, 1971; Holtzman et al., 1969; Christensen et al., 1971; Gershon, 1970; Davis et al., 1972; Carlini et al., 1970; Garriott et al., 1967; Abel, 1970; Ferraro et al., 1971; Grisham and Ferraro, 1972; Scheckel et al., 1968; Robichaud et al., 1973). This suggests that the cannabinoids may have multiple modes of action depending on their endogenous concentration.

Perhaps the tetrahydrocannabinols' bimodal form of action can be related to a differential affinity for receptors (presumably in the brain) underlying the hyperexcitable and depression states, respective-

ly. One could postulate that those receptors involved with the hyperexcitable state would have a low equilibrium constant (high affinity) for THC while those involved with the cataleptic behavior would possess a higher equilibrium constant (lower affinity). In this way, the biphasic effects induced by the administration of tetrahydrocannabinol would be directly related to the administered dose and the concomitant levels of THC absorbed (See Grand Discussion.).

This points out the importance of time of assay or testing of the experimental animal after the administration of the cannabinoids. Also, the dosage may effect the relative time course of these behavioral responses. At a high dose, the hyperexcitability period may be shorter or absent altogether with the animals displaying only a depression (Dewey et al., 1970; Kubena and Barry, 1970; Holtzman et al., 1969). Alternatively, very small doses of the cannabinoids, boarderlining the effective dose, may elicit a hyperexcitability response and be rapidly metabolized, diluted prior to the development of depression.

Interestingly, many biochemical and physiological parameters have been shown to follow a biphasic response with respect to cannabinoid dose. The administration of low or high doses or concentrations of Δ^9-THC, Δ^8-THC and marihuana extracts to a variety of animals or <u>in vitro</u> preparations produces biphasic responses in EEG pattern (Bose et al., 1964), smooth muscle contraction of vas deferens induced by ACH (Gascon and Peres, 1973), human blood pressure (Perez-Reyes et al., 1971), rat rectal body temperature (Sofia, 1972), corticosterone uptake by hippocampus (Drew and Slagel, 1973), mitochondrial oxygen consumption (Bino et al., 1972), brain NE and 5-HT

levels (Holtzman et al., 1969; Ho et al., 1972), brain cyclic AMP levels and adenyl cyclase activity (Dolby, 1974) and conditioned behavior responses (Davis et al., 1972; Ferraro et al., 1971; Grisham et al., 1972). These biphasic effects may account for much of the variation seen in the behavioral and physiological responses in various animals to the cannabinoids. The biphasic effects will be covered more thoroughly with the cannabinoids effect on cyclic AMP levels in the mammalian brain in Chapter II and Chapter III.

Behavioral Tolerance to the Cannabinoids

Tolerance is the process of behavioral habituation or extinction of a particular conditioned behavior to the repeated administration of a drug. This process of behavioral tolerance may be due to alterations in drug metabolism, disposition or excretion with continued use, the alteration of the receptor sites in the target tissues such that they become unresponsive or less sensitive to the drug over time or the repatterning of operant behavior to a state dependent learning which approaches or exceeds the rate of learning in the "normal" state.

Numerous investigators have shown that by the repeated administration of Δ^9-THC, Δ^8-THC or crude marihuana extracts to the dog, pigeon, rat, monkey, mice and man that within 3-9 days (unconfirmed in man), depending on the dosage, suspension vehicle and route of administration and other factors, a marked behavioral tolerance has developed to the point of no decrement in response to various operant paradigms. The duration of tolerance may persist for months or mere days after the last injection or administration depending on the initial series of tolerance inducing doses, the larger the dose, the longer the duration of the tolerance phenomena (McMillian et al.,

1972; Carlini, 1968; McMillian et al., 1970; Carlini et al., 1970; Thompson et al., 1971; Pirch et al., Silva et al., 1968; Ferraro and Grisham, 1972a; Harris et al., 1972; Weil et al., 1968; Dewey et al., 1972; Grilly et al., 1973; Potvin and Fried, 1972; Abel et al., 1972; Newman et al., 1972; Davis et al., 1972; Webster et al., 1973; Domino, 1971; Bueno and Carlini, 1972). Not all of the behaviorally tested parameters display a tolerance effect, those behavioral patterns which are easily altered by relearning are the easiest to effect while those processes normally under autonomic control are not generally adaptable (i.e. heart rate, glycogen stores), although there are some exceptions (hypothermia, EEG) (Pirch et al., 1972; Abel et al., 1972).

Other than the metabolic theory of tolerance and the others previously mentioned, recently, an immunological theory has been proposed. Since the time course of the immune response is on the order of a week and the development of tolerance takes a similar length of time, perhaps there is a correlation. Nahas et al. (1973) reported that when some mice were injected with Δ^9-THC as an antibody inducer and the cells from the spleen subsequently isolated in culture and incubated with THC, that there was a stimulation of mitosis by THC in the pretreated animals while the controls showed no blastogenic response. Therefore THC and perhaps the related cannabinoids may serve as effective haptens while bound to the blood lipoproteins in vivo. Thus the variation in response to the cannabinoids by various animals may reflect the rate of immunological blastogenesis and most of the tolerance reports are accounted for by this hypothesis. This is the only report on the cannabinoids which found an increased cell proliferation in the presence of THC (See Nuclear Metabolism and

Cannabinoids.).

Recently, Abel (1973) has presented preliminary data which suggests that physiological or behavioral (righting reflex) tolerance to very high doses of Δ^9-THC (60 mg/kg) in frogs occurs after 5 days of repeated administration. This implies that Δ^9-THC alone is responsible for the phenomena of tolerance since the cannabinoids are not believed to be metabolized in the frog due to the lack of microsomal cytochrome P_{450} oxidoreductase system (See Metabolism.).

It should be noted that a few investigators did not observe tolerance in the chimpanzee on operant behavioral tasks (Ferraro and Grilly, 1971) or in mice on aggressive behavior (ten Ham et al., 1973) upon the administration of Δ^9-THC daily for about one month. Therefore other than these investigators reports and possibly a few others, the phenomena of tolerance seems to be widely occurring.

Interetingly, Jaffe and Baum (1971), Brown (1971) and Drew and Miller (1973a) present some evidence which suggests that Δ^9-THC, Δ^8-THC or hashish resin may inhibit the process of habituation (decreased response to an unrewarded stimulus) of locomotor behavior in rodents.

Reverse Tolerance

Many investigators have observed that chronic marihuana smokers seem to require less drug for a definite intoxication than naive smokers and some other data also suggests a cumulative increase of behavioral alteration produced by the long term administration of the cannabinoids to non-human animals (Meyer et al., 1971; Phillips et al., 1971; Lemberger et al., 1971). Although there is more evidence to support the concept of tolerance than reverse tolerance, these studies

do demonstrate the extreme variability of the response to the administration of the cannabinoids. It has been hypothesized that this reverse tolerance is actually a function of the process of liver and lung enzyme induction, such that the chronic smoker would metabolize the cannabinoids at a faster rate to the active metabolites (11-OH-THC, See Tables VIII and IX.) than elimination and dilution would allow when compared to naive smokers (Lemberger, 1972; Mechoulam, 1970). To further speculate, perhaps the initial effect of certain active cannabinoids is the primary induction of the enzymes responsible for the conversion of THC to 11-OH-THC, resulting in a negative tolerance since 11-OH-THC has equal or greater activity as THC. With the continued use, a secondary phase ensues which involves the induction of the "remainder" of the cannabinoid metabolizing enzymes which generally render the 11-OH-THC less or inactive by further hydroxylations. Therefore after total induction of the cannabinoid metabolizing enzymes, true tolerance is observed (Dolby, 1973; See Grand Discussion.).

The fact that other investigators have not observed reverse tolerance in man (Hollister, 1971) may be accounted for by the state of the experienced users prior to the study, the potency of the marihuana preparations used during the study and the primary induction time of the naive users.

CHAPTER II

EFFECT OF INJECTION OF THE CANNABINOIDS ON MAMMALIAN CYCLIC AMP SYSTEMS

Rationale

The process of gathering information concerning the molecular mechanisms of marihuana action is complicated by the numerous naturally occurring cannabinoids present in marihuana, some of which may possess considerable pharmacological activity (See Chapter I.). Furthermore, the interpretation of this data is limited by our state of knowledge of the biochemical and biophysical processes involved in maintaining life. It follows then, that all of the molecular mechanisms of marihuana action in mammals (man) will not be elucidated until sufficient data on basic biological processes has accumulated.

In order to approach the problem of marihuana mechanisms most efficiently, given our current level of "biological competance", one must first find a parameter that has been implicated with many cellular and organismic processes at various levels. Fortunately, cyclic AMP and its metabolizing enzymes have been shown to have some regulatory capacity over glycolysis, gluconeogenesis, lipolysis, lipogenesis, RNA and protein synthesis, regulation of hormone action via the second messenger (cyclic AMP), synaptic transmission and nerve maintenance, overt behavior and hundreds of other processes concerned with the cell and organism homeostatis (Greengard et al., 1972; 1973; Robison et al., 1971; 1971a). Therefore, by using the levels of cylic AMP in various tissues as an index of cannabinoid action, provides a starting point for marihuana action mediated through the cyclic nucleotide regulatory systems.

Introduction

Numerous investigators have demonstrated that both of the tetrahydrocannabinol isomers (Δ^9-THC and Δ^8-THC) and at least one of their metabolites, 11-OH-(Δ^8, Δ^9)THC, are the major compounds responsible for the psychomimetic action of marihuana in mammalian systems (See Chapter I.). Although the molecular mechanism of THC action remains an enigma, evidence has accumulated suggesting that the effects of Cannabis may be mediated at least in part by alterations in biogenic amines in the central nervous system (CNS). Moderate doses of THC or Cannabis extracts in rodents have been reported to produce an elevation in brain levels of serotonin (5-HT) (Bose et al., 1964; Ho et al., 1972; Holtzman et al., 1969; Sofia et al., 1971; Welch et al., 1971) and a depression of norepinephrine (Ho et al., 1972; Holtzman et al., 1971; Schildkraut et al., 1971), 5-hydroxyindole acetic acid and normetaephrine (Ho et al., 1971), although there is much conflicting data concerning these aspects of cannabinoid action (See Biogenic Amines in Chapter I.). In view of the importance of these biogenic amines as neurohumors affecting behavior and mood, it seems reasonable to speculate that these changes in brain amines are in some way involved in the behavioral effects of marihuana.

Some biogenic amines are now though to transmit their effects via an activation of the membrane-associated enzyme adenyl cyclase, resulting in the synthesis of cyclic adenosine 3',5'-monophosphate (cyclic AMP) (Kakiuchi et al., 1968; 1968a; Klainer et al., 1962; Krishna et al., 1970; Shimizu et al., 1970; Weiss et al., 1970) (See Grand Discussion.).

This hypothesis is based mainly on experiments in which brain

slices have been incubated with norepinephrine and were subsequently found to contain increased levels of cyclic AMP, although some cell-free preparations have also been reported where adenyl cyclase activity can be stimulated by catecholamines (von Hungen et al., 1973). Furthermore, in some experimental situations it has been possible to demonstrate that changes in neural discharge rates produced by norepinephrine can be mimicked by direct application of cyclic AMP (Hoffer et al., 1972), thus directly implicating cyclic AMP as a mediator of the effects of biogenic amines on nerve cells. Further support for this conclusion comes from studies which have shown that administration of dibutyryl-cyclic AMP directly into the mammalian brain causes a variety of behavioral changes, including increased locomotor activity, catatonia and convulsions (Gessa et al., 1970; Herman, 1973; Weiner and Olson, 1973).

From these types of data it appears that cyclic AMP may be intimately involved in mediating the effects of brain amines on behavior. In view of the reported changes in both biogenic amines and behavior induced by the administration of active ingredients of marihuana such as Δ^9-THC, it seemed appropriate to attempt to determine whether some of the effects of this drug are also mediated by changes in cyclic AMP.

The following experiments in this chapter will deal with the effect of the cannabinoids after their in vivo administration to mice on cyclic AMP levels in various tissues and on adenyl cyclase and phosphodiesterase activity assayed in vitro from these tissues.

Materials

1-(-)-trans-Δ^9-tetrahydrocannabinol, 11-OH-Δ^9-tetrahydrocannabinol and cannabinol were kindly provided by Dr. M. Braude at the Center for Studies of Narcotic and Drug Absue, National Institute of Mental Health. ^{3}H-Δ^9-THC (109 uC/mg), ^{3}H-cyclic adenosine 3',5'-monophosphate (24.1C/mM) and ^{14}C-ATP (16 mC/mM and 50 mC/mM) were obtained from New England Nuclear Corporation. Pyruvate kinase was purchased from Cal-Biochem Corporation. The following was purchased from Sigma Chemical Corporation: DEAE-cellulose (medium mesh), propranolol, β-estradiol, l-arterinol (norepinephrine), serotonin, unlabeled cyclic AMP, cyclic GMP and King Cobra snake venom. The DEAE-cellulose was cleaned prior to use by standard acid-base treatment procedures. AG1-X2 (200-400 mesh) anion exchange resin (chloride form) was purchased from BIO-RAD Laboratories. Ammonium sulfate (enzyme grade) was purchased from Schwartz/Mann Company. All of the chemicals were of reagent grade and all glassware used was previously acid washed.

The purity of the cannabinoids was periodically tested by thin layer chromatography (TLC) on Silica Gel G with hexane/acetone (3/1) followed by vizualization with 0.2% Fast Blue B salt in dilute sodium hydroxide. Isotopic cannabinoids were also examined for their purity by TLC on Silica Gel G followed by scintillation counting of discrete regions of the developed chromatogram. The purity of both the labeled and unlabeled cannabinoids was always found to have 92% or greater homogeneity by our techniques. The purity of the labeled cyclic nucleotides were examined by descending chromatography on Whatman #40 paper with n-propanol:conc. ammonia:H_2O (6:3:1) and visualized by ultraviolet irradiation followed by scintillation counting. Purity of better than 91% was consistently observed.

Methods

Isolation of Cyclic AMP Dependent Protein Kinase from Beef Muscle

The cyclic AMP dependent protein kinase was isolated from bovine forelimb muscle employing the procedure of Miyamoto et al. (1969). Four pounds of beef forelimb (brachioadialis) muscle was procured from a local slaughterhouse and transported to the laboratory within 40 minutes in ice. Upon arrival, two pounds of muscle at a time was homogenized in 3 volumes (w/v) of 4 mM EDTA (pH=7.0) at 2°C for 2 minutes in a 6 liter capacity Waring blender at full speed. The pooled homogenate was then centrifuged at 27,000g for 30 minutes at 4°C and the resulting precipitate discarded. The pH of the supernatant was then adjusted to 4.8 by the dropwise addition of 1 N acetic acid and stirred for 10 minutes at 2°C. This process allows for the acid precipitation of various unwanted proteins which are then removed by recentrifugation at 27,000g for 30 minutes at 4°C. The resulting supernatant was then adjusted to pH=6.5 with 1 M KH_2PO_4/HPO_4-2 mM EDTA (pH=7.2) while stirring continuously at 2°C. Upon completion of the titration, 32.5 grams of $(NH_4)_2SO_4$ (enzyme grade) was slowly added per 100 ml. of supernatant and stirred for an additional 30 minutes at 2°C. The ammonium sulfate precipitate was collected by centrifugation at 16,000g for 20 minutes at 4°C and the supernatant discarded. The precipitate was redissolved in 6% of the crude volume (240-300 ml.) in 5 mM KH_2PO_4/HPO_4-2 mM EDTA (pH=7.0) and dialysed against 20 volumes of the same buffer for 14 hours at 2°C with two changes of the external dialysis medium. Upon completion of dialysis, the dialysate was centrifuged at 27,000g for 30 minutes at 4°C and the resulting pre-

cipitate discarded. The supernatant was collected and an aliquot was taken for protein analysis by the Lowry method (1951). In order to purify the kinase from the supernatant, the total supernatant (53 ml.) is applied to a 5 x 60 cm. column of DEAE-cellulose previously equilibrated with 5 mM KH_2PO_4/HPO_4-2 mM EDTA (pH=7.0) at 2°C. The column was eluted with a step gradient of 5 mM, 100 mM and finally 300 mM KH_2PO_4/HPO_4-2 mM EDTA (pH=7.0) at 2°C while monitoring the 11 ml. fractions with a miniature ultraviolet spectrophotometer-chart recorder set at 280 nm. In order to determine which fractions have the greatest specific activity for cyclic AMP binding, 200 ul. from every third fraction collected (480 fractions total) was incubated at 2°C for 2 hours with 4.15×10^{-10} moles/ml of ^{3}H-cyclic AMP. After the incubation period, 1.0 ml. of ice cold 20 mM KH_2PO_4/HPO_4-0.01 uM BSA (pH=6.2) was added to the reaction tubes, followed by a brief vortexing, and a 5 minute reequilibration period at 2°C. Each of the assay tubes were then filtered through a multiple membrane filtration apparatus on which the protein-^{3}H-cyclic AMP complex is collected on 0.45 micron pore size nitrocellulose filters. Each of the reaction tubes were washed twice with 2.5 ml. of the phosphate buffer (cold) to ensure complete recovery of the complex. The nitrocellulose filters were then washed twice with 5 ml. portions of the ice cold phosphate buffer, in order to clear any unbound labeled cyclic AMP from the filter. The filters were then dried at 80°C and counted for tritium in toluene scintillation cocktail at 32% efficiency. Tube fractions 300-375 were pooled from peak I and tubes 410-480 were pooled from peak II due to their high specific activity for the labeled cyclic AMP. The kinase preparations (peak I and II) were assayed for protein by the

Lowry method (1951) and stored at -90°C in volumes of 0.5 ml. with the protein concentration of 0.8 ug/ul and 0.46 ug/ul, respectively until required for the Gilman cyclic AMP assay. At this time, the kinase preparation (peak I) was diluted with distilled water to 0.04 ug/ul and 25 ul. of this solution added to the assay, yielding 1 ug total protein per assay tube.

Procedure for Cyclic AMP Binding Protein Homogeniety Determination

Polyacrylamide disc gel electrophoresis was performed according to a modified method of Weber and Osborn (1969). Electrophoresis was carried out in a 7.5% sodium dodecylsulfate-polyacrylamide gel in order to examine the purity of the protein kinase cyclic AMP binding protein from peak I which served as the protein source for the Gilman assays. 10 micrograms of the kinase preparation was applied to the polymerized gels after being mixed with Bromophenol blue marker in 0.13 M Tris-HCl (pH=8.1). The gels were electrophoretically chromatographed by the application of a current of 2.5 ma/gel until the marker reached the bottom of the gel (8-10 hours at 2°C). The gel was then removed and the protein visualized by the application of Coomassie blue staining solution followed by destaining in methanol-acetic acid solution.

Preparation of the Kinase Inhibitor

Appleman et al. (1966) found an inhibitor of kinase phosphorylation present in muscle which had the capacity of enhancing the binding of cyclic AMP to the kinase. The inhibitor was isolated and incorporated into the Gilman assays in order to increase the assays

sensitivity for cyclic AMP detection. Its isolation procedure is as follows.

1 kg of beef forelimb muscle is homogenized in a Waring blender in 10 mM TRIS-HCl buffer (pH=7.5) yielding 2.3 liters of homogenate. The homogenate is then boiled for 10 minutes and then filtered through two layers of cheeze cloth and then through scintered glass funnel. 1260 ml. of a brown fluid is recovered and 1/9 of this volume of 50% trichloroacetic acid is added and stirred for 30 minutes at room temperature. The precipitate is collected by centrifugation at 15,000g for 15 minutes. The precipitate is then dissolved in a small volume of distilled water (pH=7.0; adjusted with 1 N NaOH) and dialysied against distilled water over might at room temperature. The dialysate becomes a brown gel from this process and is centrifuged at 10,000g for 20 minutes yielding 125 ml. of a brown fluid which serves as the source of the inhibitor for the Gilman assay.

Protein Binding Assay for Cyclic AMP

The cyclic AMP dependent protein kinase was isolated from bovine forelimb muscle employing the procedure of Miyamoto et al. (1969) through the DEAE-cellulose chromatography step. Cyclic AMP was assayed according to a modified protein binding method of Gilman (1970). Briefly, a standard curve was generated by incubating various concentrations of unlabeled cyclic AMP (0-500 nM) with 16.6 nM ^{3}H-cyclic AMP, 1 ug protein kinase binding protein (peak I from DEAE-cellulose column), 10 ul of inhibitor as isolated by Appleman et al. (1966) and 50 mM sodium acetate (pH 4.0) in a volume of 0.1 ml for 2 hours at 2-4^{o}C. Concomitantly, unknowns were incubated with all of the preceding com-

ponents with the exception of unlabeled cyclic AMP. After 2 hours, 1 ml. of ice cold 20 mM KH_2PO_4/HPO_4-0.01 uM BSA (pH=6.2) was added to each tube, vortexed and allowed to stand for an additional 5 minutes at 2^oC. The contents of each tube was then filtered through a multiple membrane filtering apparatus with two 2.5 ml. washes of the reaction vessel with phosphate buffer, followed by two 5 ml. washes of buffer passed through the 0.45 micron mitrocellulose filters. The filters were then dried and counted in toluene scintillation cocktail at 32% efficiency. An IBM 360 computer was employed to generate a regression equation by the method of least squares for the standard curve and the unknown samples were solved for picomoles of cyclic AMP per assay tube employing the regression equation. A typical double logarithmic standard curve is presented in Figure 12 for the purposes of clarification.

Assay of Adenyl Cyclase

Adenyl cyclase activity was measured by a modification of the method of Drummond and Duncan (1970). After sacrifice by either decapitation or microwave irradiation, the mouse brains were homogenized in 5 volumes (w/v) of cold 10 mM TRIS-HCl (pH=7.5). The total volume of the assay mixture was 150 ul and contained the following: 40 mM TRIS-HCl (pH=7.5), 8 mM theophylline, 15 mM $MgSO_4$, 20 mM phosphoenolpyruvate, 2 mM cyclic AMP, 5.5 mM KCl, 130 ug/ml pyruvate kinase, 0.4 mM ^{14}C-ATP (16 mC/mM) and 300 ug (protein) of brain homogenate. The reaction was initiated by addition of homogenate and the tubes incubated with agitation for 10 minutes at 37^oC. The reaction was terminated by boiling in a water bath for 4 minutes. The tubes were

centrifuged at 2,000g for 5 minutes and 0.1 ml of the supernatant was spotted on Whatman #40 chromatography paper and developed by descending technique with n-propanol: concentrated ammonia: water (6:3:1) along with standard cyclic AMP. The developed chromatogram was visualized under ultraviolet light and the cyclic AMP regions cut out and counted in Bray's scintillation cocktail at 35% efficiency after standing at room temperature for 12 hours to insure complete solubilization of label.

Assay of Phosphodisterase

Phosphodiesterase was assayed by a modification of the method of Brooker et al. (1968). The entire assay was conducted in a new glass scintillation vial containing 0.15 ml of the following reaction mixture: 8 pmoles ^{3}H-cyclic AMP, 120 mM TRIS-HCl (pH=8.0), 0.67 mg/ml snake venom (King Cobra), 0.67 mg/ml human serum albumin, 5 mM 2-mercaptoethanol, 2.5 mM EGTA (ethylene-bis(oxethylenenitrilo)tetra-acetic acid), 120 mM $MgCl_2$, 0.12 mM 5'-AMP and ∿70 ug of brain homogenate made up in 10 mM TRIS-HCl (pH=8.0). After varying periods of incubation at 37°C, 0.8 ml of 50% settled volume of an aqueous slurry of AG1-X2 200-400 mesh anion exchange resin was added to the vials, which were then reincubated for an additional 10 minutes to absorb the unreacted cyclic AMP. Ten ml of Bray's scintillation cocktail was then added and the free radioactive adenosine representing hydrolyzed cyclic AMP determined by liquid scintillation counting (efficiency=35%).

Injection of Animals and Extraction of Cyclic AMP from Mouse Brain

CF1-Carworth, male 30-45 gram mice were used throughout all the investigations. They were housed 3-5 per cage at 24°C, photoperiod 16 hours on with food and water ad libitum. Mice were randomly chosen, weighed and injected intraperitoneally between 9-12 a.m. with Δ^9-THC solutions made up in 0.2 ml of 0.15 M NaCl - 1% Tween-80. Experimental animals received Δ^9-THC doses in the range of 0.1 to 50 mg/kg, while control animals received injections of vehicle only. Thirty minutes after injection the mice were sacrificed by 30 seconds of microwave irradiation as described by Schmidt et al. (1971; 1972). The animals were decapitated, the heads chilled and the brain dissected into the medulla oblongata, cerebellum, hypothalamus and remainder (labeled cortex) as described in Glowinski et al. (1966).

Each brain region was homogenized in 1-3 ml of 10% trichloroacetic acid (TCA) with 10 strokes of a glass teflon homogenizer at 7,000 rpm. The resulting homogenate was centrifuged at 10,000 g for 20 minutes and the supernatant collected. The homogenate pellet was extracted with 5 volumes of 20% ethanol in ether prior to a modified Koch-Putnam (1971) biuret protein determination using 1:3 proportion of 1 N sodium hydroxide to biuret reagent. Duplicate aliquots of the supernatant from each brain region (o.1-0.3 ml) were extracted 4 times with 20 volumes of ether (saturated with 1.0 N HCl) to remove the TCA prior to the assay of cyclic AMP. Extraction was performed by vortexing for 30 seconds in 12 ml acid-washed conical tubes. The extracted aqueous aliquot was dried in a vacuum desicator overnight and resuspended in 50 mM sodium acetate buffer at the time of the assay of cyclic AMP. Tracer ^{3}H-cyclic AMP added to sample brain regions

and carried through the procedure showed no counts soluble in the ether phases and a cyclic AMP recovery of approximately 90%.

Injection of Animals and Extraction of Cyclic AMP from Mouse Liver

CF-1 Carworth male mice were housed as previously described. They were injected with 0.25 mg/kg of Δ^9-THC (i.p.) in the vehicle as previously prepared and the controls were injected with an equal volume of suspension vehicle. This one acute dose was chosen due to the fact that it produces a marked elevation of cyclic AMP in the mouse brain after 30 minutes and is reasonably physiological. (See Minimum Effective Dose in Chapter I.). Thirty minutes postinjection, the mice were sacrificed in the microwave oven with 30 seconds of exposure. The livers were removed, weighed to 2 grams and homogenized in 1.5 volumes of (w/v) 10% trichloroacetic acid, followed by two washes of the teflon-glass homogenizer with 1 ml. of the homogenizing solution yielding a final volume of 5 ml. containing 2.0 grams of homogenized liver. The homogenate was thoroughly vortexed and centrifuged at 10,000g for 20 minutes. One milliliter of the resulting supernatant was extracted 6 times with 5 volumes (v/v) of acidified water-saturated ether. Each extraction consisted of vortexing the supernatant with the imiscible ether phase for 30 seconds in a 12 ml. acid washed conical tube followed by the removal of the ether phase by aspiration. Twenty-five microliters of the extracted supernatant was assayed for cyclic AMP in duplicate as previously described by the Gilman method. The wet weight of the livers served to correct the cyclic AMP levels to a weight specific basis.

Injection of Animals and the Isolation of Adenyl Cyclase

CF-1 Carworth, 31 gram, male mice were randomly chosen and injected with 0.25 mg/kg, 10 mg/kg of Δ^9-THC, CBN or suspension vehicle as described previously. Thirty minutes later, the mice were decapitated and the whole brains removed and homogenized separately in 5 volumes (w/v) of ice cold 10 mM TRIS-HCl (pH=7.5) with a loose Dounce homogenizer for 10 passes. Ten microliters of this homogenate ($\sim$ 300 ug protein by Lowry method, 1951) served as the adenyl cyclase enzyme source and initiated the adenyl cyclase assay as previously described.

It should be noted that 8 mM theophylline and 2 mM unlabeled cyclic AMP is present in the incubation medium during the adenyl cyclase assay such that we are measuring the synthesis of cyclic AMP and not the steady state levels reflecting the relative activities of both phosphodiesterease and adenyl cyclase. In order to confirm theophylline inhibition and unlabeled cyclic AMP competition of phosphodiesterase for the adenyl cyclase formed labeled cyclic AMP from our brain preparations and under our conditions, 0.01 uC of ^{3}H-cyclic AMP (24.1 C/mM) was added to the reaction mixture (the ^{14}C-ATP was omitted in this case) incubated and chromatographed along with a blank similar to the standard assay.

Finally, to see if the effects of the cannabinoids are mediated through some β-adrenergic mechanism in the CNS, the β blocker, propranolol was also added to certain reaction mixtures.

Chronic Injection of Animals and Assay of Phosphodiesterase

In order to examine the effects of the chronic treatment of Δ^9-THC on the in vitro activity of phosphodiesterase (PDE), one pair of

32 gram CF-1 Carworth male mice were injected daily for 27 days between 9-12 a.m. with 2 mg/kg of Δ^9-THC (i.p.) and concomitantly a similar pair was injected with the vehicle only (vehicle as described previously). On the 28th day, the mice were sacrificed by decapitation and the paired mouse brains pooled and carried through the PDE isolation as described in Chapter III and assayed for activity using the PDE assay as previously described.

Recovery of Injected THC in Mouse Brain

In order to determine the amount of THC which actually reaches the brain after intraperitoneal injection, recovery studies were performed after intraperitoneal injections of a moderately high dose radioactive THC (7.14 mg/kg of ^{3}H-THC, Sp. Act. = 109 uC/mg). Thirty minutes postinjection the animals were sacrificed by exposure to microwave irradiation for 30 seconds, and the brain regions removed and homogenized twice with 5.0 ml. portions of ethyl acetate using a Polytron homogenizer for 20 seconds at maximum speed. The resulting homogenate was centrifuged at 850g for 5 minutes and the ethyl acetate phases pooled and evaporated under nitrogen. The soluble counts were taken up in a small volume of ethanol, spotted on Silica Gel G thin layer chromatography plates along with standards of Δ^9-THC, and developed with hexane/acetone (3/1). The developed plates were cut in 1 cm^2 blocks and counted in toluene scintillation cocktail at 32% efficiency.

Results

Isolation of the Cyclic AMP Binding Protein

The absorbance-^{3}H-cyclic AMP binding profile of the fractions eluted from the DEAE-cellulose chromatography column is given in Figure 11. As can be seen, fractions from 300-375 and 410-480 have the highest specific activity for the binding of cyclic AMP, therefore these fractions peak I (300-375) and peak II (410-480) were pooled separately and thus provide the source for the cyclic AMP assay as described under the Methods.

Purity of the Cyclic AMP Binding Protein

SDS-polyacrylamide gel electrophoretic pattern of the protein kinase fraction (peak I) shows that at least 24 proteins of various molecular weights are present in the fraction. Thus the cyclic AMP binding assay reflects the mean binding of all of the heterogenous cyclic AMP binding proteins present in this fraction.

Protein Binding Assay for Cyclic AMP

The preparation of the cyclic AMP binding protein which we obtained was found to have a dissociation constant of 7×10^{-9} M, and at saturation bound 0.6 pmoles of radioactive cyclic AMP per ug protein (peak I). The standard curves obtained were found to have a standard deviation of less than 8% when unlabeled cyclic AMP concentrations were in the range of 0.8 pmoles to 5 pmoles per 0.1 ml. of assay mixture (See Figure 12; total cyclic AMP/tube - 1.66 pmoles). Standard curves with unlabeled cyclic GMP as competitor for radio-

active cyclic AMP binding showed this nucleotide to be only 1/30 as effective as unlabeled cyclic AMP in competing for 1 ug of the binding protein. Other nucleotides such as cyclic UMP and cyclic CMP were found to compete even less (1/60) than cyclic GMP.

Fixation of Cyclic AMP Levels by Microwave Irradiation

Numerous investigators have reported that when animals are sacrificed by decapitation, endogenous cyclic AMP levels in brain are elevated 2-10 fold within 1-3 minutes (Schmidt et al., 1971; 1972; Gilman, 1970). To prevent such post-morten changes from occurring, we employed microwave irradiation as a technique for sacrificing animals. As can be seen from the data in Table XII, this technique inactivates both adenyl cyclase and phosphodiesterase, thus preventing the dramatic changes in cyclic AMP levels seen after decapitation.

Recovery of ^{3}H-THC in Brain after Intraperitoneal Injection

Since other investigators have reported relatively poor absorption of THC after intraperitoneal injection (Ho et al., 1971), we performed some recovery experiments with radioactive THC in order to determine how much of our injected dose was reaching the brain. Thirty minutes after intraperitoneal injection of 7.14 mg/kg of ^{3}H-THC, 0.07% of the total radioactivity was recoverable in the brain and 0.84% recoverable from the liver. Assuming that 80% of the wet brain and liver weight is water, this converts to a concentration of approximately 4.8×10^{-4} M in the brain and 2.1×10^{-3} M in the liver. These concentrations are higher than the normal range of THC and its metabolites found in the blood and brain of mammals as calculated from

other studies using various routes of administration and employing behavioral tests as criteria for the effective dose (See Plasma-Tissue Concentrations in Chapter I.). Although the absorbed concentration of the cannabinoids is quite high, it does indicate adequate incorporation of this moderately high dose into the body tissues.

Effects of THC on Brain Levels of Cyclic AMP

The effects of injecting various doses of Δ^9-THC on whole brain levels of cyclic AMP are summarized in Figure 13. As can be seen, the predominant effect of Δ^9-THC and its metabolites found in 30 minutes on cyclic AMP levels is an elevation (50-160%) above controls at doses between 0.1 and 1.0 mg/kg, and a depression (30-60%) of cyclic AMP levels at doses between 2.0 and 10 mg/kg. Superimposed on this general trend is a statistically significant variation in cyclic AMP levels in the low dose range which involves a primary stimulation (0.1 mg/kg), then a depression (0.25-0.5 mg/kg), followed by a secondary stimulation (1.0 mg/kg). At the highest dose of Δ^9-THC tested (50 mg/kg), cyclic AMP levels were not significantly altered.

Table XIII summarizes the results of statistical analyses (See Appendix.) performed to determine the significance of the observed trends. As can be seen, the elevations of cyclic AMP levels observed with doses of Δ^9-THC from 0.1 to 1.0 mg/kg, and the depressions of cyclic AMP observed with doses of Δ^9-THC from 2.0 and 10.0 mg/kg are all significant ($P = .025-.0005$). In this context, it should be noted that measured levels of cyclic AMP in control animals varied some 120% in experiments performed at different times on different batches of

animals. Although the source of this variability is unknown (perhaps some daily or circadian rhythm), it emphasizes the need to run a new set of control animals with each new experiment, since only in this way can the effects of the drug treatment be reliably determined. Therefore, each individual experiment was performed with several doses and a control group, and the data normalized to the arithmetic mean of the pooled control groups (See Appendix for calculation procedures.).

In order to determine whether the observed effects of THC administration on whole brain cyclic AMP levels could be localized to any particular brain areas, cyclic AMP assays were performed on dissected regions of the brain. As is shown in Table XIV, the cortex, medulla and cerebellum follow the same general biphasic pattern as that of whole brain, except the stimulation of cyclic AMP by Δ^9-THC becomes less significant at doses of 0.25 to 1.0 mg/kg in these regions when compared to that of whole brain. Nevertheless, there is a persistantly significant elevation of cyclic AMP in these regions at 0.1 mg/kg of Δ^9-THC. The values for hypothalamus did not exhibit a clear pattern, although the statistically most significant points (.25 mg/kg and 2.0 mg/kg) suggest a trend in the opposite direction, namely depression of cyclic AMP followed by elevation.

Effect of Δ^9-THC on Liver Cyclic AMP Levels

Table XV gives the levels of cyclic AMP found in these liver preparations, thirty minutes postinjection of Δ^9-THC. No significant effect is seen, indicating that 0.25 mg/kg of Δ^9-THC and metabolites formed in thirty minutes are incapable of altering the mouse liver cyclic AMP levels while the same dose shows a dramatic elevation of

mouse brain cyclic AMP levels.

Effects of the Injected Cannabinoids on the In Vitro Activity of Adenyl Cyclase from the Mouse Brain

The results of these experiments are presented in Table XVI. As can be seen, 0.25 mg/kg of Δ^9-THC consistently produced a significant ($p=0.03-0.0005$) elevation of adenyl cyclase activity in the mouse brain, 30 minutes postinjection, thus supporting the *in vivo* experiments where an elevation of cyclic AMP levels is observed at low doses. Only in one of the three experiments did 10 mg/kg Δ^9-THC cause a significant depression of adenyl cyclase activity. In one of the three experiments, 0.3 mM propranolol prevented the 0.25 mg/kg Δ^9-THC enhancement of adenyl cyclase activity.

Table XVII shows that CBN was without effect on the *in vitro* activity of adenyl cyclase at the two doses tested and propranolol does not alter the activity of adenyl cyclase in these animals previously injected with CBN.

In regard to the activity of phosphodiesterase during the adenyl cyclase assay, it was found that 8 mM theophylline was an adequate inhibitor of phosphodiesterase only when 2 mM unlabeled cyclic AMP was present as a competitive inhibitor of labeled cyclic AMP turnover. Similar results were seen by Drummond and Duncan (1970) with guinea pig heart muscle adenyl cyclase preparations.

Phosphodiesterase Activity from Mice Chronically Treated with THC

The animals injected chronically for 27 days with 2 mg/kg of Δ^9-THC did not differ significantly from the control animals receiving the injection vehicle only. The enzyme isolated from the drug treated

animals was capable of being inhibited over time by the addition of 48 uM β-estradiol but were not stimulated in the presence of 3.2 mM CaCl (as reported by Miki et al., 1972; Kariuchi et al., 1973). The control PDE activity was capable of regulation by both of these compounds, similar to Figure 15 in Chapter III.

Discussion

In Vivo Effects of the Cannabinoids on Cyclic Nucleotide Synthesis and Steady State Levels in the Mouse Brain

The present results clearly demonstrate that administration of Δ^9-THC exerts an overall biphasic effect on brain levels of cyclic AMP, with doses less than 2.0 mg/kg inducing an elevation of cyclic AMP levels and doses of 2.0 mg/kg and greater causing a depression of cyclic AMP. The existence of a secondary stimulation of cyclic AMP levels at 1.0 mg/kg in the whole brain indicates a complex interaction of Δ^9-THC with the cyclic AMP regulatory systems, and suggests that more than one mode of action may be involved (See Grand Discussion.). Additionally, the *in vitro* activity of adenyl cyclase, 30 minutes after the *in vivo* administration of Δ^9-THC lends further support for the observed trends in the endogenous cyclic AMP levels in the mouse brain. Low doses of Δ^9-THC (0.25 mg/kg) causes a consistently significant elevation of adenyl cyclase activity while high doses (10 mg/kg) generally depresses the adenyl cyclase activity. Although the high doses of Δ^9-THC do not consistently reduce the *in vitro* adenyl cyclase activity from mouse brain homogenates, it should be pointed out that the mouse brains were homogenized in 5 volumes of buffer

prior to assay thus diluting the effective absorbed cannabinoid concentration several fold. By examination of Figure 13, it can be seen that the elevation of brain cyclic AMP at 0.25 mg/kg is 75% greater than that of controls, while at 10 mg/kg, the depression observed in endogenous cyclic AMP is only 60% of that of control with much more variation around the mean. This suggests that the dilution of the cannabinoid concentration following 10 mg/kg Δ^9-THC would have more of an effect on the adenyl cyclase activity than the dilution of the cannabinoids following 0.25 mg/kg Δ^9-THC since 0.1 mg/kg also produces a large elevation in brain cyclic AMP levels.

These general results can be correlated with observations of other investigators on the effects of Δ^9-THC on brain levels of biogenic amines. For example, Holtzman et al. (1969) observed that serotonin levels were increased and norepinephrine levels were decreased by intraperitoneal injections of Δ^9-THC in the dose range of 5-50 mg/kg. Using intravenous injection, Ho and co-workers (1972) also found that relatively high doses of THC cause an elevation of serotonin and depression of norepinephrine levels. However, with smaller doses (less than 2 mg/kg) they observed the opposite effects, namely a decrease in serotonin and an elevation of norepinephrine. Thus the presently observed biphasic effect of Δ^9-THC and its metabolites formed in 30 minutes on cyclic AMP levels is parallel to the effects of Δ^9-THC on brain norepinephrine levels, and is inversely related to THC-induced changes in serotonin levels.

Biphasic effects of THC have also been observed on other parameters. For example, Sofia (1972) has reported a biphasic effect of THC on rectal temperature in rats, with low doses (0.5-1.0 mg/kg) pro-

ducing hyperthermia and high doses (4-8 mg/kg) causing a marked hypothermia. Biphasic effects of THC on behavioral responses have also been reported, with low doses causing excitation and increased response rate, while higher doses produce decreased motility and suppression of response rate (Davis et al., 1972; Ferraro et al., 1971; Grisham et al., 1972; See Biphasic Action in Chapter I.).

At this point is is interesting to note that serotonin containing neurons (Raphe neurons, corpus striatum) are associated with relaxation and slow wave sleep patterns (EEG) (Jouvet, 1969; McGeer, 1971) while NE containing neurons (reticular formation, locus coeruleus) are generally associated with alertness and aroused state of awareness (McGeer, 1971). Thus the present biphasic effects observed with cyclic AMP levels in the CNS, and other investigators' observations on brain biogenic amine levels correlate directly with behavior states and particular brain regions associated with these behavioral states.

The similarity of these reported biphasic effects of THC on biogenic amines, temperature regulation, and behavior with the presently reported observation of a biphasic effect of THC on brain cyclic AMP levels suggests that these findings may be all interrelated. Especially significant is the observed correlation with behavioral responses, since it is changes in behavior induced by THC which are most directly relevant to any discussions of the effects of marihuana in man. These data suggest, then, that the Δ^9-THC induced changes in cyclic AMP may be involved in the behavioral effects of this drug.

There is some direct support for this conclusion available in the studies of Gessa et al. (1970), who demonstrated that micro-

injection of dibutyryl-cyclic AMP into the lateral ventricles or the hypothalamus produces psychomotor stimulation, while injection into the recticular formation causes catatonia. It is interesting to note that these behavioral patterns are similar to those described by several investigators as occurring over time after a high initial dose of THC namely excitation followed by catatonia or depression (Christensen et al., 1971; Holtzman et al., Loewe, 1946; See Biphasic Action and Behavior.). Other indirect evidence suggesting a relationship between psychological state and cyclic AMP can be found in the reports that urinary excretion of cyclic AMP is elevated in manic patients and lowered in patients with severe depression (Abdulla et al., 1970; Paul et al., 1970; See Grand Discussion.).

The present data raise the obvious critical question as to the mechanism via which Δ^9-THC induces an alteration of cyclic AMP levels. One can distinguish at least two general levels at which THC might be acting. One possibility would be an indirect effect resulting from an alteration in biogenic amine metabolism and/or transport. Since biogenic amines are known to stimulate the formation of cyclic AMP (See Grand Discussion.), any changes in biogenic amines caused by Δ^9-THC could in turn affect cyclic AMP levels. In addition to this relatively indirect mode of action, the other general way in which THC might be acting would be directly on the enzymes involved in cyclic AMP formation and breakdown, namely adenyl cyclase and phosphodiesterase.

In regard to the first possibility, namely an indirect mechanism of action, evidence is now available which suggests that Δ^9-THC can inhibit the uptake and turnover of serotonin and norepinephrine and

some of their amino acid precursors in brain particulate fractions (Sofia et al., 1971; Truitt and Anderson, 1971; See Biogenic Amines in Chapter I.). Data is also available which suggests that THC and some of its metabolites can also alter the activity of the biogenic amine synthesizing enzymes (See Biogenic Amines.) and thus their endogenous levels in the brain (See Grand Discussion.).

Although the in vitro activity of adenyl cyclase can be altered by the preadministration of low or high doses of Δ^9-THC, it is not known whether or not the cannabinoids are acting directly on the adenyl cyclase system. The chronic administration of 2 mg/kg of Δ^9-THC for 27 days did not alter the rate of hydrolysis of ^{3}H-cyclic AMP over that of controls, although some degree of allosteric regulation (Ca^{++} stimulation) appears to be lost either due to the cannabinoids effect on phosphodiesterase assembly in vivo, the interaction of its subunits or structural organization of the phosphodiesterase macromolecule during isolation.

Since resolution of the level of cannabinoid induced alterations in cyclic AMP levels may be of major importance for the understanding of marihuana action, experiments along these lines are presented in Chapter III.

CHAPTER III

THE IN VITRO EFFECT OF SEVERAL CANNABINOIDS ON THE ACTIVITY OF ADENYL CYCLASE AND PHOSPHODIESTERASE FROM MOUSE BRAIN

Introduction

Since both in vivo and in vitro studies have shown that Δ^9-THC and its metabolites formed in 30 minutes, but not cannabinol (or its metabolites) can alter adenyl cyclase activity and the levels of brain cyclic AMP when injected intraperitoneally, the following experiments were designed in order to determine the possible sites of action of Δ^9-THC, 11-OH-Δ^9-THC and CBN in the mammalian brain with respect to adenyl cyclase and PDE.

Materials

The materials used for this investigation were as previously described in Chapter II, except ^{14}C-adenine (55.5 mC/mM) was purchased from International Chemical and Nuclear Corporation and its purity by chromatographic techniques was found to be 95%.

Methods

Isolation of Adenyl Cyclase from the Mouse Brain

Animals were housed and fed as previously described. Male, 30 gram CF-1 Carworth mice were decapitated and the whole brain removed and homogenized with a loose Dounce glass homogenizer in 5 volumes (w/v) of ice cold 10 mM TRIS-HCl (pH=7.5) with 10 passes. Ten microliters of this homogenate ($\sim$ 300 ug by Lowry method, 1951) was added to the adenyl cyclase assay as previously described. The assays were

incubated for ten minutes at 37°C with either Δ^9-THC, 11-OH-Δ^9-THC, CBN or ethanol (control) at various concentrations in the presence of absence of 5-HT, NE, β-estradiol, KF or the β blocker, propranolol. The reaction was terminated by boiling the reaction tubes for 3 minutes. One tenth of one milliliter of the supernatant obtained by centrifugation was chromatographed and counted by standard scintillation procedures as previously described.

Preparation of Brain Slices and Incubation Conditions

Thirty-five gram, male CF-1 Carworth mice were housed as previously described. The mice were decapitated, the whole brain dissected free and chopped into 300 micron slices lonitudinally with a McIlwain Tissue Chopper at 2°C, such that all of the gross structures of the brain (cerebrum, cerebellum, hypothalamus and medulla) are represented in each slice. Slices not meeting the criteria were discarded. The brain slices were then added to 3 ml. of cold Krebs-Ringer Phosphate buffer system (pH=7.4) with 5 mM glucose (KRPBS) previously equilibrated with 95% O_2-5% CO_2, along with 256,160 cpm of ^{14}C-adenine. This reaction mixture was incubated for 15 minutes at 37°C in an 95% O_2-5% CO_2 atmosphere in order to ensure the complete incorporation of labeled adenine into the brain slices (empirically determined by saturation analysis). At the end of this preincubation period, various concentrations of Δ^9-THC, 11-OH-Δ^9-THC with or without 5-HT, NE or propranolol were added to the tubes, voretexed and incubated for an additional 20 minutes at 37°C with the O_2-CO_2 atmosphere. Each treatment was run in duplicate. After incubation the reaction vessels were plunged into an ice bath and immediately thereafter centrifuged at

850g at 2°C for 10 minutes. The supernatant was discarded and the pellet resuspended in 2 ml. of ice cold KRPBS and respun at 850g for 10 minutes. After washing out the unincorporated labeled adenine the resulting pellet was homogenized with 0.5 ml of KRPBS with a glass-teflon homogenizer. Fifty microliters of the homogenate was counted in Brays scintillation cocktail at 87% efficiency in order to correct for the pool size of the ^{14}-C adenine and metabolites incorporated into the slices. The remainder of the homogenate was boiled for 3 minutes in a water bath followed by centrifugation at 850g for 10 minutes in order to remove the particulate matter. One hundred microliters of the resulting supernatant is spotted on Whatman #40 chromatography paper. Superimposed on this spot is 10 ul. of a 0.1 M cyclic AMP solution in order to facilitate the ultraviolet visualization of cyclic AMP region after development for 16 hours with n-propanol: concentrated ammonia: water (6:3:1). The cyclic AMP regions of the chromatogram were cut out and chopped into small squares and counted in Brays scintillation fluid at 83% efficiency after sitting 12 hours to allow sufficient solubilization of the label.

In Vitro Assay of Phosphodiesterase in the Presence of Various Compounds

The phosphodiesterase (PDE) assay was performed as previously described except the animals which provided the source of the PDE were treated as follows. In the first experiment, the CF-1 Carworth mice were decapitated and the brains removed and homogenized in 5 volumes (w/v) of 10 mM TRIS-HCl (pH=8.0) at 2°C. The homogenate was centrifuged at 30,000g for 30 minutes in order to remove the particulate matter. The supernatant was collected and brought to 50% saturation

with saturated solution of enzyme grade, ammonium sulphate. The precipitate formed was collected by centrifugation at 30,000g for 10 minutes. The pellet was redissolved in 5 volumes per gram of the original wet weight in 60 mM TRIS-HCl-5 mM 2-mercaptoethanol and dialysed for 14 hours against two changes of 20 volumes of this buffer at 2°C. A Lowry (1951) protein analysis was determined on the PDE enzyme source. This PDE preparation is basically the same as that described by Brooker et al. (1968). The PDE was incubated with 20 nM Δ^9-THC, 11-OH-Δ^9-THC, CBN, ethanol (3.2% final concentration; control), 48 uM β-estradiol, 3.2 mM CaCl for 0, 5, 10, 15, 20 and 30 minutes at 37°C and the reaction terminated at this time as previous described. In the second experiment, identical procedures were followed except 212 uM Δ^9-THC, 11-OH-Δ^9-THC, CBN or control was used along with 48 uM β-estradiol and 3.2 mM CaCl. All of the assays were performed in quadruplicate or quintuplicate for each time point and conditions.

Results

In Vitro Effect of the Cannabinoids on the Activity of Adenyl Cyclase Isolated from the Mouse Brain

The C^{14}-ATP concentration and the ATP generating system exceeds the rate of formation of cyclic AMP by adenyl cyclase for a period of 20 minutes, such that the available ^{14}C-ATP for adenyl cyclase does not become rate limiting during the 10 minutes incubation period. The corrected recovery of labeled cyclic AMP from the total reaction mixtures following descending chromatography is about 85%.

Δ^9-THC when incubated with mouse brain homogenate preparations at various concentrations generally does not affect the converstion

of ^{14}C-ATP to ^{14}C-cyclic AMP under the conditions described (See Table XVII.). A slight elevation of adenyl cyclase activity occurs when Δ^9-THC is 2 uM in concentration. Although this effect is not highly significant (P=0.03), it may suggest a specific cannabinoid interaction with adenyl cyclase and its postulated allosteric regulatory receptors or perhaps an alteration in the neurolemma structural configuration such that adenyl cyclase is enhanced only with uM concentration ranges.

11-OH-Δ^9-THC does not seem to affect adenyl cyclase activity in any consistent manner. The variation in enzyme activity by this drug is quite large, especially at the 20 uM, however no statistically significant alteration of adenyl cyclase activity from control levels is observed (See Table XVII.). Furthermore, CBN at low endogenous concentrations (20 nM) seems to enhance the adenyl cyclase activity but is ineffective at higher concentrations (See Table XVII.).

Interactions between the Biogenic Amines and the Cannabinoids on Adenyl Cyclase Activity from Mouse Brain Homogenates

The results of these experiments are presented in Table XVIII. One uM serotonin was without effect on the activity of adenyl cyclase from these mouse brain homogenates, suggesting that either a disruption of the structural relationships between the regulatory protein associated with adenyl cyclase or that the serotonin concentrations used was too low to elicit an alteration of adenyl cyclase activity. Ten mM KF was found to stimulate adenyl cyclase from the mouse brain preparation, indicating that at least the catalytic unit for this enzyme complex is viable. Two hundred twelve uM of Δ^9-THC, 11-OH-Δ^9-THC and CBN did not alter the 5-HT insensibility of adenyl cyclase preparation.

On the other hand, NE produced a marked enhancement of adenyl cyclase activity from the same mouse brain preparation, indicating that the structural relationships of the adenyl cyclase complex are still in tact and capable of response. This raises the hypothesis that either the 5-HT binding subunit of the adenyl cyclase complex is lost or altered by homogenization or requires higher concentrations of serotonin to elicit a response (See Discussion.).

The interactions between the cannabinoids and norepinephrine induced enhancement of adenyl cyclase are very complex as can be seen from Table XVIII. It appears that 11-OH-Δ^9-THC and CBN at high concentrations are antagonistic to Δ^9-THC when NE is mediating the activity of the adenyl cyclase. Twenty nM THC in the presence of 1 uM NE does not alter the NE stimulation (P=0.40) while higher doses (2 uM and 212 uM Δ^9-THC) significantly potentiate the NE induced enhancement of adenyl cyclase (P=0.03,0.0005). On the other hand, 20 nM 11-OH-Δ^9-THC and 20 nM as well as 212 uM CBN, in the presence of 1 uM NE, also enhance the NE induced response (P=0.0005) but as 11-OH-Δ^9-THC is increased in concentration by 100 fold (2 uM) it does not alter the NE induced enhancement of adenyl cyclase (P=0.15). When both CBN and 11-OH-Δ^9-THC concentrations are very high (212 uM) the NE potentiated stimulation of adenyl cyclase is depressed significantly (P=0.0005).

Finally, β-estradiol did not significantly alter the NE induced stimulation of adenyl cyclase or the basal activity of adenyl cyclase (See Table XVIII.) implying specificity as to the cannabinoid response.

Effect of the Cannabinoids on ^{14}C-Adenine Incorporation into ^{14}C-Cyclic AMP in Mouse Brain Slices

The results are presented in Tables XIX. It appears that when NE or 5-HT concentrations are very low (166 nM), the cannabinoids are relatively without effect on adenyl cyclase activity, as was observed in previous experiments when no exogenous biogenic amines were added to the adenyl cyclase assays with mouse brain homogenates (See Table XVII.). However when, the biogenic amine levels (NE and 5-HT) are raised to 166 uM, both displayed a marked enhancement of ^{14}C-cyclic AMP formation from the labeled ATP pool (P=0.025,0.015; See Table XIX.). High concentrations of Δ^9-THC (318 uM) alone did not effect the formation of cyclic AMP as compared to control (P=0.15; See Table XIX.). This confirms previous experiments with the in vitro adenyl cyclase assays with mouse brain homogenates in the presence of cannabinoids alone. Three hundred eighteen uM Δ^9-THC in the presence of 166 uM NE showed no significant effect on the NE potentiation of adenyl cyclase activity (See Table XIX.). Although at first, this seems contradictory to the previous experiments with NE induced adenyl cyclase activity when Δ^9-THC was at 212 uM and a significant enhancement of adenyl cyclase activity was seen (See Table XVIII.). The Δ^9-THC concentration in the present experiment was 318 uM and the NE concentration was 166 times more concentrated than previously employed. Also the trend of the adenyl cyclase assays in the presence of NE when Δ^9-THC is approaching high concentrations is that toward control levels (See Figure 14.).

It is interesting to note that 166 uM 5-HT was capable of enhancing adenyl cyclase activity in these brain slices but was not capable of eliciting this response in mouse brain homogenates. This may

be related to either the larger concentrations of serotonin used in these experiments or perhaps the fact that brain slices retain more structural integrity than homogenates, thus leaving the receptor intact. Other investigators have noted the poor ability of 5-HT to stimulate adenyl cyclase in various homogenates and brain preparations (Rall, 1971; Robison et al., 1970).

Finally, high concentrations of Δ^9-THC is capable of enhancing sertonin stimulated adenyl cyclase activity in these brain slices implying a similar mechanism at work with the serotonin system as with the NE system (See Table XIX.).

It should be noted that PDE was not inhibited by theophylline or unlabeled cyclic AMP competitor in these studies, implying that PDE even in the uninhibited state is not capable of counteracting the interactions of the cannabinoid and the biogenic amines producing higher levels of cyclic AMP. This supports the in vivo observations in Chapter II.

In Vitro Effect of the Cannabinoids on PDE Activity from the Mouse Brain

As can be seen from Figure 15, β-estradiol inhibited PDE activity while CaCl stimulated its activity. None of the cannabinoids at either high (212 uM) or low (20 nM) concentrations altered the PDE activity from that of controls (See Figure 16.). Additionally, the animals injected chronically for 27 days with 2 mg/kg of Δ^9-THC did not differ significantly from the controls receiving the injection vehicle only (See Chapter II.). The only significant difference among the three experiments is that CaCl was incapable of stimulating the PDE activity from those mice chronically treated with THC, although

β-estradiol was equally as effective as previously stated.

Discussion

In summary then, low concentrations (nanomolar) of the cannabinoids in the absence of exogenous NE and perhaps 5-HT may slightly enhance adenyl cyclase activity, although this effect is variable and not highly significant with Δ^9-THC and 11-OH-Δ^9-THC.

Interestingly, Christensen et al. (1971) found that when CBN was injected intracranially into mice, that its activity nearly paralleled that of Δ^8 and Δ^9-THC, while an intraperitoneal or intravenous injection was without effect on the mouse behavior. When CBN was incubated with brain homogenates at low concentrations (nanomolar with no exogenously added biogenic amines) a significant enhancement of adenyl cyclase activity was seen (P=0.023), while our *in vivo* experiments with the intraperitoneal injections of CBN followed by the assay of adeny cyclase *in vitro* showed no significant effect, thus confirming Christensen and co-workers observations at the molecular level.

This data presented in Chapter III gives rise to a very interesting hypothesis of cannabinoid action in the CNS which can ultimately be related to overt behavior on the experimental animal (See Grand Discussion.).

It has been well established that the lung and liver both have a high capacity for the metabolism of Δ^9-THC to 11-OH-Δ^9-THC while, the brain is very poorly developed in this aspect (Christensen et al., 1971; Dolby, 1973; See Metabolism.). Therefore, given that Δ^9-THC is converted with time to 11-OH-Δ^9-THC which then redistributes it-

self to the major metabolizing organs throughout the rest of the body, displacing Δ^9-THC. The following hypothesis is present to explain the biphasic effect of the cannabinoid on overt behavior.

It has been established that low doses of Δ^9-THC when administered to a variety of animals (man also) causes a hyperexcitable state and perhaps an enhanced motor activity while high doses of Δ^9-THC produce a short hyperexcitable state followed by a depression and catatonia (See Behavior and Grand Discussion.).

As can be seen from Table XVIII, low doses of Δ^9-THC does not alter NE stimulation of adenyl cyclase. However as it is metabolized to 11-OH-Δ^9-THC by lung and liver, low concentrations of 11-OH-Δ^9-THC potentiate the NE stimulation of adenyl cyclase giving rise to higher levels of cyclic AMP which have been implicated with hyperexcitable states (See Grand Discussion and Chapter II.).

Since a small dose of Δ^9-THC was administered to the animal, 11-OH-Δ^9-THC concentrations will never exceed but very low levels and will subsequently be further metabolized and eliminated yielding only a hyperexcitable state as seen in overt behavior.

A high initial dose of Δ^9-THC itself is stimulating to NE potentiation of adenyl cyclase and the turnover of THC to 11-OH-Δ^9-THC in the early phase (i.e. when 11-OH-Δ^9-THC is in low concentrations) is also stimulating on NE induced adenyl cyclase activity, thus producing a hyperexcitable state in the animal perhaps by alterations in neural discharge rates in certain neuronal pathways (See Grand Discussion.). As metabolism of Δ^9-THC to 11-OH-Δ^9-THC continues, Δ^9-THC is displaced from brain, redistributed and thus terminating its effect on NE potentiation of adenyl cyclase (i.e. 20 nM Δ^9-THC is

without effect). As the THC is made available for metabolism, the 11-OH-Δ^9-THC levels are elevated and thus the brain concentration increases over time producing a reduced enhancement, followed by no effect and finally a depression of NE induced adenyl cyclase potentiation. This depression of adenyl cyclase activity lowers the endogenous cyclic AMP levels which have been correlated with depression of behavior and catatonia (See Grand Discussion.).

GRAND DISCUSSION

The purpose of this discussion is to interrelate the biochemical and behavior observations in a variety of animals produced by the cannabinoids and possible implications with the cyclic adenosine monophosphate metabolizing system. It is hoped that these discussions will generate further research interest on the actions of the cannabinoids and cyclic AMP systems.

Relationships among Cyclic AMP, Nerve Function and Biogenic Amine Action

Since the discovery that adenyl cyclase (AC) and phosphodiesterase (PDE) have very high specific activities in the mammalian central nervous system and can be localized by fractional centrifugation techniuqes mainly with the synaptosomal regions (DeRobertis et al., 1967; Robison et al., 1970; von Hungen et al., 1973; Florendo et al., 1971), the hypothesis that adenyl cyclase complex may be involved in synaptic transmission has emerged.

Although the evidence is quite conflicting, the following compounds are considered to be possible neurotransmitters in the mammalian central nervous system and in some cases the peripheral nervous system: dihydroxyphenylethylamine (dopamine), dihydroxyphenylalanine (dopa), norepinephrine (NE), epinephrine (E), serotonin (5-HT), γ-aminobutyric acid (GABA), γ-amino-β-hydroxybutyric acid (GABOB), glycine, glutamic acid, aspartic acid, histamine, prostaglandins, substance P, acetylcholine (ACH), ergothioniene, cyclic AMP, γ-aminobutyrocholine, γ-aminobutyrylhistidine, γ-butyrobetaine, γ-guanidinobutyric acid, acetylcarnitine and perhaps many of their metabolites (Cooper et al.,

1970; Fernstrom et al., 1974).

Of these listed compounds, only dopamine (Brown and Makman, 1972; von Hungen et al., 1973; Kebabian et al., 1972; Rall, 1971; Jard et al., 1972), NE, E, histamine, ACH, glutamic acid, GABA and serotonin (Kakuchi et al., 1968; 1968a; 1969; Shimizu et al., 1970; Rall and Gilman, 1971; Rall, 1971; Chasin et al., 1971; 1973; von Hungen et al., 1973; Perkins, 1970; Robison et al., 1970; Cramer et al., 1973; Weiss et al., 1968) have been shown to generally enhance the formation of cyclic AMP in nervous tissue homogenates, brain ganglia cultures and in some cases, brain slices. Additionally, depolarizing agents (ouabain, batrachotoxin etc.), various electrolytes (K^+, Mg^{++}, NH_4^+), methylxanthines (caffine, theophylline), adenosine and some of its metabolites and electrical stimulation of brain slices have been shown to effect the activity of adenyl cyclase or phosphodiesterase and thus alter the levels of cyclic AMP in these brain preparations (Shimizu et al., 1970; Sattin and Rall, 1970; Rall and Gilman, 1970; Rall, 1971; Kakiuchi et al., 1969).

From these and other data, it can be inferred that cyclic AMP metabolizing system appears to be intimately involved with the mediation of the action of the so named putative neurotransmitters, at least in the CNS. Direct support for this concept comes from studies of Hoffer and co-workers (1972), who have presented evidence that indicates that cyclic AMP is capable of mimicking, thus potentially mediating the NE induced hyperpolarization and decreased discharge rate of the cerebellular located Purkinje neurons. Additional support for cyclic AMP's direct role in the mediation of dopaminergic synaptic transmission in the superior cervical sympathetic ganglia

has also been found (Kebabian et al., 1971; McAfee et al., 1971; Greengard et al., 1972a; Libet, 1970; Libet et al., 1971).

The adenyl cyclase complex in the brain is believed to be an aggregate of the adenyl cyclase catalytic unit, several phospholipids (perhaps prostaglandins), Ca^{++}, Mg^{++} and other cations and several regulatory lipo and/or glycoproteins which reside in the synaptic membrane regions of neurons and glial cell interfaces (von Hungen et al., 1973; Rethy et al., 1971; Robison et al., 1971; Smythies, 1972; Levey, 1971). These regulatory proteins are of prime interest when discussing the action of the biogenic amines, which are believed to transmit their effects to adenyl cyclase catalytic unit via allosteric interactions with their respective regulatory receptor proteins (von Hungen et al., 1973; Kebabkian et al., 1972; Robison et al., 1971).

These regulatory receptors of the adenyl cyclase complex have been roughly classed into α and β-adrenergic receptors, based on their ability to alter adenyl cyclase activity in the presence of various biogenic amines. Thus if a particular biogenic amine can alter adenyl cyclase activity and its effects can be blocked by the addition of propanolol or a similar acting compound, then the receptor is classed as β-adrenergic. On the other hand, if a particular biogenic amines' action on adenyl cyclase can be blocked by the addition of phentolamine or related compound, the receptor is classed as α-adrenergic receptor. Generally, those biogenic amines which can bind to the β-receptors of the adenyl cyclase complex, enhance or stimulate the formation of cyclic AMP, while α-adrenergic receptor compounds, decrease adenyl cyclase activity (Robison et al., 1971; Perkin, 1973; Gilman and Minna,

1973). There are many exceptions and complicating factors surrounding the α-β receptor theory, however for our purposes the general description will suffice. Although the data is contradictory (Chasin et al., 1971; Correspondant, 1974), it may generally be stated that NE and perhaps 5-HT transmit their effects to adenyl cyclase catalytic unit via interactions with the β-adrenergic receptors, while those of dopamine may at least in part be transmitted by the α-adrenergic receptors in certain neuron types (ganglia) (Kebabian et al., 1972; McAfee et al., 1972; Deguchi, 1973).

Given that the biogenic amines are involved with synaptic transmission and their effects may possibly be mediated through the adenyl cyclase complex, then where do the cannabinoids manifest their action during this process. In order to communicate this hypothesis, a general model for synaptic transmission, based on information from sympathetic nerve studies and axon-dendritic investigations in the central and peripheral nervous systems, is presented first.

In the absence of the cannabinoids, the neurotransmitter secretion-excitation process between two hypothetical neurons in the CNS may proceed in the following manner. An action potential generated in the primary neuron, upon reaching its axon terminus will alter the intracellular levels of Ca^{++} either by liberating it from the synaptic mitochondrial pool or by enhancing the neurolemmas permeability to Ca^{++} (perhaps by an adenyl cyclase mechanism controlling K^{+} fluxes; de Belleroche and Bradford, 1972) at specialized junction between the glial cells and the neurons (Rasmussen, 1970; Rubin, 1970; Katz et al., 1969; Wooten et al., 1973). The rise in intracellular Ca^{++} then couples the migration of the synaptic vesicles (containing the neuro-

transmitter) perhaps with a contractile protein found in the axons, neurostenin (neurin is similar to actin and stenin is similar to myosin of muscle). It has been established that stenin is localized mainly with the synaptic vesicles membranes and neurin is mainly associated with the plasma membranes (Berl et al., 1973). Thus Ca^{++} would provide the ionic link between these two proteins in a similar manner as the interaction of Ca^{++} with the skeletal muscle contractile proteins. Therefore, upon binding of Ca^{++} between the brain contractile proteins, the vesicle is brought in contact with the presynaptic membrane and then liberates its contained neurotransmitter in a quantum fashion into the synaptic cleft (Kirpekar et al., 1967, sympathetic nerves). The fate of the neurotransmitter at this point is variable, some may be absorbed (active transport) by the adjacent glial cells, some to be inactivated by the synaptic cleft localized enzyme, catechol-O-methyltransferase, some to be actively transported into the presynaptic neuron and reabsorbed into the vesicles or metabolized by the mitochondrial associated, monoamine oxidase and some diffusing across the synaptic cleft to binding with the β-receptor (in the case of NE). Upon binding of the neurotransmitter (NE) to the β-receptors, an allosterically induced change in the conformation of the β-receptor-adenyl cyclase catalytic phospholipid complex is transmitted such that the synthesis of cyclic AMP from intracellular ATP is initiated or enhanced. The resultant rise in the postsynaptic cellular cyclic AMP eventually reached the K_{eq} of a particular protein kinase(s) (Johnson et al., 1971a) which is then activated (or inhibited; Kish, 1973) due to the release of its catalytic unit from negative regulation. The activated kinase may now phosphorylate

various membrane localized proteins (Maeno et al., 1971; Weller and Rodnight, 1971; Heald, 1961) which selectively open channels Na^+ and K^+ (perhaps other ions, Cl^-) conductance across the membrane thus eliciting an action potential (or hyperpolarization, depending on the neuron) in the postsynaptic neuron. A postsynaptic intracellular phosphatase could then cleave the phosphates from the "channel protein" and thus orderly terminate the Na^+ and K^+ conductances, respectively and restore the protein to its former state.

Now that we have established a hypothetical model of excitatory (as opposed to inhibitory) synaptic transmission based on the available data (Van der Kloot and Kita, 1974; Marx, 1972), we can now speculate on the sites of action of the cannabinoids during this process.

As previously stated, low doses of marihuana or THC produce hyperexcitable and increased motor activity in a variety of animals, while high doses of marihuana or THC produce an initial hyperexcitable state, followed by a depression and perhaps catalepsy (See Chapter I - Biphasic Effects on Behavior.). This behavior was correlated with the levels of cyclic AMP excreted in the urine of manic patients (high cyclic AMP) and depressed patients (low cyclic AMP; Paul et al., 1970, Adulla et al., 1970) as well as more directly related experiments involving the microinjection of dibutyrl-cylic AMP into various regions of the cat and rat brain which produced an aroused hyperstate, depression or catatonia depending on the region of injection (Gessa et al., 1970; Herman, 1973; See Cyclic AMP and Behavior; Grand Discussion.). Thus we established some correlation between the elevations of brain cyclic AMP and hyperexcitable

state of behavior as well as depression of brain cyclic AMP and a behavioral depression and catatonia. These evidences set the stage for the proposed model of cannabinoid action in the central nervous system.

The incubation of low concentrations (nanomolar) of Δ^9-THC in the presence of NE was shown not to alter the response of adenyl cyclase to NE alone, while low concentrations (nanomolar) of 11-OH-Δ^9-THC was found to enhance the effect of NE. Therefore after the administration of low doses of Δ^9-THC (or marihuana) and its incorporation into the brain, Δ^9-THC itself does not potentiate synaptic transmission coupling with adenyl cyclase, however as the THC is metabolized by the lung and liver to 11-OH-Δ^9-THC, the 11-OH-Δ^9-THC levels are being elevated with time, and thus displacing the THC in the brain, it begins to enhance the NE potentiation of adenyl cyclase (M concentrations) at the level of the synapse and thus raise the postsynaptic intracellular cyclic AMP levels perhaps causing alterations in nerve discharge rate, which then produces an overall hyperexcitable state of behavior depending on the neurons and brain regions affected. (This could possibly explain why low doses of 11-OH-Δ^9-THC seem to have a more rapid onset of action than low doses of Δ^9-THC. Perez-Reyes et al., 1973). As the Δ^9-THC and 11-OH-Δ^9-THC are further metabolized, their endogenous concentrations begin to fall below that of the level required for activity and the hyperexcitable state passes.

Upon the administration of high doses of Δ^9-THC (or marihuana) and its incorporation into the brain, the immediate effect of the Δ^9-THC would be an elevation of postsynaptic cyclic AMP in those neurons whose synapses are mediated by NE and perhaps 5-HT via the same or

similar process described previously. This is due to the fact that moderate doses or levels of Δ^9-THC enhances NE potentiation of adenyl cyclase (See Table XVIII.). Additionally, the initial metabolism of Δ^9-THC to 11-OH-Δ^9-THC would also potentiate the high concentrations of Δ^9-THC's effect on NE induced adenyl cyclase activity such that the hyperexcitable state could even be more pronounced. As more Δ^9-THC is displaced and metabolized, the Δ^9-THC concentration would drop to levels which produce no effect on the NE-adenyl cyclase coupling, while the now high concentrations of 11-OH-Δ^9-THC would either begin to depress the release of NE from the presynatpitc nerve ending, alter the interactions of NE with the β-receptors or even begin to interact with the α-receptors, resulting in the decreased formation of cyclic AMP postsynaptically (See Table XVIII.). As the levels of cyclic AMP fall, a change in the neural discharge rate may occur, causing the depression and cataleptic state observed during the latter phases of intoxication after the administration of high or moderate doses of Δ^9-THC or marihuana, depending on the particular neuronal tracts affected.

As previously pointed out, CBN does not alter adenyl cyclase activity when injected intraperitoneally (only in <u>in vitro</u> brain homogenates). This confirms Christensen and others (1971) observations, that CBN appears functionally inactive in mice when injected systemically, while intracranial injections produce activity similar to Δ^9-THC. Based on this data, the effects of CBN when injected intraperitoneally can be ignored, at least in terms of the cyclic AMP metabolizing systems. Should additional data emerge demonstrating that CBN when administered by smoking (vis. human subjects) does produce alterations in

brain cyclic AMP levels similar to that of Table XVII, then its effects can be integrated into the hypothesis as follows.

Since CBN is often in considerable concentrations in marihuana (See Chapter I.), small doses of marihuana would deliver small quanitities of both Δ^9-THC and CBN to the subject. This would produce an overall hyperexcitable state, since low levels of CBN (nanomolar) in in vitro assays stimulated adenyl cyclase directly (See Table XVII). Only with high doses of marihuana would CBN (212 uM; See Table XVII.) be antagonistic to the action of Δ^9-THC. With high doses of marihuana, CBN would antagonize the initial Δ^9-THC potentiation of NE induced adenyl cyclase activity, however this effect could soon be counteracted by 11-OH-Δ^9-THC which in low concentrations would also potentiate the NE induced activity of adenyl cyclase. Thus CBN in high concentrations, could compete with Δ^9-THC when at moderate concentrations (micromolar) and 11-OH-Δ^9-THC when at low concentrations under in vitro conditions on the adenyl cyclase system.

Therefore, the action of Δ^9-THC and its metabolite, 11-OH-Δ^9-THC, on general overt behavior can be correlated with the mouse brain levels of cyclic AMP perhaps as a result of alterations in the biogenic amine release, transport or binding to various synaptic localized proteins. The model presented gives only one of the possible mechanisms for the interaction of the cannabinoids with biogenic amine function in the CNS based on the observed data. Cannabinoid induced alterations in biogenic amine precursor uptake, synthesis, degradition, transport, packaging into synaptic vesicles, interactions with the regulatory proteins associated with adenyl cyclase and a host of other actions may well be occurring to various degrees depending on the animals physiol-

ogical and behavioral state.

Finally, if the cannabinoids potentiate or depress synaptic transmission coupling in the CNS, their brain steady state levels or synthesis need not be affected to mimic the "syndrome(s)" of excessive adrenergic activity. Additionally, the dosage of the cannabinoids, their absorbtion rate and most of the aforementioned factors contributing to the variability in response of animals to these drugs could alter the time relations and perhaps even the behavioral sequence of this proposed model. Thus, the reported variation in physiological and behavioral response to the cannabinoids seems more reasonable since the levels of cyclic AMP and its subsequent biochemical actions are fluxuating from moment to moment. The cannabinoids could "catch" the cyclic AMP regulatory system in nearly an infinite number of states.

Cyclic AMP and Biogenic Amine Synthesis

Several studies have demonstrated an induction of pineal serotonin N-acetyltransferase (synthesis of melatonin) to be associated with catecholamine induced elevations in pineal cyclic AMP levels, which is capable of being blocked by propranolol (Deguchi, 1973; Klein et al., 1970). Additionally, the regulation of tyrosine hydroxylase (rate limiting enzyme in the pathway for catecholamine synthesis) activity in cultured mouse neuroblast cells has been shown to be directly related to the cyclic AMP levels within these cells (Waymire et al., 1972; Prasad et al., 1972; Richelson, 1973). Similar results were obtained during earlier studies with adrenal tissue (Kvetnansky et al ., 1971) and later by Guidotti and Costa (1973). Goldstein and others (1973) found that dibutyryl-cyclic AMP produced a dose dependent el-

evation in ^{14}C-dopamine synthesis from ^{14}C-tyrosine in rat brain slices. They also found an enhanced uptake of tyrosine in the presence of dibutyryl-cyclic AMP into these brain slices. Finally, Forn et al. (1972), Klein et al. (1970), Shein et al. (1969), Tagliamonte and others (1970; 1971) found that dibutyryl-cyclic AMP or cyclic AMP stimulated sertonin synthesis and tryptophan transport into rat brain slices.

Thus it appears from this evidence, that the alteration in cyclic AMP levels induced by the cannabinoids (our data) may have a direct effect on the induction or increased activity of the biogenic amine synthesizing enzymes in the CNS as well as the transport of their substrates into the neurons. This may alter the steady state levels of the biogenic amines in the CNS and thus generally increase the appearant adrenergic activity between adjacent neurons and cause the elevation of postsynaptic cyclic AMP levels. Interestingly, many investigators have reported an increase in several biogenic amine levels, their synthesizing enzymes as well as the alteration of the biogenic amines' precursor uptake in the CNS, after the administration of the cannabinoids (See Biogenic Amines in Chapter I.). It is tempting to speculate that these cannabinoid effects on the biogenic amine systems in the mammalian brain are in fact mediated via a cyclic AMP mechanism under certain conditions.

Finally, the cyclic AMP linked induction of liver serine dehydratase, tyrosine aminotransferase (in vivo) and in cultured hepatoma cells (Stellwagen, 1972; Jolicoeur et al., 1971; Jost et al., 1972) and the cyclic AMP inhibitory effect on liver drug metabolizing enzyme activity (Weiner et al., 1972; 1972a) provide further evidence of the intimate

evation in ^{14}C-dopamine synthesis from ^{14}C-tyrosine in rat brain slices. They also found an enhanced uptake of tyrosine in the presence of dibutyryl-cyclic AMP into these brain slices. Finally, Forn et al. (1972), Klein et al. (1970), Shein et al. (1969), Tagliamonte and others (1970; 1971) found that dibutyryl-cyclic AMP or cyclic AMP stimulated sertonin synthesis and tryptophan transport into rat brain slices.

Thus it appears from this evidence, that the alteration in cyclic AMP levels induced by the cannabinoids (our data) may have a direct effect on the induction or increased activity of the biogenic amine synthesizing enzymes in the CNS as well as the transport of their substrates into the neurons. This may alter the steady state levels of the biogenic amines in the CNS and thus generally increase the appearant adrenergic activity between adjacent neurons and cause the elevation of postsynaptic cyclic AMP levels. Interestingly, many investigators have reported an increase in several biogenic amine levels, their synthesizing enzymes as well as the alteration of the biogenic amines' precursor uptake in the CNS, after the administration of the cannabinoids (See Biogenic Amines in Chapter I.). It is tempting to speculate that these cannabinoid effects on the biogenic amine systems in the mammalian brain are in fact mediated via a cyclic AMP mechanism under certain conditions.

Finally, the cyclic AMP linked induction of liver serine dehydratase, tyrosine aminotransferase (in vivo) and in cultured hepatoma cells (Stellwagen, 1972; Jolicoeur et al., 1971; Jost et al., 1972) and the cyclic AMP inhibitory effect on liver drug metabolizing enzyme activity (Weiner et al., 1972; 1972a) provide further evidence of the intimate

role of cyclic AMP and liver enzyme function. This may have important ramifications in cannabinoid action and metabolism.

Cyclic AMP and Behavior

Weiner and Olson (1973), Herman (1973), Breckenridge (1970) and Gessa and coworkers (1970) have found that the intraventricular, (intraperitoneal) or microinjection of dibutyryl-cyclic AMP into various regions of the mammalian brain produces marked alterations in animal behavior including hyperactivity, hyperthermia, depression and catalepsy depending on the dosage and region of application.

Interestingly, many investigators have observed a correlation between doses of the active cannabinoids and changes in overt behavior (See Behavior in Chapter I.). Low doses of marihuana or THC generally cause hyperexcitability and a general increase in locomotor activity as well as an elevation in whole brain cyclic AMP levels and adenyl cyclase activity. High doses of marihuana or THC produce an initial hyperactive state followed by a depression of activity and catatonia, which correlates with our data of a decreased adenyl cyclase activity and lower levels of cyclic AMP in the mouse brain. Paul et al. (1971) have observed a correlation between the shift of mental patients from the depression phase (depressed mood, seclusiveness, non-verbal) to the manic phase (increased locomotor activity, verbosity) and a 2-3 fold elevation in the excretion of urinary cyclic AMP. Worthy or mention is that cyclic AMP did not remain elevated during the entire manic period but only on the switch day. Bunney et al. (1965) has also correlated an elevation of NE excretion on the day prior to the shift from depression to manic states, however the increased NE excretion

continued throughout the entire manic episode. Mania and general increased motor activity is associated with an excess of brain biogenic amines at the synapses (Schildkraut et al., 1965). Along these lines, when mice are treated with L-dopalanine and other monoamine oxidase inhibitors, there is an elevation in brain cyclic AMP levels due to the inhibition of biogenic amine degradation. The behavioral patterns of the mice under the influence of these inhibitors are hyperactivity and agressiveness (Paul et al., 1971). Depression is usually correlated with low levels of brain biogenic amines (Schildkraut et al., 1965).

Thus alterations of brain cyclic AMP levels may serve as a trigger event, since Paul et al. (1971) as well as more direct biochemical data have shown that the response of the cyclic AMP generating system to a hormonal or other stimulus is usually transitory, while the subsequent actions initiated by the alterations in intracellular cyclic AMP levels may last several hours or days (Haynes et al., 1959; Ho and Sutherland, 1971; Robison et al., 1970; Farese et al., 1969).

Finally, Phillips et al. (1971a) found that low doses of Δ^9-THC potentiated caffeines' stimulant effect on mouse behavior, while high doses of Δ^9-THC reduced the stimulant effect of caffeine. This provides further support for the observed biphasic effects of Δ^9-THC and its metabolites formed in thirty minutes on overt behavior and its involment with cyclic AMP systems.

Additional correlations between the cannabinoids and other forms of overt behavior mediated through the cyclic AMP system can be seen from Booth (1972). The implantation of a 500 ug capsule of dibutyryl-cyclic AMP into the mammillary body of the hypothalamus produces hyperphagia in immature rats and 22.5- 25 ug of dibutyryl-cyclic AMP injec-

ted into the hypothalamus of mature rats can elicit the same increase in food intake (Booth, 1972; Gessa et al., 1970). Additional experiments by Booth (1972) show that dibutyryl-cyclic AMP injections into the hypothalamus can cause either an increased food intake or a decreased food intake in rats depending on the dosage and site of injection.

From this data it may be infered that the increased food intake observed in humans and other animals during intoxication with marihuana (Abel, 1971a; See Cannabinoid and Nutrition in Chapter I).) may be mediated via elevations in hypothalamic cyclic AMP levels. This would account for the seemingly paradoxical observation that the cannabinoids do not alter blood glucose levels but do cause hyperphagia in some subjects. Additionally, cyclic AMP does not appear to regulalate glycogen metabolism in the brain as in the liver (Kakiuchi and Rall, 1968; Goldberg et al., 1969; Robison et al., 1970), so the cannabinoids are probably not altering brain glucose levels in the region of the hypothalamus but are acting directly on the hypothalamic cyclic AMP systems perhaps via the biogenic amines.

Neto and Carlini (1972) have found that the administration of Dopa potentiated the Δ^9-THC induced agression in starved rat behavior. Garelis and Neff (1974) found that the administration of Dopa into rats caused a significant increase in the levels of cyclic AMP in the caudate nucleus region of the rat brain. This data implies a relationship between the caudate nucleus, dopaminergic activity and various neural disorders (manic depressive psychosis,Parkinson's disease, Huntington's disease; Cools, 1972) in the central nervous system which also correlates with dramatic changes in overt behavior. This may provide additional

evidence for the possible involvement of cyclic AMP and marihuana induced behavioral patterns. Perhaps marihuana could induce some sort of mental dysfunction (psychosis or neurosis) in certain individuals who are particularly susceptable to brain amine or cyclic AMP level alterations, thus accounting for some of these reports (Allentuck Bowman, 1946; Grinspoon, 1972).

Finally, in Chapter I, it was stated that the Arabs considered Cannabis a potent aphrodisac. Interestingly, p-chlorophenylalanine (potent inhibitor of tryptophan hydroxylase, 5-HT synthesis) when administered to rats causes an enhanced sexual activity (Ferguson, 1970; Tagliamonte et al., 1969) which is correlated with low levels of brain 5-HT. Perhaps the cannabinoid induced changes in brain 5-HT via a cyclic AMP mechanism (See Cyclic AMP and Enzyme Induction in Grand Discussion) may underlie the expression of the sexual drive and may account for some of the reported aphrodisac actions of marihuana.

Behavioral and Physiological Tolerance to the Cannabinoids

The phenomena of behavioral tolerance may also involve the cyclic AMP generating system. Support for this concept comes from the investigations of Ho, Loh and Way (1972). They present data which demonstrates that the acute or repeated administration of cyclic AMP to morphine dependent mice, enhanced the rate of tolerance developement to the analgesic effects of morphine, as measured by the response to a noxious stimulus. Interestingly, the maximum effect of the injected cyclic AMP was not manifested immediately after its administration but 6 or more hours later, again suggesting that the elevation of cyclic AMP serves as a trigger event for the development of behavioral or

physical tolerance. Studies by Contreras and others (1972) imply a similar antagonism between cyclic AMP and morphine analgesia. Whether or not this process of tolerance development and cyclic AMP may be at work during the development of tolerance to the action of the cannabinoids and marihuana, remains to be established.

More direct support for the involvement of cyclic AMP in the mediation of tolerance may be seen from Uzunov et al. (1973). They demonstrated that acutely administered NE can induce a single isoenzyme form of PDE from tumor derived cultures of rat brain astrocytes. This PDE enzyme induction was blocked by propranolol and cyclohexamide. This data suggests that an increased adrenergic activity (perhaps via cannabinoid alterations in biogenic amine levels or synthesis or cannabinoid action on biogenic amine coupling with adenyl cyclase) can feedback and reregulate the steady state levels of cyclic AMP in these cells. Therefore, the continued use of marihuana (perhaps even after the acute administration) may cause an increase in brain NE and other biogenic amines, which then induces a high specific activity form of PDE such that the enhanced levels of adenyl cyclase activity (caused by biogenic amine-cannabinoid interactions) may be counteracted by the action of this specific PDE. Since PDE activity in fibroblasts was also shown to be enhanced by the addition of dibutyryl-cyclic AMP or PGE_1 (Manganiello et al., 1972; D'Amlento et al., 1972), these results suggest that cyclic AMP can induce the synthesis of its own degradation enzymes, even in normal cell lines, such that the homeostatic levels of cyclic AMP within these cells can be maintained at the "proper" levels for maximum response.

Finally, an increase in sympathetic nerve activity results in a

decreased sensitivity of the NE-adenyl cyclase system, while a decreased sympathetic nerve activity produces an increase in the NE-adenyl cyclase system sensitivity (Weiss, 1971; Kakiuchi and Rall, 1968; Weiss, 1969; Sattin and Rall, 1970). Since NE was shown to induce the formation of a particular PDE isoenzyme form, this lends additional support for some sort of feedback process regulating synaptic sensitivity. Chronic injections of the cannabinoids and their effect on cyclic AMP levels and PDE activity in the brain during the development of true tolerance will be critical in resolving the phenomena of behavioral tolerance as it relates to marihuana.

Cyclic AMP, Protein Kinases, RNA and Protein Synthesis and Memory

Several investigators have found a correlation between the available substrate concentration for RNA synthesis (orotic acid, UMP and CMP by brain hippocampal injection) and the consolidation of learned behavior (rats in Y-maze; lighted and dark alley) into long term memory (Ott et al., 1972; Pohle and Matthies, 1971; Ott et al., 1973). The administration of these pyrimidines caused a delay in behavioral extinction in the absense of negative reinforcement, suggesting that RNA transcription and translation may be involved in the aquisition of long term memory. Presumably, once the long term "memory marcomolecules" have been formed, behavioral extinction becomes more difficult. More direct evidence for this involvement comes form conditioning experiments employing actinomycin D and cyclohexamide (transcription and translation inhibitors, respectively) concurrently or just after the learning paradigm. Generally a marked reduction in subsequent behavioral performance is noted (Geller et al., 1970; Barondes, 1970), although relearning is

not usually affected (Shashoua, 1968; Glassman, 1969). These data imply a direct relationship between RNA precursors and their subsequent processing and the consolidation of a learned event into long term memory.

Many investigators have observed that cyclic AMP can play an important role in the regulation of RNA synthesis (Dokas et al., 1971; 1973; Adiga et al., 1970; Shashoua, 1971; Holtta et al., 1973; Jost and Sahib, 1971; Langan, 1968) and protein synthesis (Leung et al., 1971; Boucher et al., 1973; Bosmann, 1971; Adiga et al., 1971; Malkin et al., 1969) in many cell types. Some evidence suggests that this action of cyclic AMP on transcription and translation may be mediated via alterations of protein kinase activity at the nuclear and cytoplasmic level (Kish, 1973; Schmidt and Sokoloff, 1973; Langan, 1973; Gaballah et al., 1971).

Therefore the observed alterations in mouse brain cyclic AMP levels, as a result of cannabinoid administration (THC) may change the activity of certain protein kinases which in turn would phosphorylate various key regulatory proteins associated with the nuclear or cytoplasmic machinery involved in the transcription and translation and thereby alter the acquisition of memory, its consolidation and the extinction of non-reinforced behavior (See Behavior in Chapter I.).

In partial support of the hypothesis that alterations in brain cyclic AMP caused by the cannabinoids may effect RNA synthesis and the acquisition of behavior can be seen from studies by Izquierdo and Nasello (1973a). They found that moderate doses of CBD (3.5 mg/kg i.p.) blocked the increase in rat brain hippocampal RNA concentration induced by the electrical stimulation of the hippocampus normally

associated with this process. This cannabinoid also impaired the acquisition of conditioned avoidance behavior in these rats.

Furthermore, Dittrich et al. (1973) have presented evidence that Δ^9-THC administered to human subjects produced temporal disintegration (impairment of short term memory) as well as its consolidation into long term memory. Admittedly, much more research along these lines is required in order to confirm these inferences between cyclic AMP and memory and behavior acquisition. It is hoped that these speculations will generate interest by other researchers to investigate the effects of the cannabinoids on these cyclic AMP dependent regulatory processes.

Cyclic AMP and Hormone Action

Cyclic AMP has been shown to mediate the intracellular actions of numerous hormones and in some cases, releasing factors in their respective target tissues (viz. second messenger hypothesis; Robison et al., 1971; 1971a; Greengard et al., 1972; 1973; Borgeat et al., 1972; Pastan and Perlman, 1971; Wellmann and Schwabe, 1973). The fact that the administration of the cannabinoids causes a depression of LH release from pituitary (Nir et al., 1973) a suppression of I^{131} release from thyroid (Lomax, 1971), an elevation of blood corticosterone levels presumably by elevations on blood ACTH (Kubena et al., 1971), an inhibition of E and PGE induced stimulation of lung cell cyclic AMP (Kelly et al., 1973) and an inhibition of PGE synthesis (Burstein, 1973), all tend to suggest that the cannabinoids can induce alterations in the target tissues' response to these circulating hormones or alter the release of these hormones from certain secretory

centers.

In view of the fact that the cannabinoids can change brain cyclic AMP levels, it seems reasonable to infer that the cannabinoids may be mimicking certain phases of the negative feedback process regulating the release of ACTH, LRH (LH), and TSH from the anterior pituitary-hypothalamic axis. Depending on the metabolic state of the hormone or releasing factor cell machinery, its response to changing cyclic AMP levels may be the enhancement or inhibition of hormone release.

Cyclic AMP and Smooth Muscle Function

Several investigators have observed a decreased twitch response as well as other alterations in smooth muscle parameters while subjected to various concentrations of the cannabinoids (See Chapter I.). Data has been accumulated suggesting that α and β adrenergic mechanisms via cyclic AMP mediation underlie the coupling of smooth muscle excitation-contraction and relaxation processes (Triner et al., 1971; Vulliemuz et al., 1970). If the cannabinoids alter adenyl cyclase activity in smooth muscle (as occurs in brain), then perhaps cannabinoid induced alterations in the adenyl cyclase system are in some way responsible for the biphasic effect on contractility (low concentrations; Gascon and Peres, 1973) and for the decreased contractility and the interference with the ACH induced contraction process. Further investigations will no doubt resolve the mechanisms participating in this process.

Cyclic AMP, DNA Synthesis, Cell Growth and Tumor Formation

Many tumor and non-tumor derived tissue cultured cells have been

observed to display a decreased incorporation of ^{3}H-thymidine into DNA as well as a decreased DNA synthesis in the presence of dibutyryl-cyclic AMP while undergoing the mitotic cycle (Yang and Nas, 1971; Van Wijk et al., 1972; 1973; Masui et al., 1970). High levels of cyclic AMP or dibutyryl-cyclic AMP also inhibits the growth of various cell lines (Otten et al., 1971; Ryan and Heidrick, 1968) and may even play a decisive role in the transformation of normal cells to tumor-like cell lines, since the application of cyclic AMP can cause reversal of tumor-like characteristics or inhibit their cell growth (Heidrick and Ryan, 1970; Reddi et al., 1972; Hsie et al., 1971; Sheppard, 1971; Keller, 1972; Gericke et al., 1969; Korinek et al., 1973).

For comparison, studies by others have shown that marihuana smoke as well as the cannabinoids can cause a decreased mitotic index (proliferation rate) and a decreased ^{3}H-thymidine incorporation into the DNA of these dividing cultured cells (Neu et al., 1970; Martin, 1969; Zimmerman et al., 1973; Nahas et al., 1974). These data raise the possibility that cyclic AMP is involved in the processing of DNA during the cell cycle and that this process may be altered by the administration of the cannabinoids or marihuana. The ramifications of this to the marihuana user are far reaching and could be subject to great speculation.

On the positive side, the cannabinoids or synthetic derivatives may have a theraputic value in the treatment or prevention of certain types of tumors or carcinoma that are particularly sensitive to prolong alterations in cyclic AMP levels.

APPENDIX I OF FIGURES

Figure 1. Anatomy of Cannabis sativa L.

a) seed with seed coat
b) seed coat removed; two cotyledons (seed leaves) and hypocotyl (embryonic stem) and radicle (embryonic root) constituting mature embryo
c) maturing embryo with endosperm
d) radicle eruption from seed
e) radicle and hypocotyl eruption
f) cotyledons erupting from seed coat
g) about 5-9 days since sowing; note first true leaves emerging from between cotyledons
h) young plant
i) section from stem of female plant with leaves (5-7 leaflets) and flowering inflorescences
j) individual female flower consisting of bract (1) which surrounds the ovary (2) from which project two stigmas
k) section from stem of male plant with leaves and flowering inflorescences
l) individual male flower with 5 sepals (1) and 5 stamens (2)
m) individual stamen showing anthers which hold and release the pollen

Drawings a-h from Hegi, 1958 and i-m from Stearn, 1970

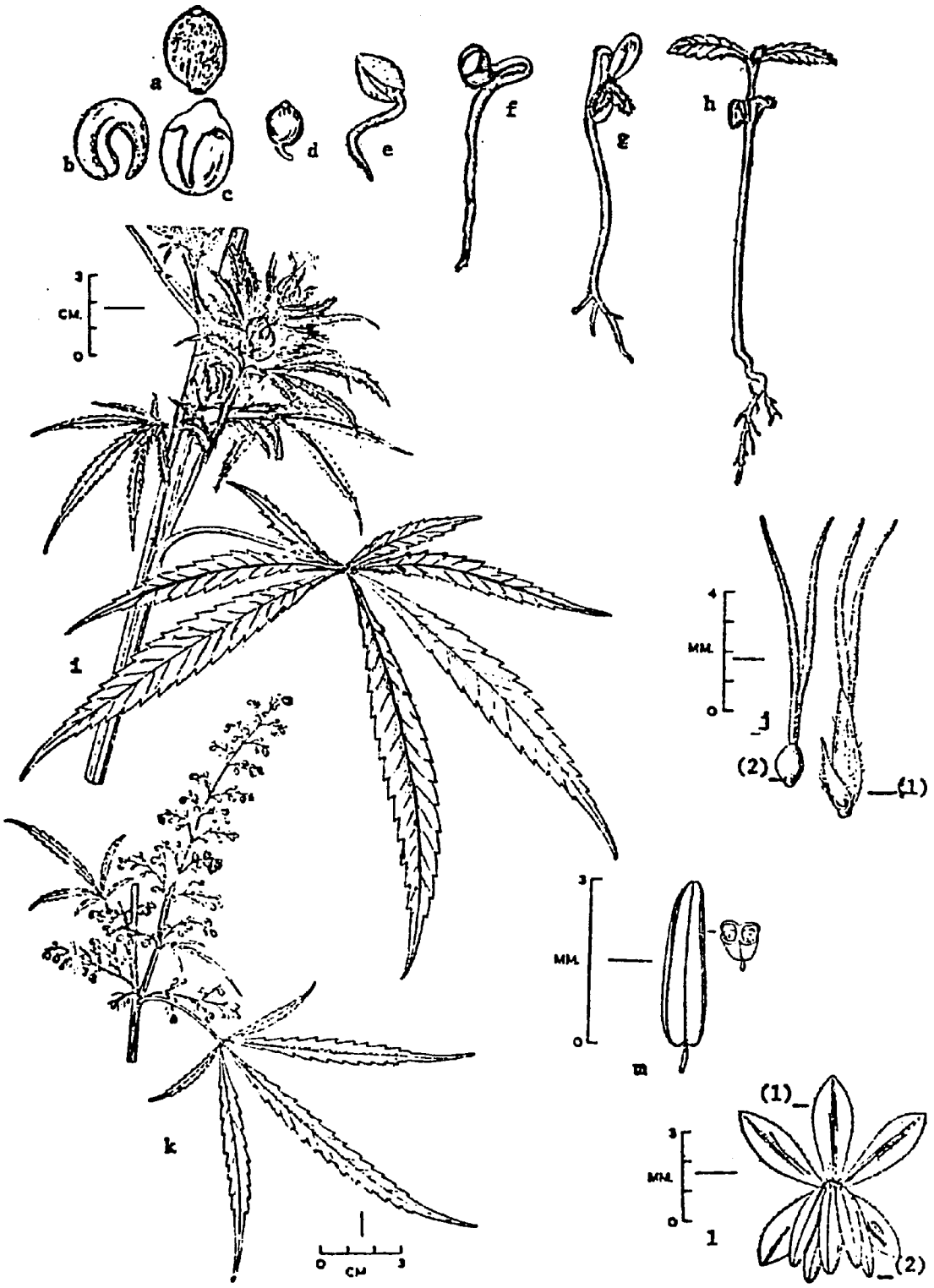


Figure 1

Figure 2. Microanatomy of Cannabis sativa L.

Part A. Cross section of a female fruiting bract

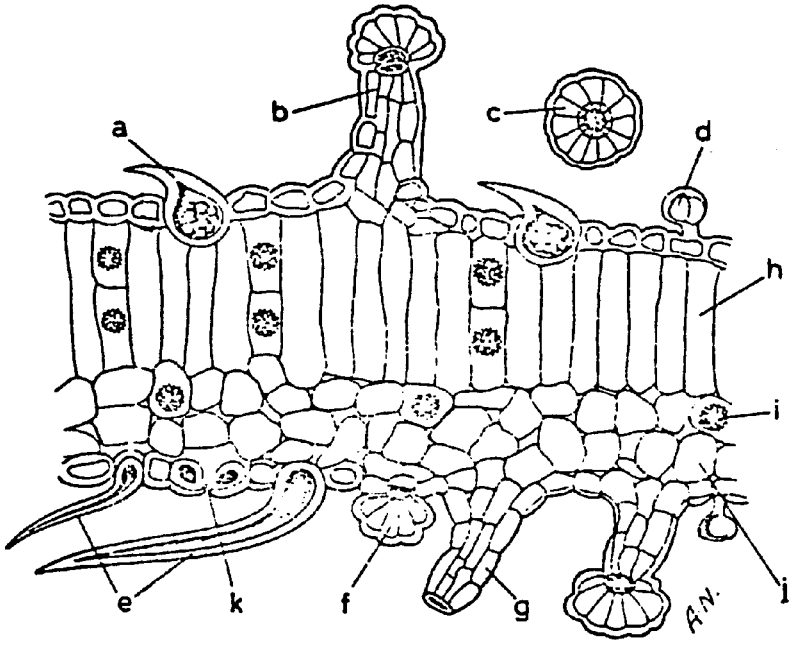
a) small upper epidermal trichome with cystolith of $CaCO_3$
b) large glandular hair composed of many cells (resin secretion)
c) head of one of the large glandular hairs
d) small glandular hair with bicellular head and unicellular stalk
e) large thick walled trichome on lower surface usually without crystals
f) developing glandular hair (large)
g) stalk of large glandular hair
h) palisade cell (columnar chloroplast bearing cell) perhaps with rosette crystal of calicum oxylate
i) mesophyll (thin walled cell with chloroplasts) with crystal cluster of calicum oxylate
j) mesophyll without crystals
k) reniform stoma

Drawing from Nordal, 1970

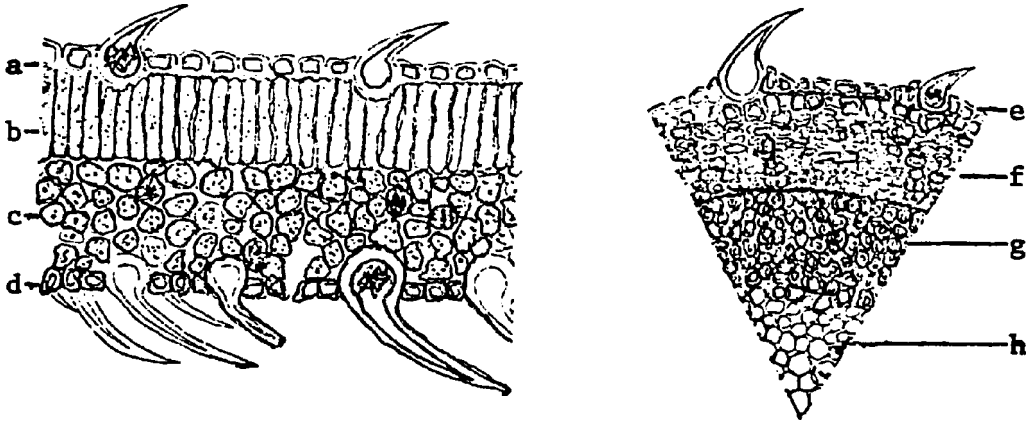
Part B. Transverse section through leaf and stem of Cannabis sativa

a) upper epidermis of leaf with trichomes perhaps with $CaCo_3$
b) palisade cells with chloroplast granules in cytoplasm
c) mesophyll with Ca oxylate crystals present in some cells and chloroplast granules in others
d) lower epidermis with stoma and trichomes containing cytoliths
e) epidermis of stem with short trichomes
f) collenchyma cells (supportive functions) of stem
g) fibrovascular layer
h) pith (parenchyma and other cells) of stem

Drawing from Ballard, 1915



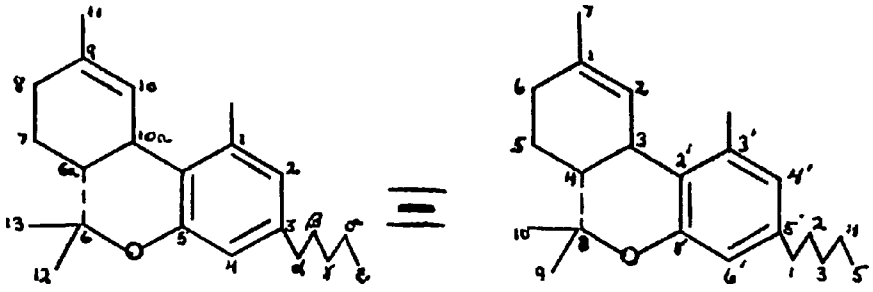
Part A



Part B

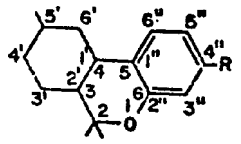
Figure 2

Figure 3. Chemical Nomenclature
These are the numbering systems used for the formal nomenclature to describe the cannabinoids. Figure 3a. shows the numbering system used throughout this thesis. This numbering system is not strictly correct when dealing with the non-pyran cannabinoids (open O-containing heterocyclic ring), therefore equilvalent systems are presented in order to convert the cannabinoid nomenclature used in this thesis to more formal systems. Lower figures from Brown, 1972.

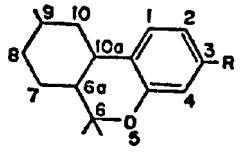


DIBENZOPYRAN (C) SYSTEM

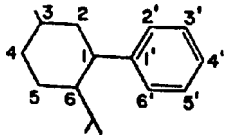
MONOTERPINOID SYSTEM



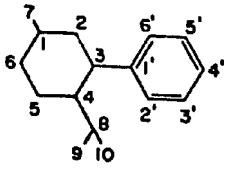
PYRAN



DIBENZOPYRAN



BIPHENYL



TERPINE

Figure 4. Farmilo's proposed synthesis of the cannabinoids in the plant. (See text under Biogenesis).

Figure 5. Mechoulam's proposed biosynthesis for the cannabinoids. This synthesis model was very adequate in proposing the pathway for the synthesis of the pentyl-homologs of the cannabinoids. More recently, methyl and propyl homologs (3 position) have been reported to be present in the plant. Figure 6 establishes an additional pathway to account for all of the current cannabinoids while still maintaining the relationships to Mechoulam's proposed biogenesis.

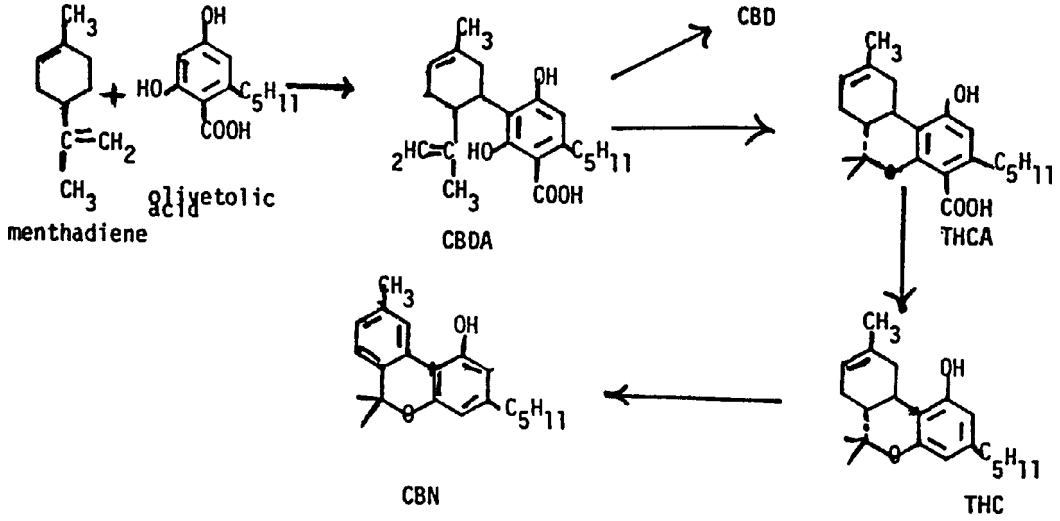


Figure 4

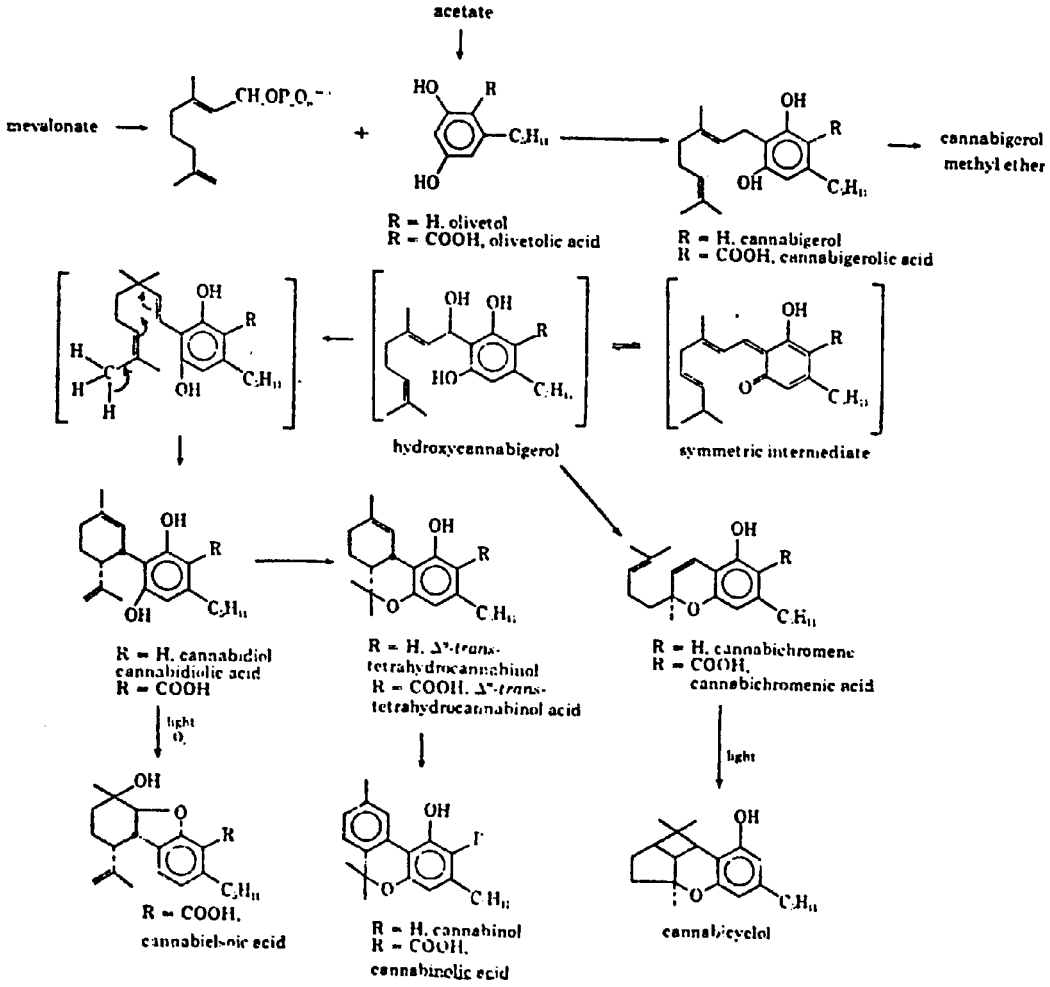


Figure 5

Figure 6. Biosynthesis of the cannabinoids
This synthesis scheme accounts for all of the naturally occurring cannabinoids. Acetyl-CoA and malonyl-CoA are the precursors for the biosynthesis of the aromatic phenol, orsellinic acid (Neish, 1964) which then condenses with geranyl pyrophosphate forming the precursor for the remaining cannabinoids, cannabigiorcinic acid (CBGA-C_1). As can be seen from the figure, CBGA-C_1 can freely rotate about the covalent bond indicated. This will allow for the A and B acid forms of some of the cannabinoids upon cyclization in later steps. Although CBGA-C_1 has not yet been identified in Cannabis extracts, the enzyme responsible for its conversion to hydroxy-CBGA-C_1 may have a high affinity for this particular homolog or an isozyme specific for this form may drive the reaction to the right more so than with CBGA-C_3 and CBGA-C_5. Spontaneous decarboxylation or a decarboxylase could give rise to the neutral forms of the cannabinoids by being routed through the acetate condensation and reduction pathway which adds acetate units to the side chain. In this way, the methyl, propyl and pentyl cannabinoids can all be accounted for in the plant material at various concentrations depending on the equilibrium constants of the respective enzymes involved for each particular plant variant. Δ^8-THC (C_1,C_3,C_5) can be formed in the plant from CBD (C_1, C_3,C_5) in the presence of an acid catalysed reaction. This could even be an "unintentional" side product. Dimer and polymerized cannabinoids can be accounted for due to the reactivity of phenolic rings and double bonds present in certain cannabinoids; these reactions remain to be established.

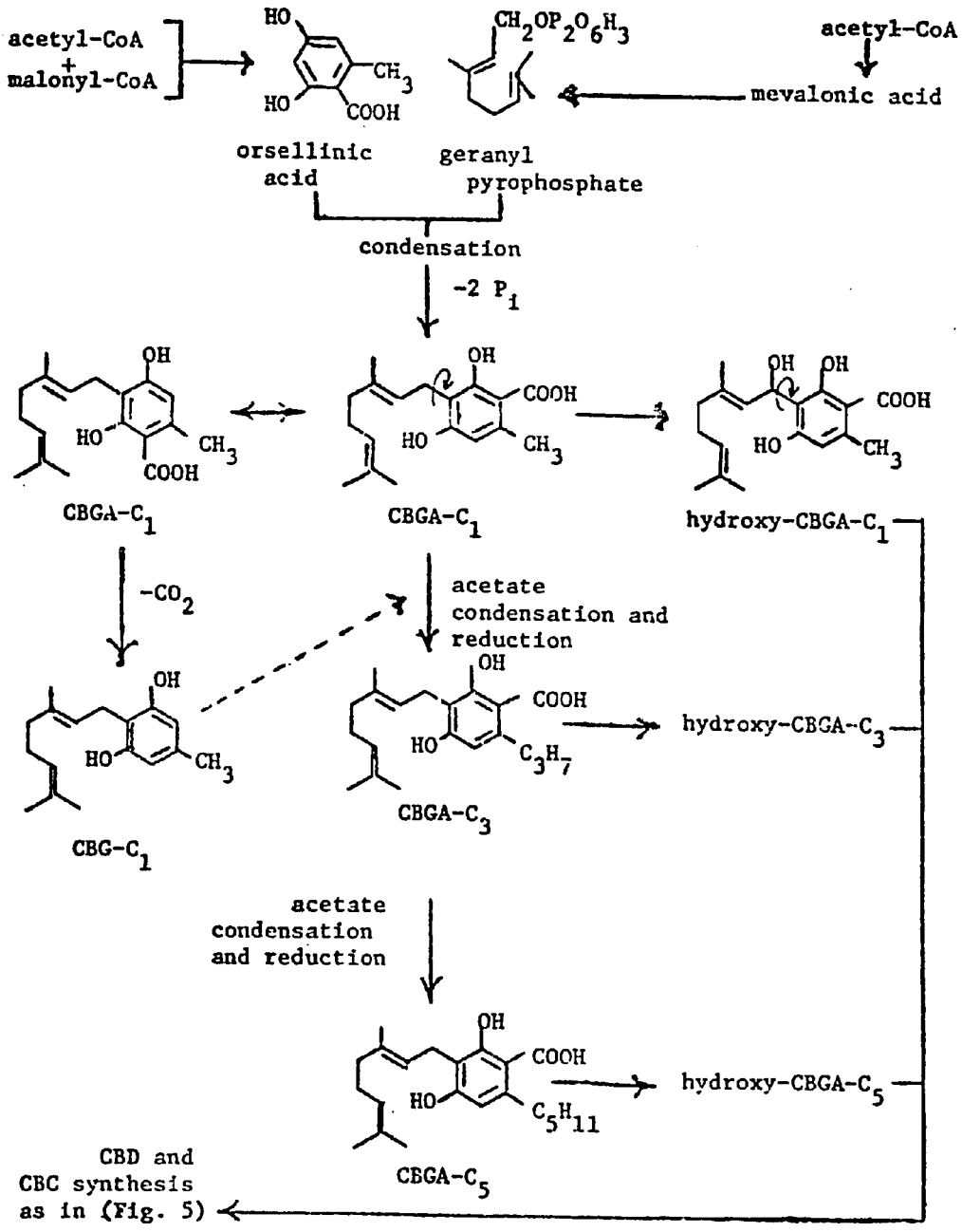
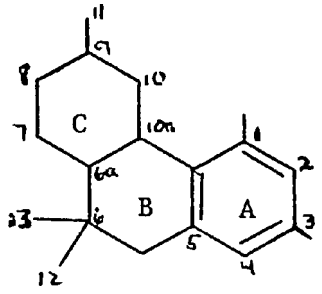

acetyl-CoA
+
malonyl-CoA
orsellinic acid
geranyl pyrophosphate
acetyl-CoA
mevalonic acid
condensation
-2 P_i
CBGA-C_1
CBGA-C_1
hydroxy-CBGA-C_1
-CO_2
acetate condensation and reduction
CBG-C_1
CBGA-C_3
hydroxy-CBGA-C_3
acetate condensation and reduction
CBGA-C_5
hydroxy-CBGA-C_5
CBD and CBC synthesis as in (Fig. 5)

Figure 6

Figure 7. Model cannabinoid
This figure shows the structure of the model cannabinoid which is referenced in the structure-activity relationships under chemistry in the text.



MODEL CANNABINOID

Figure 7

Figure 8. This table gives the metabolic transitions of Δ^9-THC which occurs in several animals. This is not necessarily the sequence of reactions, for they have not been formally determined. This figure is to be used in conjunction with Table VIII. 8α-OH-Δ^9-THC has not yet been reported in the literature as a metabolic of Δ^9-THC, although I suspect it will soon be isolated.

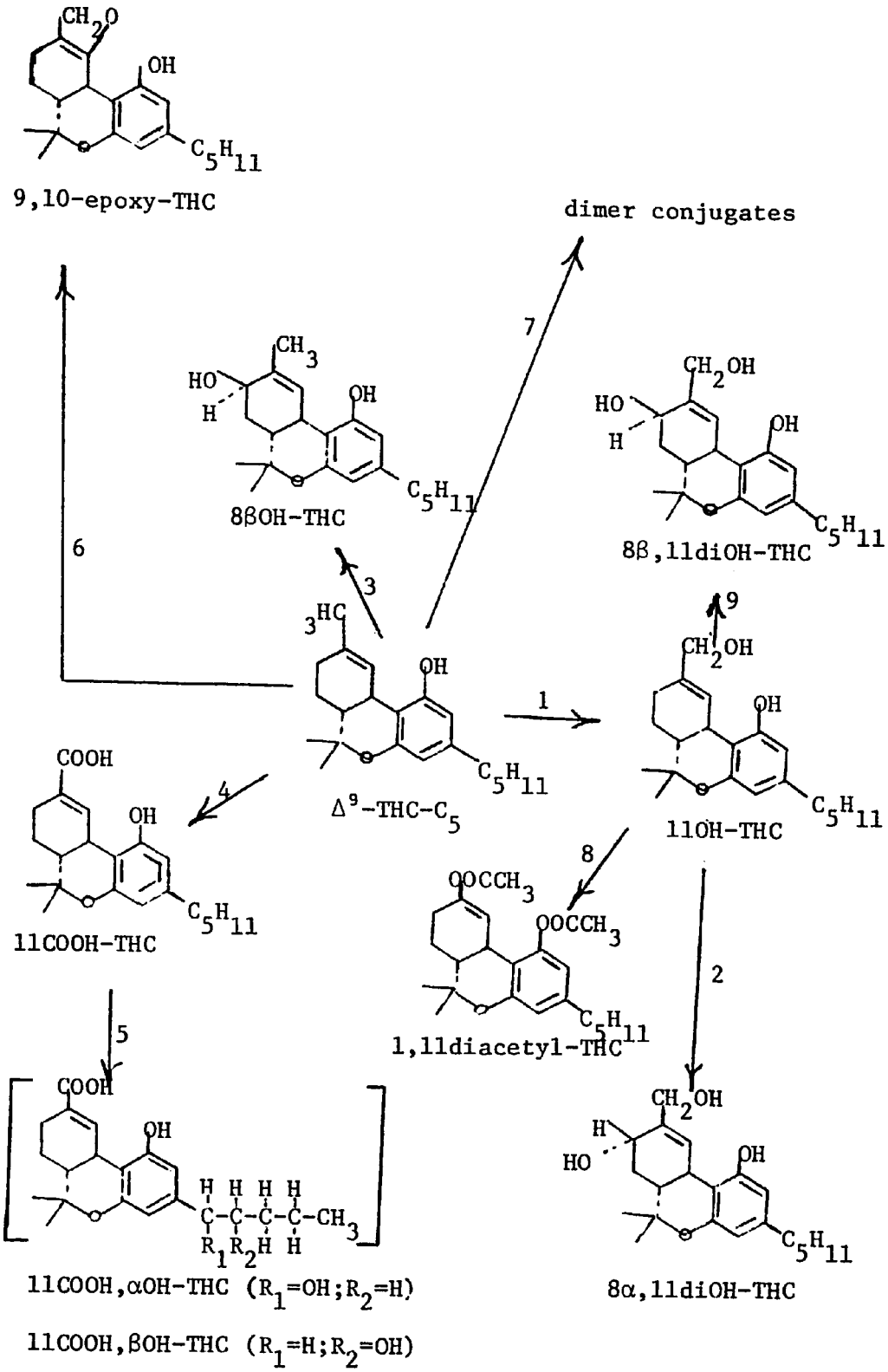

9,10-epoxy-THC
dimer conjugates
8βOH-THC
8β,11diOH-THC
Δ9-THC-C5
11OH-THC
11COOH-THC
1,11diacetyl-THC
11COOH,αOH-THC (R1=OH;R2=H)
11COOH,βOH-THC (R1=H;R2=OH)
8α,11diOH-THC
1
2
3
4
5
6
7
8
9

Figure 8

Figure 9. The metabolites of Δ^8-THC recovered from various animals. This table is to be used in conjunction with Table IX.

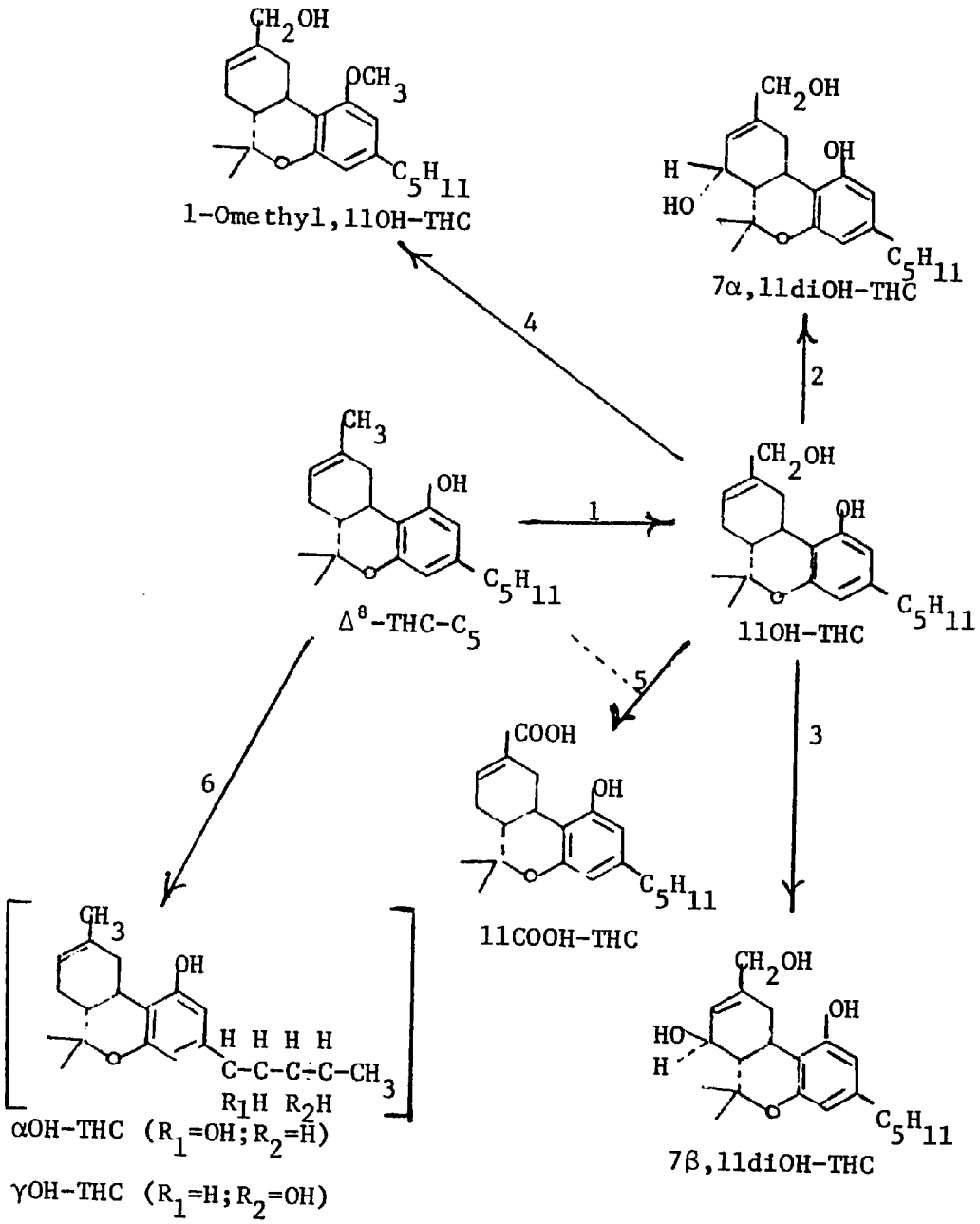
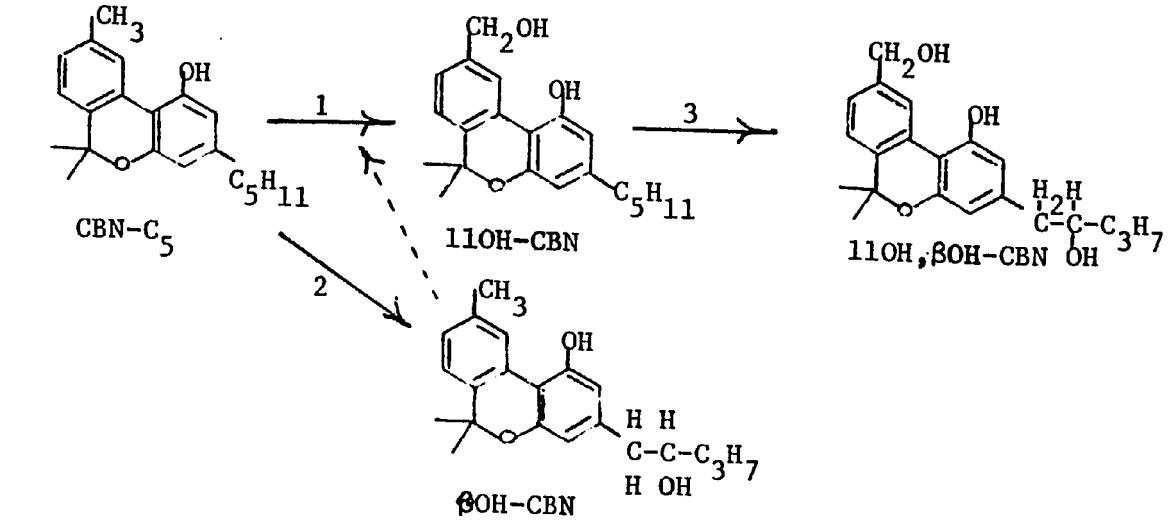

1-Omethyl,11OH-THC
7α,11diOH-THC
Δ⁸-THC-C5
11OH-THC
11COOH-THC
7β,11diOH-THC
αOH-THC (R1=OH;R2=H)
γOH-THC (R1=H;R2=OH)
1
2
3
4
5
6

Figure 9

Figure 10. The metabolism of CBD and CBN
This figure should be used in conjunction with Table X.


CH3
OH
C5H11
CBN-C5
1
CH2OH
OH
C5H11
11OH-CBN
3
CH2OH
OH
H2H
C=C-C3H7
OH
11OH,βOH-CBN
2
CH3
OH
H H
C-C-C3H7
H OH
βOH-CBN

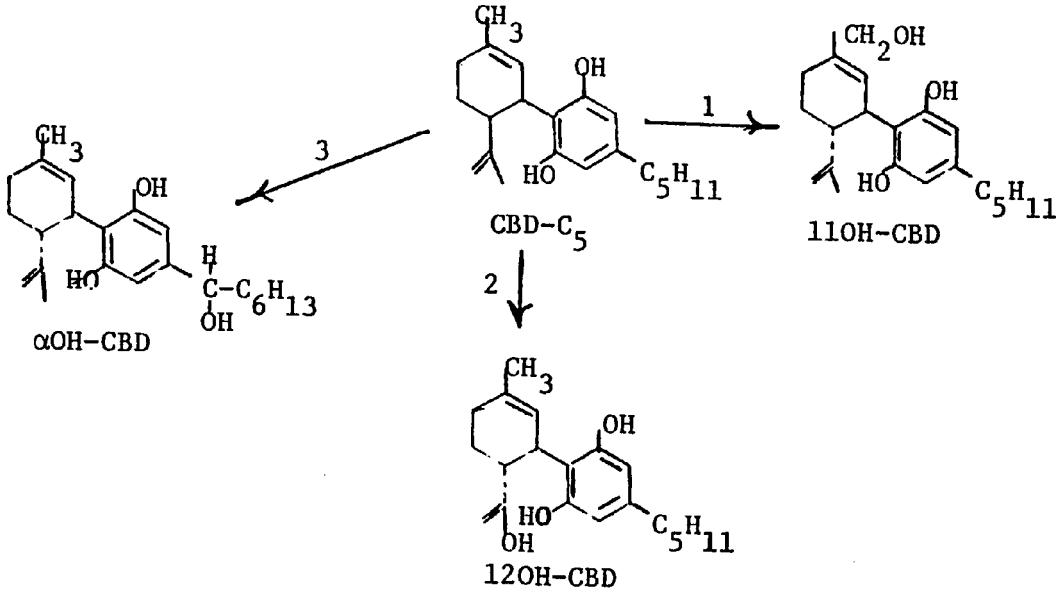

CH3
OH
HO
C5H11
CBD-C5
1
CH2OH
OH
HO
C5H11
11OH-CBD
3
CH3
OH
HO
H
C-C6H13
OH
αOH-CBD
2
CH3
OH
HO
OH
C5H11
12OH-CBD

Figure 10

Figure 11. The absorbance-^{3}H-cyclic AMP binding profile from the DEAE-cellulose ion exchange chromatography fractions. The procedures are described in the text under Methods. Fractions from 300-375 were pooled from peak I and fractions from 410-480 were pooled from peak II due to their high specific activity. Peak I kinase preparation served as the binding protein source during the cyclic AMP assays.

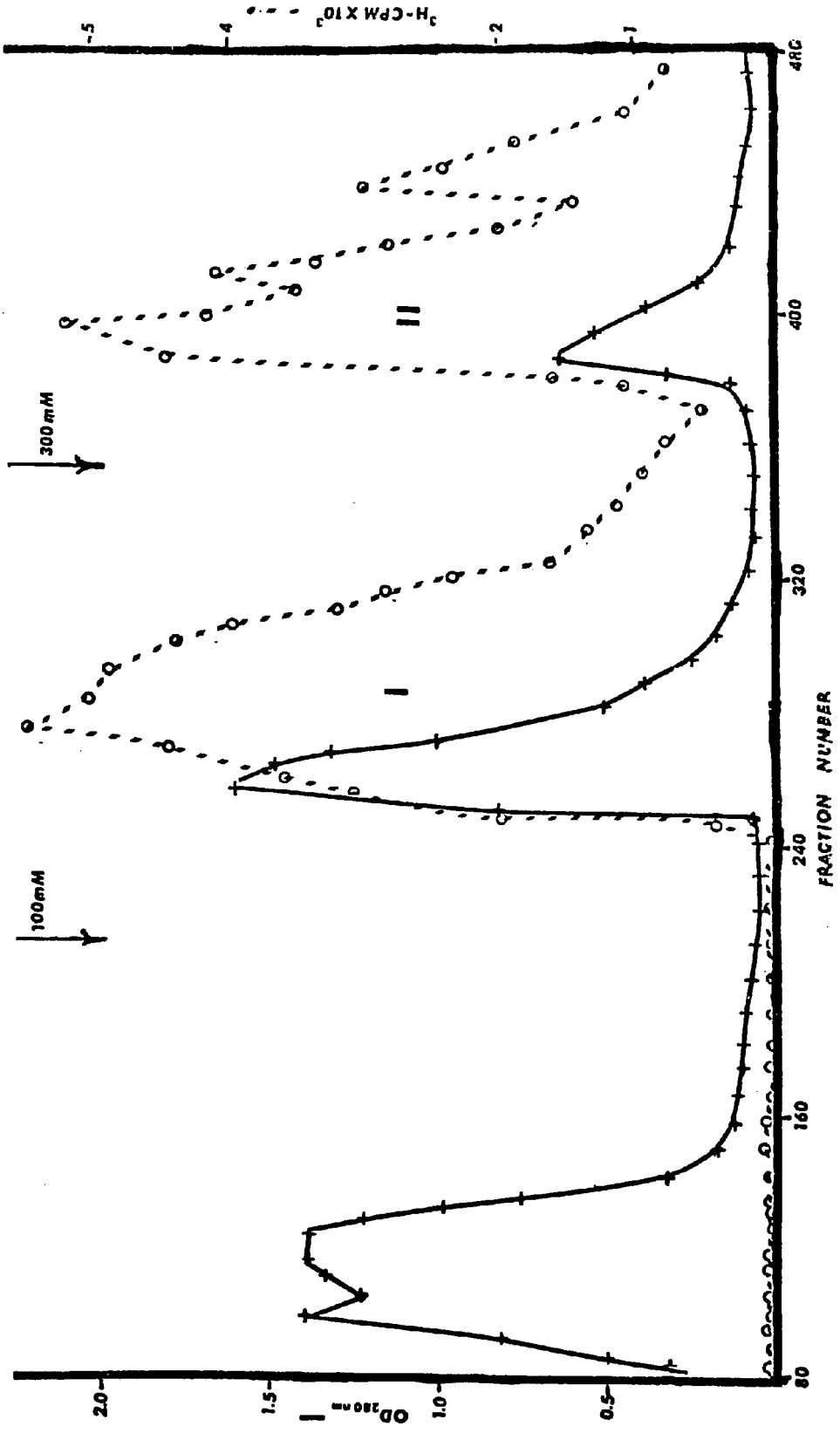


Figure 11

Figure 12. A typical double logarithmic standard curve generated by incubating 1 ug of the protein kinase (peak I) with 1.66 picomoles of ^{3}H-cyclic AMP in the presence of unlabeled cyclic AMP competitor at 0, 0.24, 0.40, 0.80, 4.0, 8.0, 16.0, 24.0, 32.0 and 40.0 picomoles/0.1 ml. in 50 mM sodium acetate (pH=4.0) and 10 ul of the inhibitor for 2 hours at 2^{o}C. The bound ^{3}H-cyclic AMP was collected on nitrocellulose filters and counted for tritium in toluene scintillation cocktail as described in the text. Bars represent mean of duplicate assays ± 1 standard deviation.

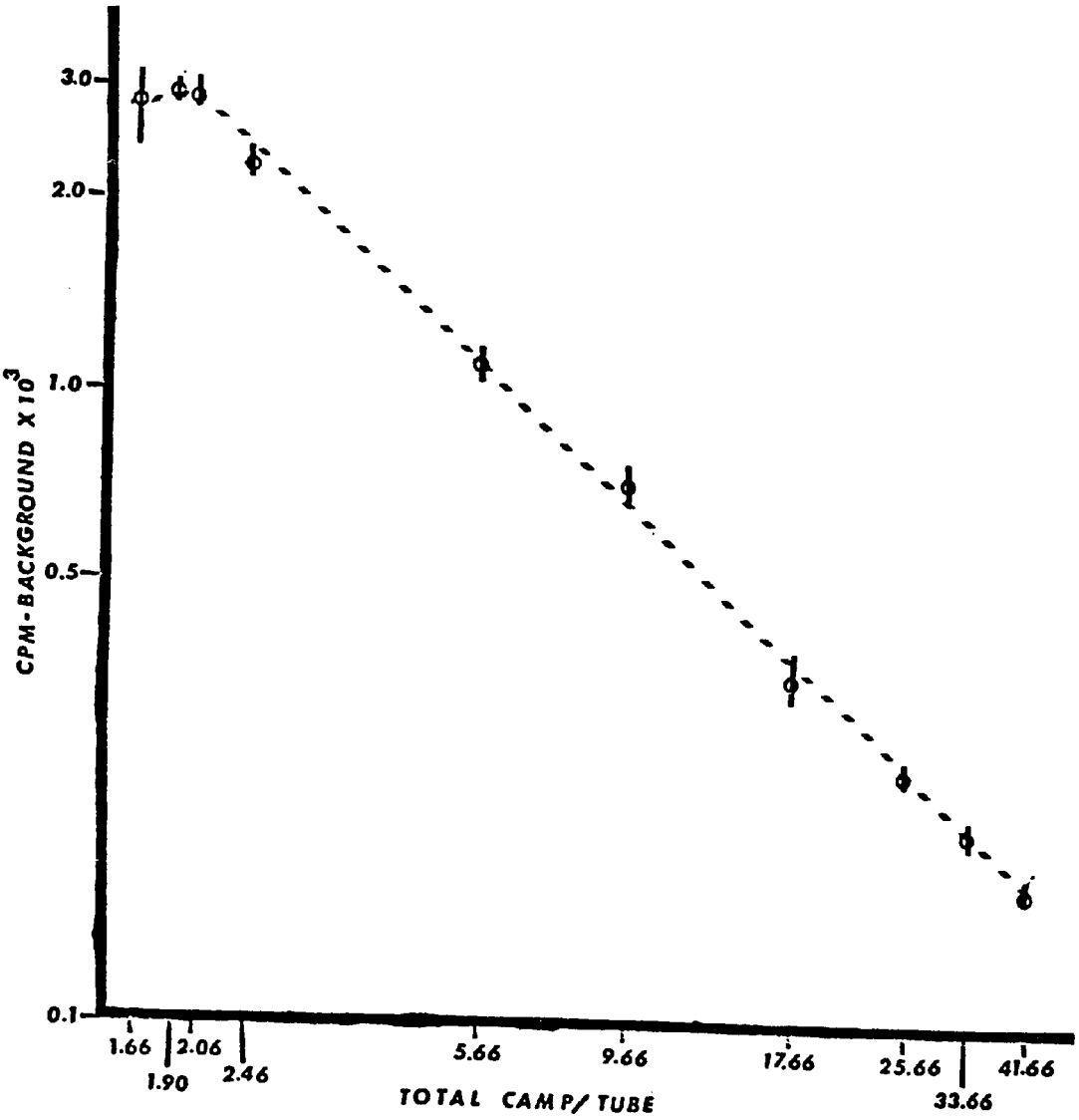

3.0
2.0
1.0
0.5
0.1
CPM-BACKGROUND X 10^3
1.66
1.90
2.06
2.46
5.66
9.66
17.66
25.66
33.66
41.66
TOTAL CAMP/ TUBE

Figure 12

Figure 13. Change in brain levels of cyclic AMP as a function of injected dose of Δ^9-THC. Data are expressed as percentage change from control values, with the verticle bars representing standard deviations.

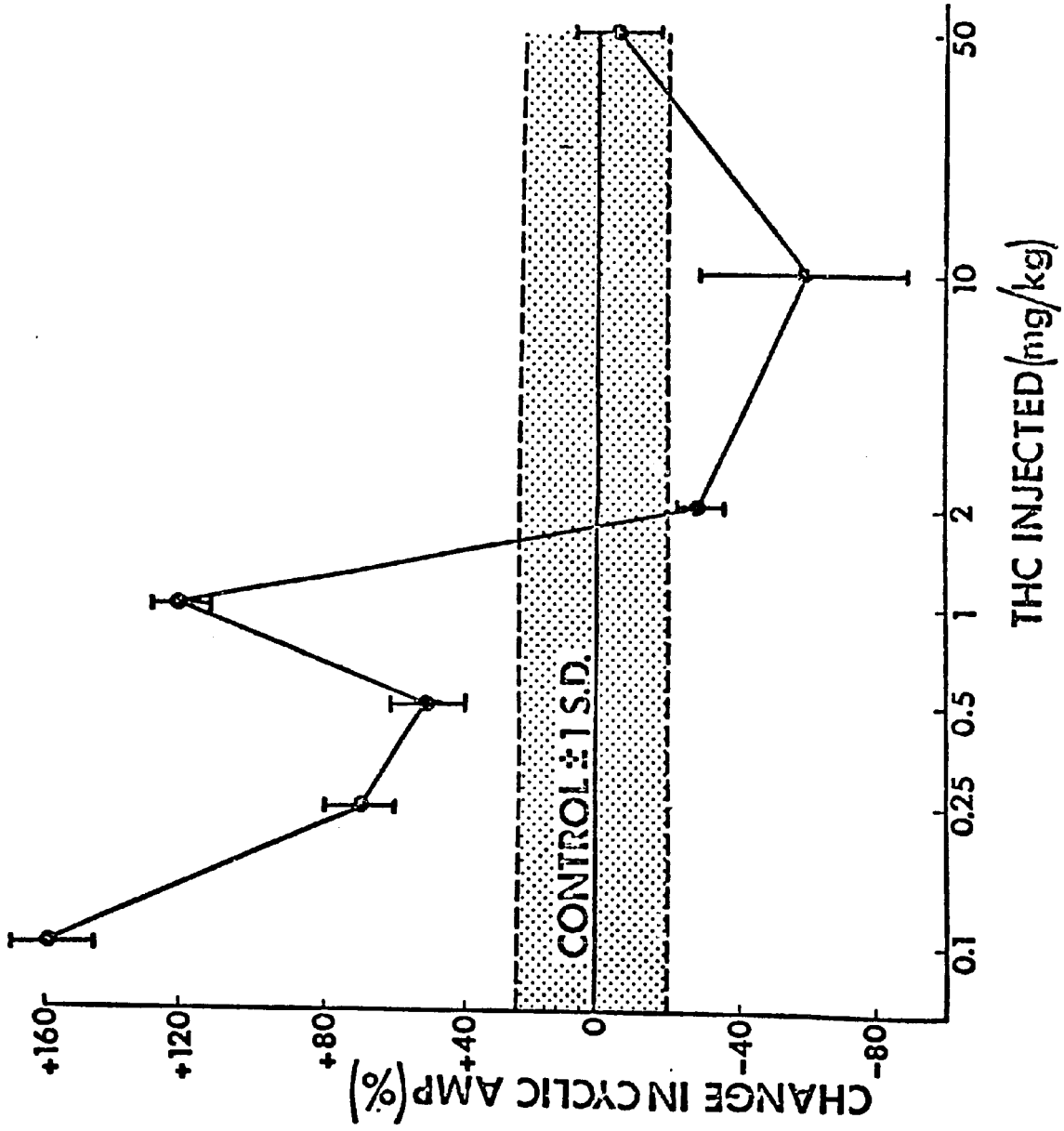

CHANGE IN CYCLIC AMP (%)
+160
+120
+80
+40
0
-40
-80
CONTROL ± 1 S.D.
0.1
0.25
0.5
1
2
10
50
THC INJECTED (mg/kg)

Figure 13

Figure 14. The graphic representation of Table XVI. The effects of Δ^9-THC administered *in vivo* on the ability of *in vitro* isolated adenyl cyclase to convert ^{14}C-ATP to ^{14}C-cyclic AMP. Experiment was repeated three times with different sets of animals.

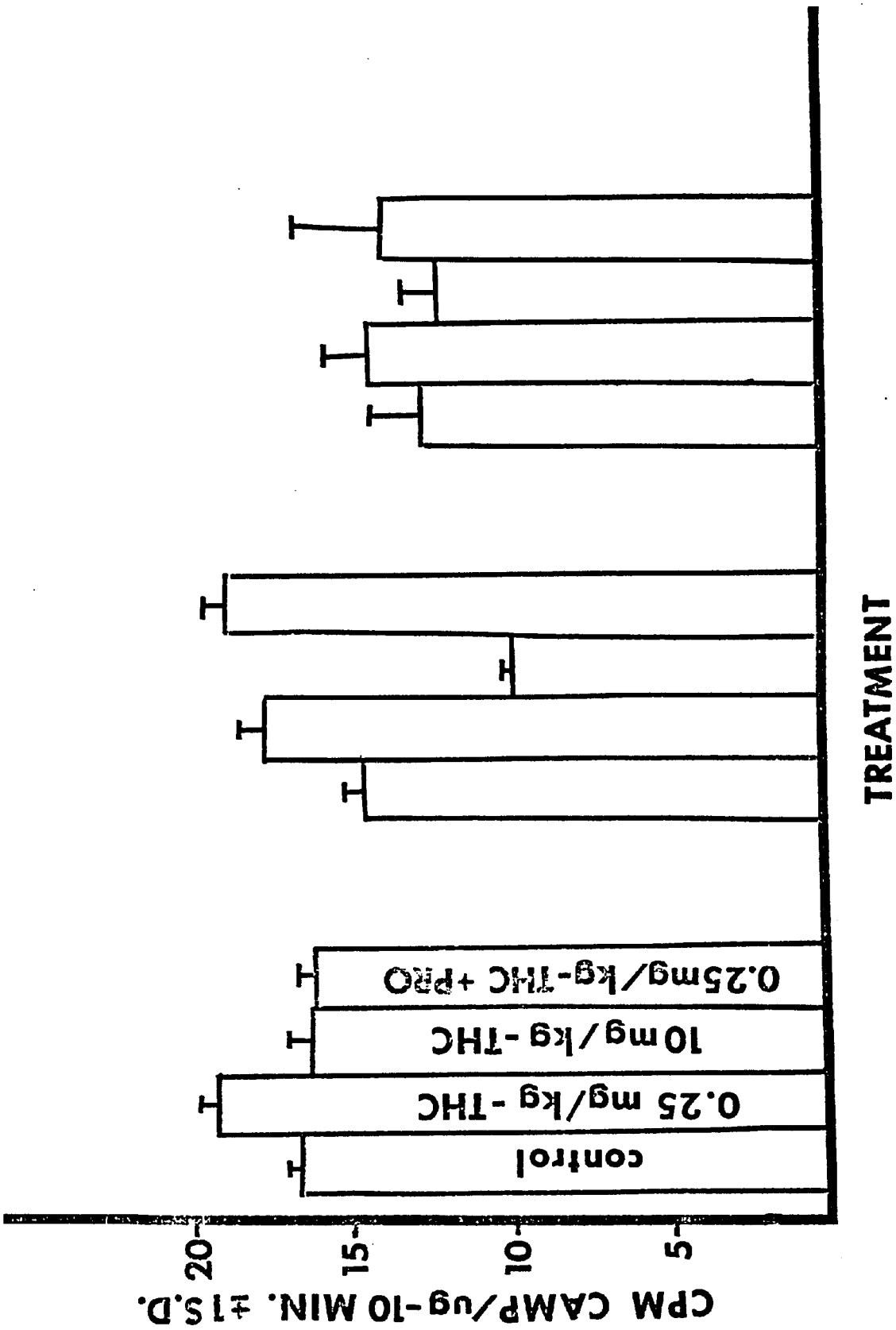

CPM CAMP/ug-10 MIN. ±1 S.D.
20
15
10
5
control
0.25 mg/kg -THC
10mg/kg -THC
0.25mg/kg-THC +PRO
TREATMENT

Figure 14

Figure 15. The effect of β-estradiol and CaCl on the activity of PDE from mouse brain assayed in vitro over time. Ordinate represents ^{3}H-cyclic AMP hydrolysis and the abscissa time (minutes) control (—o—) 3.2 mM CaCl (--+--) 48 uM β-estradiol (--□--). The bars represent range of quintuplicate assays. CaCl produces stimulation while β-estradiol inhibits PDE activity.

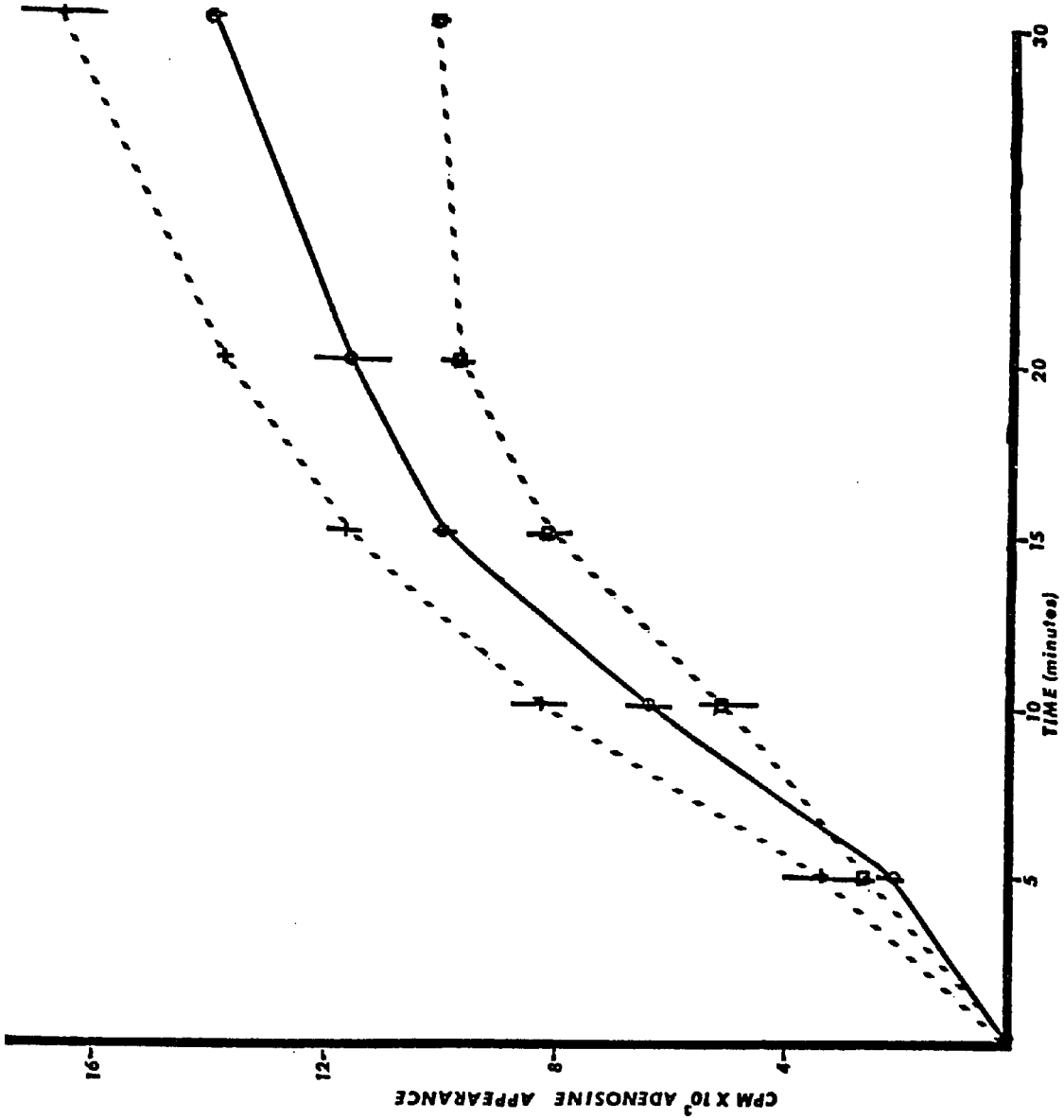

CPM X 10³ ADENOSINE APPEARANCE
16
12
8
4
TIME (minutes)
5
10
15
20
30

Figure 15

Figure 16. The effect of Δ^9-THC, 11-OH-Δ^9-THC and CBN on the activity of mouse brain PDE assayed in vitro. Cannabinoid concentrations of 20 mM and 212 um were carried out in two separate experiments. No significant effect is observed with 212 um concentrations of any of the cannabinoids although much variation exists (Figure 16). When the cannabinoids were incubated at 20 nM, the form of the curve all coincided with that of the control (not reproduced here) with very little variation. Control (--o--); Δ^9-THC (--□--); 11-OH-Δ^9-THC (--◇--); CBN (--+--). The vertical bars represent the range about the mean.

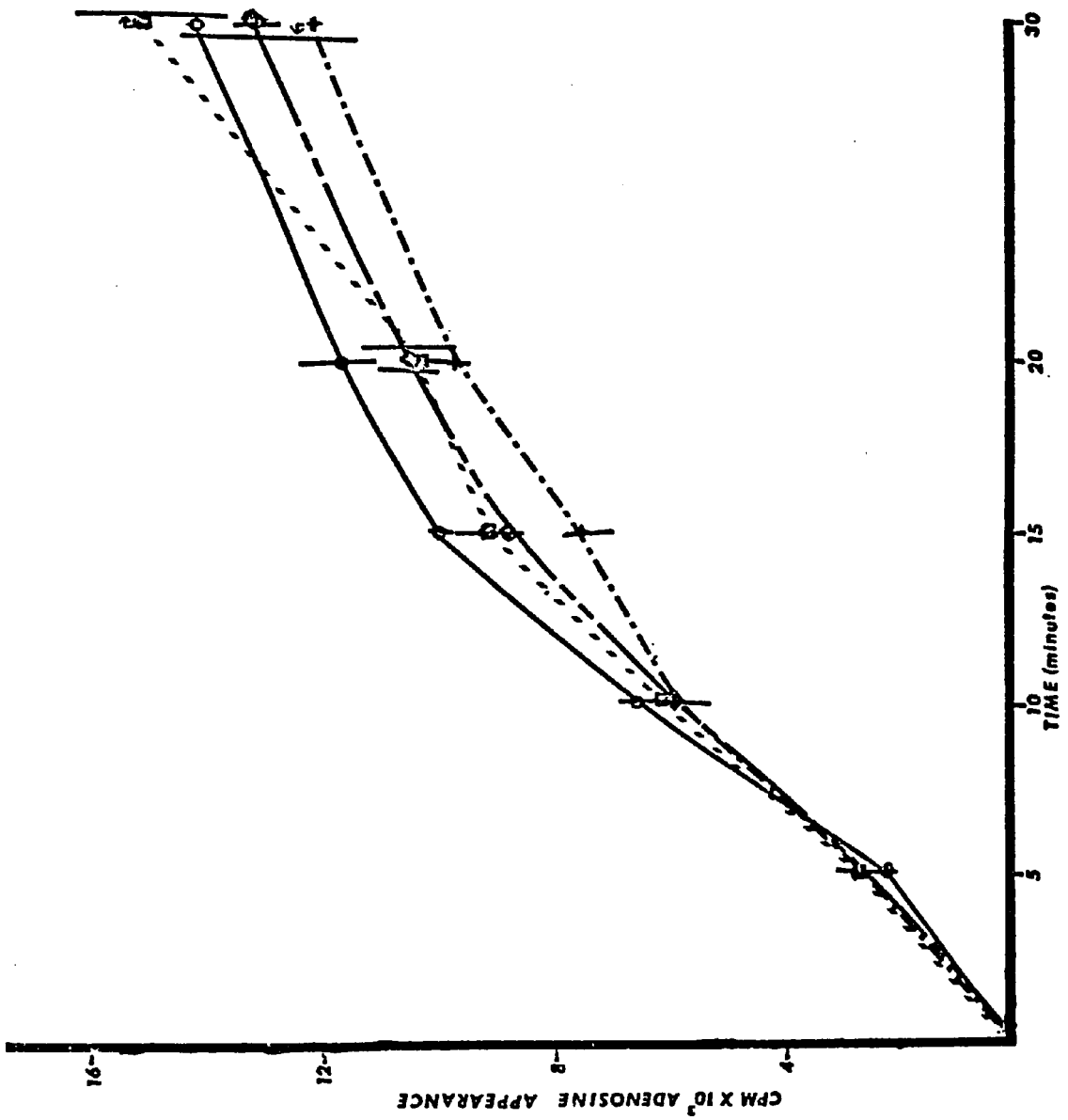

CPM X 10³ ADENOSINE APPEARANCE
16
12
8
4
TIME (minutes)
5
10
15
20
30

Figure 16

APPENDIX II OF TABLES

Table I. Naturally occurring cannabinoids

This table gives all of the naturally occurring cannabinoids isolated and characterized to date. The trivial names for the cannabinoids have for the most part been derived from the base parent aromatic alcohol within the dibenzopyran ring structure. Therefore cannabidiol, cannabinol and tetrahydrocannabinol common nomenclature stems from the olivetol moiety; cannabidivarol, cannabivarol and tetrahydrocannabivarol from divarinol and cannabidiorcol, cannabiorcol and tetrahydrocannabiorcol from the orcinol moiety. This convention was proposed by Vree, Breimer, van Ginneken and van Rossum (1972) and will be used in the following tables. The abbreviations of the cannabinoids that have not been conventionalized and will be represented by the letters at the phonetic accents in the word followed by the suffix as to the side chain at the 3 position ($-C_1$,$-C_3$,$-C_5$). The chemical numbering used in the table is not strictly classical but is a compromise for clarity of comparisons and consistancy. The psychoactivity index is based on Δ^9-THC-C_5 as the standard criteria of potency and has been arbitrarily set at 1.0. This number represents the ratio of the mean dose in mg/kg for a variety of animals which causes "pronounced behavior alterations" to the minimum dose of Δ^9-THC required to produce the same degree of alteration. Unfortunately, many of these are subjective estimates. The citations give the source of synthesis, structure, properties, isolation and pharmacological testing of each of the cannabinoids.

Table I

NATURALLY OCCURING CANNABINOIDS

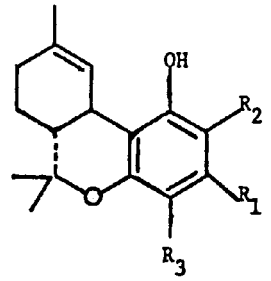
Trivial Name (abbreviation)	Structure	Psychoactivity Index and Properties	Citation
			
(-)-Δ^9-trans-tetrahydrocannabinol (Δ^9-THC-C_5)	R_1=C_5H_{11} R_2=H R_3=H	P.I.=1.0; insoluble in H_2O; soluble in most organic solv.; $[\alpha]_D^{CHCl_3}$= -175°; [UV_λ^{max}(nm)= 210,275,282]; MW= 314.5; viscous yellow-red oil at 25°C; high affinity for glass	Gaoni and Mechoulam,1964 Razdan and Handrick,1970 Mechoulam,Braun et al.,1967 Mechoulam and Gaoni,1964 Isbell et al.,1967 Petrzilka and Sikemeier, 1967; Taylor et al.,1966
(-)-Δ^9-trans-tetrahydrocannabivarol tetrahydrocannabivarin (syn) (Δ^9-THC-C_3)	R_1=C_3H_7 R_2=H R_3=H	P.I.=.1-.25; less potent than Δ^9-THC-C_5 but faster acting	Vree et al.,1972 Gill et al., 1970 Merkus,1971

Table I

NATURALLY OCCURING CANNABINOIDS

Trivial Name (abbreviation)	Structure	Psychoactivity Index and Properties	Citation
(-)-Δ^9-trans-tetrahydrocannabiorcin (Δ^9-THC-C_1)	R_1=CH_3 R_2=H R_3=H		Vree et al.,1972
(-)-Δ^9-trans-tetrahydrocannabinolic acid-A (Δ^9-THCA(A)-C_5)	R_1=C_5H_{11} R_2=COOH R_3=H	P.I.=0; soluble in methanol; decarboxylation upon heating to Δ^9-THC-C_5 at 75°C or more	Yamauchi et al.,1967 Mechoulam,1970 Mechoulam and Ben-Zvi,1969. Davis et al.,1970
(-)-Δ^9-trans-tetrahydrocannabinolic acid-B (Δ^9-THCA(B)-C_5)	R_1=C_5H_{11} R_2=H R_3=COOH	P.I.=0; soluble in methanol; thermodynamically unstable, may rearrange to Δ^9-THCA(A)-C_5	Mechoulam,Ben-Zvi, et al., 1969;Mechoulam,1970

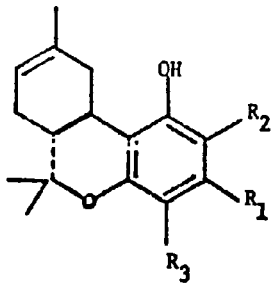


Table I

NATURALLY OCCURING CANNABINOIDS

Trivial Name (abbreviation)	Structure	Psychoactivity Index and Properties	Citation
(-)-Δ^8-trans-tetrahydrocannabinol (Δ^8-THC-C_5)	R_1=C_5H_{11} R_2=H R_3=H	P.I.= 1.0; similar properties as Δ^9-THC-C_5; $[\alpha]_D^{CHCl_3}$ = -268°	Taylor and Strojny,1960 Gaoni and Mechoulam,1966 Hively et al.,1966; Jen, Hughes and Smith,1967; Mechoulam, Braun and Gaoni, 1967; Petrzitka and Sikemeier,1967; Razdan and Handrick,1970
(-)-Δ^8-trans-tetrahydrocannabivarol (Δ^8-THC-C_3)	R_1=C_3H_7 R_2=H R_3=H		Vree et al.,1972
(-)-Δ^8-trans-tetrahydrocannabiorcin (Δ^8-THC-C_1)	R_1=CH_3 R_2=H R_3=H		Vree et al.,1972
(-)-Δ^8-trans-tetrahydrocannabinolic acid-A (Δ^8-THCA(A)-C_5)	R_1=C_5H_{11} R_2=COOH R_3=H	P.I.=0; decarboxylated to Δ^8-THC-C_5 upon heating to 75°C or more	Davis et al.,1970

Table I

NATURALLY OCCURING CANNABINOIDS

Trivial Name (abbreviation)	Structure	Psychoactivity Index and Properties	Citation
(-)-Δ^8-trans-tetrahydrocannabinolic acid-B (Δ^8-THCA(B)-C_5)	R_1=C_5H_{11} R_2=H R_3=COOH	P.I.=0; same as Δ^8-THCA(A)-C_5	Davis et al.,1970

Table I

NATURALLY OCCURING CANNABINOIDS

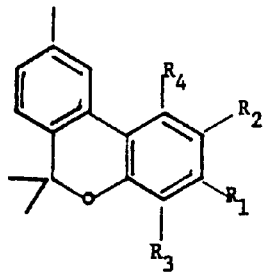
Trivial Name (abbreviation)	Structure	Psychoactivity Index and Properties	Citation
			
cannabinol ($CBN-C_5$)	$R_1=C_5H_{11}$ $R_2=H$ $R_3=H$ $R_4=OH$	P.I.=0; no euphoric properties potentiates barbiturates;some sedative action at large doses MW=310; oxidation product of Δ^9-THC-C_5	Adams, Cain and Baker,1940 Mechoulam,1970 Adams, Pease et al.,1940 Loewe,1950 Mechoulam, Shani et al.,1970 Ghosh et al.,1940 Cahn,1933
cannabivarol ($CBN-C_3$)	$R_1=C_3H_7$ $R_2=H$ $R_3=H$ $R_4=OH$	P.I.= ; oxidation product of Δ^9-THC-C_3	Vree et al.,1972

Table I

NATURALLY OCCURING CANNABINOIDS

Trivial Name (abbreviation)	Structure	Psychoactivity Index and Properties	Citation
cannabiorcin ($CBN\text{-}C_1$)	$R_1=CH_3$ $R_2=H$ $R_3=H$ $R_4=OH$		Vree et al.,1972
cannabinolic acid-A ($CBA(A)\text{-}C_5$)	$R_1=C_5H_{11}$ $R_2=COOH$ $R_3=H$ $R_4=OH$		Korte et al.,1965
cannabinol-1-methyl ether ($CBN\text{-}1\text{-}MEt\text{-}C_5$)	$R_1=C_5H_{11}$ $R_2=H$ $R_3=H$ $R_4=OCH_3$		Bercht,Lousberg et al.,1973

Table I

NATURALLY OCCURING CANNABINOIDS

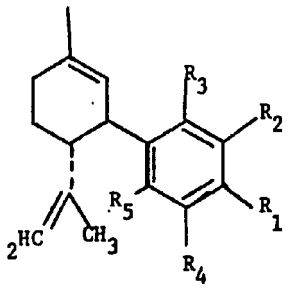
Trivial Name (abbreviation)	Structure	Psychoactivity Index and Properties	Citation
			
trans-cannabidiol ($CBD-C_5$)	R_1=C_5H_{11} R_2=H R_3=OH R_4=H R_5=OH	P.I.=0; insoluble in H_2O and hot or cold 10% NaOH; $[\alpha]_D^{28}$= -125°; converted to Δ^9-THC-C_5 upon heating or treatment with acid; antibiotic activity	Adams, Wolf et al.,1940 Mechoulam and Shvo,1963 Mechoulam and Gaoni,1965; 1966; Petrizilka et al., 1967
trans-cannabidivarol cannabidivarin (syn) ($CBD-C_3$)	R_1=C_3H_7 R_2=H R_3=OH R_4=H R_5=OH		Vree et al.,1972 Vollner et al.,1969

Table I

NATURALLY OCCURING CANNABINOIDS

Trivial Name (abbreviation)	Structure	Psychoactivity Index and Properties	Citation
trans-cannabidiorcin ($CBD\text{-}C_1$)	R_1=CH_3 R_2=H R_3=OH R_4=H R_5=OH		Vree et al.,1972
trans-cannabidiolic acid ($CBDA\text{-}C_5$)	R_1=C_5H_{11} R_2=COOH R_3=OH R_4=H R_5=OH	P.I.=0; not euphoric but has sedative effect on mammals; antibiotic action on bacteria	Schultz and Haffner,1958 Kabelik,Krejci and Santavy, 1960; Krejci and Santavy, 1955; Mechoulam and Ben-Zvi,1969
trans-cannabidiol-5-methyl ether ($CBD\text{-}5\text{-}MEt\text{-}C_5$)	R_1=C_5H_{11} R_2=H R_3=OH R_4=H R_5=OCH_3	P.I.=-; [$UV_{\lambda max}^{MeOH}$(nm)= 233;273; 280]; MW=328.2; formed from the methylation of $CBD\text{-}C_5$, $CBDA(A)\text{-}C_5$ or cyclization of $CBG\text{-}5\text{-}MEt\text{-}C_5$	Shoyama et al.,1972

Table I

NATURALLY OCCURING CANNABINOIDS

Trivial Name (abbreviation)	Structure	Psychoactivity Index and Properties	Citation
trans-cannabidiol-1-methyl ether (CBD-1-MEt-C_5)	R_1=C_5H_{11} R_2=H R_3=OCH_3 R_4=H R_5=OH		Vree et al.,1972
trans-cannabidiolic acid-1-methyl ether (CBDA(A)-1-MEt-C_5)	R_1=C_5H_{11} R_2=COOH R_3=OCH_3 R_4=H R_5=OH		Vree et al.,1972
trans-cannabidiolic acid-5-methyl ether (CBDA(A)-5-MEt-C_5)	R_1=C_5H_{11} R_2=COOH R_3=OH R_4=H R_5=OCH_3		Waller,1971

Table I

NATURALLY OCCURING CANNABINOIDS

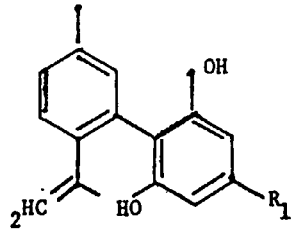
Trivial Name (abbreviation)	Structure	Psychoactivity Index and Properties	Citation
ortho-trans-cannabidiol (σ-CBD-C_5)	R_1=OH R_2=H R_3=C_5H_{11} R_4=H R_5=OH		Vree et al.,1972
cannabinodiol (CBO-C_5)	 R_1=C_5H_{11}		Vree et al.,1972
cannabinodivarol (CBO-C_3)	R_1=C_3H_7		Vree et al.,1972

Table I

NATURALLY OCCURING CANNABINOIDS

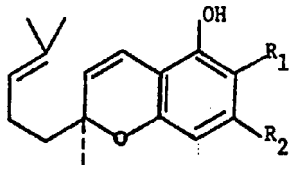
Trivial Name (abbreviation)	Structure	Psychoactivity Index and Properties	Citation
			
cannabichromene ($CBC-C_5$)	R_1=H R_2=C_5H_{11}	P.I.=0-0.01; loss of neuromuscular co-ordination in mice. decomposes to $CBL-C_5$; optically inactive	Cardillo et al.,1968 Kane and Razdan,1968 Mechoulam and Yagnitinsky, et al.,1968; Mechoulam and Shani,1970; deZeeuw et al.,1973;Claussen et al., 1966.
cannabivarichromene ($CBC-C_3$)	R_1=H R_2=C_3H_7	P.I.= - ; decomposes to $CBL-C_3$	deZeeuw,Vree et al.,1973 Vree et al.,1972
cannabichromenic acid-A ($CBCA(A)-C_5$)	R_1=COOH R_2=C_5H_{11}		Shoyama et al.,1968

Table I

NATURALLY OCCURING CANNABINOIDS

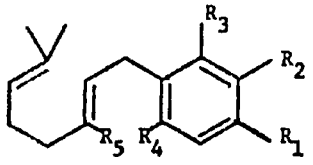
Trivial Name (abbreviation)	Structure	Psychoactivity Index and Properties	Citation
			
cannabigerol ($CBG-C_5$)	$R_1=C_5H_{11}$ $R_2=H$ $R_3=OH$ $R_4=OH$ $R_5=CH_3$	P.I.=0; some antibiotic properties	Gaoni and Mechoulam,1964 Grunfeld and Edery,1969 Kabelik et al.,1960 Schultz and Haffner,1958 Mechoulam and Yagen,1969 Mechoulam,Shani et al.,1970
cannabigivarol ($CBG-C_3$)	$R_1=C_3H_7$ $R_2=H$ $R_3=OH$ $R_4=OH$ $R_5=CH_3$		Vree et al.,1972

Table I

NATURALLY OCCURING CANNABINOIDS

Trivial Name (abbreviation)	Structure	Psychoactivity Index and Properties	Citation
cannabigerolic acid ($CBGA-C_5$)	$R_1=C_5H_{11}$ $R_2=COOH$ $R_3=OH$ $R_4=OH$ $R_5=CH_3$	P.I.=0 ; antibiotic activity on bacteria	Korte,Haag et al.,1965 Mechoulam and Ben-Zvi,1969 Kabelik et al.,1960
cannabigerol-1-methyl ether ($CBG-1-MEt-C_5$)	$R_1=C_5H_{11}$ $R_2=H$ $R_3=OCH_3$ $R_4=OH$ $R_5=CH_3$		Vree et al.,1972
cannabigerol-5-methyl ether ($CBG-5-MEt-C_5$)	$R_1=C_5H_{11}$ $R_2=H$ $R_3=OH$ $R_4=OCH_3$ $R_5=CH_3$		Yamauchi et al.,1968

Table I

NATURALLY OCCURING CANNABINOIDS

Trivial Name (abbreviation)	Structure	Psychoactivity Index and Properties	Citation
cannabigivarol-1-methyl ether (CBG-1-MEt-C_3)	R_1=C_3H_7 R_2=H R_3=OCH_3 R_4=OH R_5=CH_3		Vree et al.,1972
cannabigerolic acid-5-methyl ether (CBGA-5-MEt-C_5)	R_1=C_5H_{11} R_2=COOH R_3=OH R_4=OCH_3 R_5=CH_3		Waller,1971

Table I

NATURALLY OCCURING CANNABINOIDS

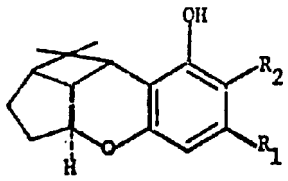
Trivial Name (abbreviation)	Structure	Psychoactivity Index and Properties	Citation
			
cannabicyclol cannabipinol(syn) ($CBL-C_5$)	$R_1=C_5H_{11}$ $R_2=H$	P.I.=0-0.05;$CBC-C_5$ or CBCA(A)-C_5 cyclizes in the presence of light to form $CBL-C_5$	Vree et al.,1972 Crombie et al.,1968 Kane and Razdan,1968 Razdan and Pars,1970 Begley et al.,1970 Mechoulam, Shani et al.,1970 Claussen et al.,1968 Shoyama et al.,1972 Mechoulam and Gaoni,1967
cannabivaricyclol ($CBL-C_3$)	$R_1=C_3H_7$ $R_2=H$		Vree et al.,1972

Table I

NATURALLY OCCURING CANNABINOIDS

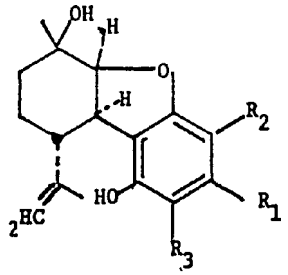
Trivial Name (abbreviation)	Structure	Psychoactivity Index and Properties	Citation
cannabicyclolic acid-A (CBLA(A)-C_5)	R_1=C_5H_{11} R_2=COOH	P.I.= -; generated from CBCA-C_5 in the presence of light; m.p.=152-5°C; optically inactive; [$IR_{\nu\ max}^{CHCl_3}$(cm^{-1})=3080-3260(OH),1665(C=O)]; [$UV_{\lambda\ max}^{MeOH}$(nm)=227.5,271,306]	Shoyama et al.,1972
trans-cannabielsoin (CBS-C_5)	 R_1=C_5H_{11} R_2=H R_3=H		Bercht,Lousberg et al.,1973

Table I

NATURALLY OCCURING CANNABINOIDS

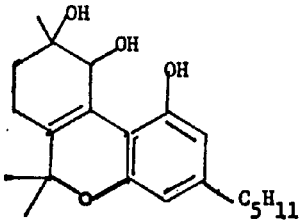
Trivial Name (abbreviation)	Structure	Psychoactivity Index and Properties	Citation
trans-cannabielsoic acid-A ($CBSA(A)-C_5$)	$R_1=C_5H_{11}$ $R_2=H$ $R_3=COOH$	P.I.=0	Shani and Mechoulam,1970
trans-cannabielsoic acid-B ($CBSA(B)-C_5$)	$R_1=C_5H_{11}$ $R_2=COOH$ $R_3=H$	P.I.=0	Shani and Mechoulam,1970
$\Delta^{6a,10a}$-tetrahydrocannabitriol ($\Delta^{6a,10a}$-TCT-C_5)			postulated

Table I

NATURALLY OCCURING CANNABINOIDS

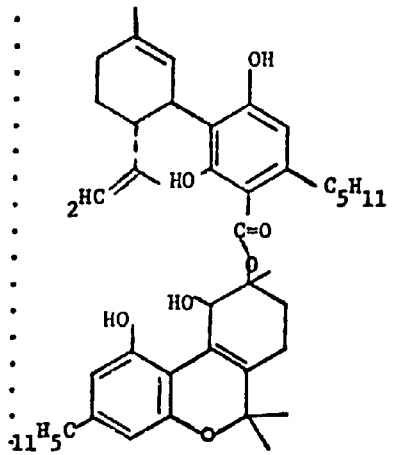
Trivial Name (abbreviation)	Structure	Psychoactivity Index and Properties	Citation
cannabitriol ester of CBDA(B)-C_5			von Spulak et al.,1968

Table I

NATURALLY OCCURING CANNABINOIDS

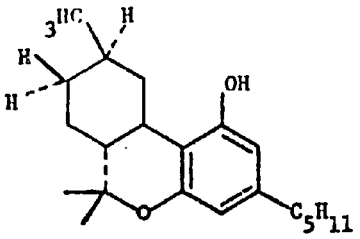
Trivial Name (abbreviation)	Structure	Psychoactivity Index and Properties	Citation
trans-hexahydrocannabinol ($HHC-C_5$)		P.I.=0.025	Edery et al.,1971 Turner et al.,1973

Table II. Cannabinoid content of marihuana and hashish
This table gives the percent by weight of the major naturally occurring cannabinoids present in Cannabis preparations as determined by gas-liquid chromatography techniques.

Table II

CANNABINOID CONTENT of MARIHUANA and HASHISH (% by weight)

Sample/ Source	% Δ^9-THC mean (range)	% Δ^8-THC mean (range)	% CBD mean (range)	% CBN mean (range)	% CBDA mean (range)	% Δ^9-THCA mean (range)	Citation
MARIHUANA							
Mexico	3.2	...	0.35	...	...	...	Sanchez et al.,1972
Thailand	2.5	...	0.44	...	...	...	" "
India	2.2	...	0.15	...	...	...	" "
Brazil	0.19	...	...	0.41	...	...	" "
Chile	0.171	...	0.116	0.013	...	...	" "
Russia	0.14	...	1.7	...	...	...	" "
Morocco	0.096	...	0.129	0.123	...	...	" "
Nigeria	0.079	...	...	0.481	...	...	" "
Turkey	0.07	...	1.5	...	...	...	" "
Geneva	0.059	...	0.129	0.017	...	...	" "
Canada	trace	...	0.103	...	...	...	" "
Chile	3.15(1.8-5.3)	0.49(0.1-1.0)	2.07(0.8-3.9)	1.56(0.8-3.1)	2.06(0.75-4.3)	...	" "
Chile	4.66(2.7-6.3)	0.07(0-trace)	3.23(1.1-5.9)	0.35(0.1-5.0)	3.43(1.9-5.8)	...	" "
Iowa	trace-0.34	...	-- (0.14-0.6)	...	--(0.25-0.33)	trace-0.20	Masoud and Doorenbos,1973
Minnesota	--(0.03-0.04)	...	--(0.07-0.11)	...	--(0.27-0.33)	trace	" "
Mexico	--(0.07-0.55)	...	...	...	...	--(0.29-1.4)	Fetterman et al.,1971a
Unknown	1.08	...	0.1	0.37	...	...	Davis et al.,1970
New Jersey	0.70	...	0.35	0.16	...	...	" "
New Jersey	0.26	...	1.00	0.19	...	...	" "
Unknown	0.79	...	0.16	0.86	...	...	" "
Unknown	0.65	...	0.13	0.83	...	...	" "
Unknown	0.76	...	0.19	1.02	...	...	" "
Minnesota	0.36	...	2.70	0.24	...	...	" "
Minnesota	0.22	...	0.85	0.14	...	...	" "
Mass.	0.32	...	1.10	0.24	...	...	" "
Unknown	0.93(.02-3.5)	--(0.0-0.02)	0.12(0.0-2.0)	0.1(0.0-0.3)	...	...	Canadian Commission,1972
Unknown	0.56(.02-3.5)	--(0.0-0.02)	0.16(.01-1.1)	0.01(0.0-.06)	...	...	" "
Thailand	4.8	...	0.14	0.12	...	...	Doorenbos et al.,1971
Panama	3.2	...	0.29	0.80	...	...	" "
Minnesota	0.038	...	1.7	0.15	...	...	" "

Table II

CANNABINOID CONTENT of MARIHUANA and HASHISH (% by weight)

Sample/ Source	% Δ^9-THC mean (range)	% Δ^8-THC mean (range)	% CBD mean (range)	% CBN mean (range)	% CBDA mean (range)	% Δ^9-THCA mean (range)	Citation
Mississippi grown marihuana :Seed source							
France	0.085	...	...	...	...	...	Waller,1969
Italy	--(.041-.068)	...	...	...	...	...	"
Mexico	--(1.31-1.47)	...	...	...	...	...	"
Sweden	0.021	...	...	...	...	...	"
Turkey	--(.05-0.4)	...	...	...	...	...	"
Mexico	--(1.0-1.2)	...	--(.075-.32)	--(.54-.59)	...	...	Fetterman et al.,1971
Turkey	--(.007-.37)	...	--(0.24-1.7)	--(0.0-0.06)	...	...	" "
Minnesota	--(.043-.054)	...	--(0.71-1.3)	--(0.0-0.01)	...	...	" "
Italy	0.32	...	1.2	0.085	...	...	" "
France	0.11	...	1.55	0.04	...	...	" "
Turkish	--(.01-.04)	...	--(0.44-.52)	...	--(0.6-0.8)	--(.02-.06)	Masoud et al.,1973
Sweden	trace	...	0.03	...	0.04	trace	" "
Italy	0.06	...	--(0.08-.21)	...	--(0.1-0.45)	--(.13-.15)	" "
France	trace	...	0.08	...	0.12	0.05	" "
India	--(0.81-2.2)	...	--(0.12-2.1)	...	...	...	Doorenbos et al.,1971
Thailand	--(2.1-3.2)	...	--(0.08-.41)	...	...	...	" "
Korea	--(0.94-1.2)	...	--(0.13-0.16)	...	...	...	" "
Iowa	0.068	...	2.7	...	...	...	" "
Russia	0.16	...	2.4	...	...	...	" "
Lebanon	1.0	...	2.0	...	...	...	" "
Mexico	--(3.7-3.9)	...	--(0.18-0.31)	--(0.11-0.22)	...	...	" "
Minnesota	0.077	...	1.6	0.14	...	...	" "
South Africa	--(1.18-1.6)	...	0.0	...	...	...	Turner et al.,1973

Table II

CANNABINOID CONTENT of MARIHUANA and HASHISH (% by weight)

Sample/ Source	% Δ^9-THC mean (range)	% Δ^8-THC mean (range)	% CBD mean (range)	% CBN mean (range)	% CBDA mean (range)	% Δ^9-THCA mean (range)	Citation
England grown marihuana:							
Seed source							
Nepal	1.6 (bracts)	...	0.8	...	...	...	Shulgin,1971
Nepal	3.4 (bracteoles)	...	2.1	...	...	...	"
Nepal	1.4 (bracts)	...	6.4	...	...	...	"
Nepal	5.8 (bracteoles)	...	3.5	...	...	...	"
Turkey	... (leaf)	...	0.3	...	...	...	"
Turkey	... (bracts)	...	0.49	...	...	...	"
Turkey	... (bracts)	...	1.7	...	...	...	"
Thailand	1.16 (leaf)	...	0.08	...	...	...	"
Confiscated HASHISH							
Ontario	4.82(1.0-14.3)	--(0.0-0.2)	5.5(1.09-12.9)	0.66(0.0-2.63)	...	...	Canadian Commission,1972
Nigeria	0.098	...	...	0.481	...	...	Korte,1970
Brasil	0.19	...	...	0.410	...	...	"
Cyprus	0.38	...	...	0.06	...	...	"
Morocco	0.096	...	0.129	0.123	...	...	"
Geneva	0.087	...	0.129	0.017	...	...	"
Canada	trace	...	0.103	...	...	...	Canadian Commission,1972
Canada	5.4(0.8-6.4)	--(0.1-0.2)	11.7(2.2-13.6)	0.3(0.0-1.0)	...	...	Davis et al.,1970
Unknown	1.08	...	0.15	0.44	...	...	" "
Unknown	11.53	...	15.84	2.66	...	...	

Table III. Methods for the qualitative determination of the cannabinoids.
This table lists the methods that have been employed to identify the cannabinoids present in marihuana and to confirm plant identity.

Table III

METHODS for the QUALITATIVE DETERMINATION of the CANNABINOIDS

Name or Reagent	Procedure	Cannabinoid/ Color / ug limit	Citation
Alloxan-H_2SO_4	solution of alloxan-H_2SO_4 is mixed with petroleum ether extract of Cannabis; probably non-specific; general field test	CBD/ bright red /-- "red oil"/ red /--	Adams et al.,1940a
Baker-Rosenkrantz histochemical localization	sections of tissue from animals pretreated with Δ^9-THC are stained with fast garnet GBC and visualized under light microscope	Δ^9-THC/ orange-red/ 5.0	Baker and Rosenkrantz,1972
Beam	5 drops of 5% ethanolic-KOH are added to an ethanol or lingroin extract of Cannabis; good field test	CBD/ blue / 0.5 CBN/ no color Δ^9-THC/ no color CBDA/ blue/ 0.5 CBG/ blue / 0.5	Beam,1911 Duquenois,1950
Blackie	solution of benzaldehyde and isobutanol is mixed with extract of Cannabis	No color	Blackie,1941 Korte,1970
Chloroform-H_2SO_4	extract of Cannabis is dissolved in chloroform and acid is added to form layer, color develops at interface	CBD/ brown-red / -- "red oil"/ brown-red / --	Adams et al.,1940a

Table III

METHODS for the QUALITATIVE DETERMINATION of the CANNABINOIDS

Name or Reagent	Procedure	Cannabinoid/ Color / ug limit	Citation
Duquenois-Negm	non-specfic field test on Cannabis extract with solution of H_2O_2 and H_2SO_4	CBDA / orange / 500 CBN / green-brown / 500 CBD / pink or red / 500 Δ^9-THC / violet / 500	Duquenois and Negm,1938 Duquenois,1950
Duquenois-Negm	field test on Cannabis extract or as developer following TLC or PC; solution of vanillin, acetaldehyde and HCl are applied to chromatograms or extracts; full development of color in 1 hour	CBD / blue-orange / 0.01 CBDA / orange / 0.01 CBN / violet / 10.0 Δ^9-THC / violet / 1.0	Duquenois and Negm,1938 Duquenois,1950 Korte,1970
Echtblausalz B (Fast Blue B Salt)	most commonly used stain for cannabinoid visualization following TLC or PC. Merkus; TLC is performed on lingroin extract redissolved in $CHCl_3$, spotted on precoated silica gel G plates impregnated with dimethylformamide and $CHCl_3$ (6:4) and dried at 25°C for 30 min. Spots are developed 3X with cyclohexane and then visualized with Fast Blue B salt in 0.5% NaOH	CBDA / orange / 0.01 R_f=0.082 CBD-C_3 / orange / 0.01 R_f=0.145 CBD-C_5 / orange / 0.01 R_f=0.273 CBN-C_3 / violet / 0.01 R_f=0.4 CBN-C_5 / violet / 0.01 R_f=0.68 Δ^9-THC-C_3 / red / 0.01 R_f=0.76 Δ^9-THC-C_5 / red / 0.01 R_f=0.90	Korte and Sieper,1964 Merkus,1971

Table III

METHODS for the QUALITATIVE DETERMINATION of the CANNABINOIDS

Name or Reagent	Procedure	Cannabinoid/ Color / ug limit	Citation
Extinction ratios	red oil test, very non-specific; based on the absorbance ratio of a red oil extract at 260/280 (nm) and at 300/310 (nm)	not visually colorimetric	Grlic,1962
Ferric chloride	dissolve a petroleum ether extract in 1 ml of ethanol and and 2 drops of 0.1% $FeCl_3$; will react with most phenols so non-specific	? / grey-green /--	Duquenois,1950
Formaldehyde-H_2SO_4	solution of H_2CO and H_2SO_4 is mixed with a lingrain extract of Cannabis ; general field test	CBD / deep red / --	Adams et al.,1940a
Forrest	incubation of the cannabinoid mixture with 1-dimethylaminonapthalene-5-sulfonyl chloride in acetone followed by TLC and UV visualization or spectrophotofluorometry of oxy-dansyl cannabinoid derivatives	CBC / yellow / 0.0005 Δ^9-THC / yellow / 0.0005 Δ^8-THC / yellow / 0.0005 $\Delta^{6a,10a}$-THC / yellow / 0.0005 synhexyl / yellow / 0.0005 CBL / yellow / 0.0005 CBN / yellow / 0.0005 CBD / yellow / 0.0005 Δ^9-11-OH-THC / pink / 0.0005	Forrest et al.,1971 Melikian and Forrest,1973

Table III

METHODS for the QUALITATIVE DETERMINATION of the CANNABINOIDS

Name or Reagent	Procedure	Cannabinoid/ Color / ug limit	Citation
Fulton	solution of 1% $FeCl_3$ in methanol and ammonium acetate is mixed with lingroin extract of Cannabis; relatively insensitive field test	CBDA / red-blue /--	Fulton,1942
Furfurole-H_2SO_4	extracts of Cannabis are diluted with 90% ethanol and mixed with 1 drop of 1% solution of furfurole and stratified with 5 ml of H_2SO_4; not specific for cannabinoids, field test	? / brown to violet brown /--	Kechatov,1959
Ghamrawy	solution of p-dimethylaminobenz-aldehyde-H_2SO_4 is mixed with an extract of Cannabis and heated on water bath, brown-red color occurs which turns blue upon addition of water	CBD / brown-red / 0.5 CBN / brown-red / 1.0 Δ^9-THC / brown-red / 1.0	Korte,1970 Duquenois,1950
Gibb	spray on a solution of 2,6-dibromo quinone-4-chlorimide in isopropyl-amine or ethanol following TLC or PC	CBD / pink / 1.0 CBN / blue-green / 1.0 Δ^9-THC / grey-violet / 1.0	Grlic,1962 Korte,1970

Table III

METHODS for the QUALITATIVE DETERMINATION of the CANNABINOIDS

Name or Reagent	Procedure	Cannabinoid/ Color / ug limit	Citation
Mechoulam	extract of Cannabis is chromatographed on silica gel (TLC) and developed in lingroin-ether (4:1) and visualized with Fast Blue B Salt in dilute NaOH	CBL / R_f=0.62 CBD / R_f=0.58 Δ^8-THC / R_f=0.57 Δ^9-THC / R_f=0.51 CBN / R_f=0.47 CBC / R_f=0.43 CBG / R_f=).42	Mechoulam,1970 Gaoni and Mechoulam,1971
Millon	petroleum ether extract of Cannabis is treated with Millon's reagent and heated in a water bath; non-specific, reacts with many phenols	CBD / red precipitate /-- "red oil" / red precipitate /--	Duquenois,1950
Opianic acid-H_2SO_4	field test for extracts of Cannabis in lingroin	CBD / bright red /-- "red oil" / bright red /--	Adams et al.,1940a
Petcoff	a centrifugational chromatography technique through silica gel mini-columns of the cannabinoids with visualization by Fast Blue B Salt	CBC / red-violet / 0.5 CBN / violet / 1.0 Δ^9-THC / red / 1.0 Δ^8-THC / red / 1.0 CBD / yellow-orange / 2.5 CBL / red / 15.0	Petcoff et al.,1971

Table III

METHODS for the QUALITATIVE DETERMINATION of the CANNABINOIDS

Name or Reagent	Procedure	Cannabinoid/ Color / ug limit	Citation
Rathenasinkam	modified acid Beam test; dissolve extract of Cannabis in 1 ml. glacial acetic acid and add 1 drop of conc. H_2SO_4 and mix well.	? / pink-rose /--	Rathenasinkam,1948
Rim test	mainly for the identification of marihuana; good field test; small amount of marihuana is cleared for microscopic examination with chlorohydrate; part of the mixture is chromatographed (TLC) and visualized with Fast Blue B Salt while the remainder is checked microscopically for key histological structures		Segelman et al.,1973
Shoyama	cannabinoids are methylated by treatment with diazomethane in benzene followed by TLC on silica gel G with hexane-$CHCl_3$-ethylacetate	methyl-CBC / R_f=0.57 methyl-CBC / R_f=0.51 methyl-THC / R_f=0.48 methyl-CBD / R_f=0.43 methyl-CBG / R_f=0.40	Shoyama et al.,1970

Table III

METHODS for the QUALITATIVE DETERMINATION of the CANNABINOIDS

Name or Reagent	Procedure	Cannabinoid/ Color / ug limit	Citation
Vanillin-H_3PO_4	petroleum ether extract of Cannabis is subjected to a freshly prepared solution of dilute vanillin-H_3PO_4; a pink coloration is produced which disappears upon addition of ethanol; non-specific; reacts with most phenols	? / pink /--	Duquenois,1950
Scaringelli	spectrophotometric identification of marihuana from a petroleum ether extract which is reconstituted in ethanol, separated into two aliquots and subjected to acid and basic conditions respectively and subsequently scanned from 360-220 nm against blanks. Based on characteristic absorbtion of the cannabinoids		Scaringelli,1961

Table IV. Methods for the quantitative determination of the cannabinoids.

Table IV

METHODS for the QUANITITATIVE DETERMINATION of the CANNABINOIDS

Method	Compounds Determined	Procedure	Citation
Antibody fluorescence-quenching	Δ^9-THC but applicable to other cannabinoids	couple THC to paraaminobenzoic acid and then bind to hemocyanin, use this as antigen in rabbit to produce antibodies. Standard curve is generated from quenching ratio vs. THC concentration; limit-10pmol/ml	Grant et al.,1972
^{13}C-nuclear magnetic resonance spectroscopy	Δ^9-THC and Δ^8-THC but applicable to other cannabinoids	analysis of charge density of electrons and chemical shifts	Wenkert et al.,1972
Duquenois-Negm	applications on pure cannabinoids only; has not been throughly investigated	same as acealdehyde-vanillin-HCl test described for qualitative test except color development is measured colorimetrically.	Duquenois,1950
Fluorescent derivatives	Δ^9-THC,Δ^8-THC,$\Delta^{6a,10a}$-THC, Δ^9-11-OH-THC,CBC,CBN,CBD, CBL and synhexyl	incubation of mixture of cannabinoids with DANS-Cl in acetone followed by TLC or PC, then spectrofluorimetric assay at 400-500 nm; limit-0.5 nanograms	Forrest et al.,1971

Table IV

METHODS for the QUANITITATIVE DETERMINATION of the CANNABINOIDS

Method	Compounds Determined	Procedure	Citation
Gas-liquid chromatography combined with mass spectrometry	25 naturally occuring cannabinoids and 45 synthetic cannabinoids are resolved	ether extraction of Cannabis material followed by GLC for separation and mass spectrometry for identification; retention times, structure and characterization of cannabinoids	Vree et al.,1972
Gas-liquid chromatography combined with mass spectrometry	identification of cannabinoid metabolites	need between 1 ug- 1 ng of metabolite for analysis	Breimer et al.,1972 Mikes,Hofmann and Waser,1971
Gas-liquid chromatography	separation and identification of synthetic cannabinoids with emphasis on cis, trans and iso forms of THC encountered during synthesis	GLC of ether solutions and information on retention times and structure	Vree et al.,1973
Gas-liquid chromatography	major cannabinoids	solid injection technique to eliminate solvent interaction and condensation reactions due to high temperatures employed	Rasmussen et al.,1972
Gas-liquid chromatography	Δ^9-THC and Δ^9-THC acids	preparation of trimethylsilyl- cannabinoid derivatives to prevent decarboxylation upon heating	Fetterman et al.,1971a

Table IV

METHODS for the QUANITITATIVE DETERMINATION of the CANNABINOIDS

Method	Compounds Determined	Procedure	Citation
Gas-liquid chromatography	naturally occuring cannabinoids with 3-propyl side chain; CBD-C_3, CBN-C_3 and Δ^9-THC-C_3. Major cannabinoids of pentyl side chain and C_{19}-C_{32} alkanes	GLC and comparasion of various supports; OV-25, OV-17, QF-1 and SE-30	deZeeuw et al., 1972a deZeeuw et al., 1973
Gas-liquid chromatography combined with electron capture detection	Δ^9-THC and Δ^8-THC but applicable to other cannabinoids	prepare pentafluorobenzoate esters of cannabinoids for GLC with eletron capture (^{63}Ni) detection to picogram level	Garrett and Hunt, 1973
Spectrofluorometric assay	Δ^9-THC but applicable to other cannabinoids which have been purified	formation of malic acid derivatives of the cannabinoids followed by excitation and spectrofluorimetry; sensitive to 0.05 ug/ml	Bullock et al., 1970
Mukhopadhyay colorimetric assay	quanititation of phenolic compounds in Cannabis extract; possible modification to assay pure cannabinoids	Standard pure cannabinoids are treated with p-nitroaniline-HCl along with unknown samples; yellow products can be measured colorimetrically	Mukhopadyay et al., 1943

Table IV

METHODS for the QUANITITATIVE DETERMINATION of the CANNABINOIDS

Method	Compounds Determined	Procedure	Citation
Vapor-phase chromatography	CBC, CBD, CBN, CBG, Δ^9-THC and Δ^8-THC; applicable to other cannabinoids		Gaoni and Mechoulam,1971 Mechoulam,1970 Farmilo and Davis,1961 Davis et al.,1963

Table V. Synthetic cannabinoids

This table lists many of the synthetic cannabinoids prepared and tested for pharmacological potency in various animals. From this data, many general structural activity relationships can be established. For easier referencing, the cannabinoids are listed as derivatives of the side groups (i.e. $\Delta^{6a,10a}$-THC-3-butane); this nomenclature is not normally recommended.

Table V

SYNTHETIC CANNABINOIDS

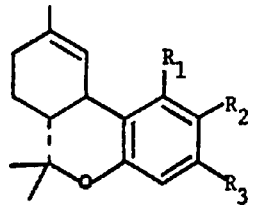
Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
			
(-)-Δ^9-cis-tetrahydrocannabinol	R_1=OH R_2=H R_3=C_5H_{11}	P.I.=0	Taylor et al.,1966 Razdan and Handrick,1970 Farenholtz et al.,1966;1967
(+)-Δ^9-trans-tetrahydrocannabinol	R_1=OH R_2=H R_3=C_5H_{11}	P.I.=0; dextrorotatory form totally inactive	Edery et al.,1971
Δ^9-trans-tetrahydrocannabinol-1-acetate	R_1=$OCOCH_3$ R_2=H R_3=C_5H_{11}	P.I.=0.1	Edery et al.,1971

Table V

SYNTHETIC CANNABINOIDS

Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
Δ^9-trans-tetrahydrocannabinol-1-diethylaminobutyrate ester ·HCl	R_1=$OCO(CH_2)_3^+NH(CH_2CH_2)_2Cl^-$ R_2=H R_3=C_5H_{11}	P.I.= ?; water soluble ester probably cleaved by esterase *in vivo* to Δ^9-THC-C_5	Howes,1970 Pars and Razdan,1971
Δ^9-trans-tetrahydrocannabinol-1-diethylaminoethane ether ·HCl	R_1=$O(CH_2)_2^+NH(CH_2CH_2)_2Cl^-$ R_2=H R_3=C_5H_{11}	P.I.= ?; water soluble ether	Howes,1970
Δ^9-trans-tetrahydrocannabinol-1-diethylaminoethane ester ·HCl	R_1=$OCO(CH_2)_2^+NH(CH_2CH_2)_2Cl^-$ R_2=H R_3=C_5H_{11}	P.I.= ?; water soluble ester probably cleaved to Δ^9-THC-C_5 by esterase *in vivo*	Pars and Razdan,1971
Δ^9-trans-tetrahydrocannabinol-3-α,β-dimethylheptane	R_1=OH R_2=H R_3=$CH(CH_3)CH(CH_3)C_5H_{11}$	P.I.=25	Loev et al.,1973

Table V

SYNTHETIC CANNABINOIDS

Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
Δ^9-trans-tetrahydrocannabinol-2-ethane	R_1=OH R_2=CH_2CH_3 R_3=C_5H_{11}	P.I.=0.17	Edery et al.,1971
Δ^9-trans-tetrahydrocannabinol-3-hexane	R_1=OH R_2=H R_3=C_6H_{13}	P.I.=2-5	Edery et al.,1971
Δ^9-trans-tetrahydrocannabinol-2-methane	R_1=OH R_2=CH_3 R_3=C_5H_{11}	P.I.=0.2	Edery et al.,1971
Δ^9-trans-tetrahydrocannabinol-1-methyl ether	R_1=OCH_3 R_2=H R_3=C_5H_{11}	P.I.=0	Edery et al.,1971

Table V

SYNTHETIC CANNABINOIDS

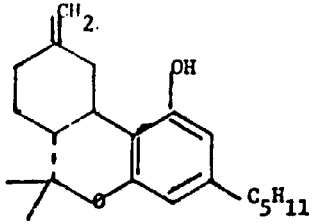
Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
Δ^9-trans-tetrahydrocannabinol-3-α-methylpentane	R_1=OH R_2=H R_3=$CH(CH_3)C_4H_9$	P.I.=0.25	Edery et al.,1971
Δ^9-trans-tetrahydrocannabinol-1-p-oxypiperdine butyrate ester ·HCl	R_1=$OCO(CH_2)_3$- ·HCl R_2=H R_3=C_5H_{11}	P.I.=0.5; desterified by microsomal esterase to Δ^9-THC-C_5; water soluble	Zitko et al.,1972
$\Delta^{9(11)}$-trans-tetrahydrocannabinol		P.I.=0.05-0.1; cis form has also been prepared	Edery et al.,1971 Fahrenholtz et al.,1966; 1967 Mechoulam,1970 Christensen et al.,1971
Δ^9-tetrahydrocannabinols		numerous cis-trans isomers and their GLC behavior	Vree et al.,1972 Vree et al.,1973

Table V

SYNTHETIC CANNABINOIDS

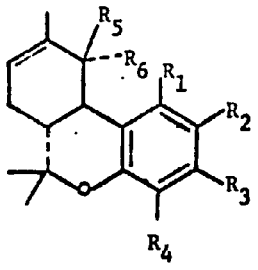
Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
			
(+)-Δ^8-trans-tetrahydrocannabinol	R_1=OH R_2=H R_3=C_5H_{11} R_4=H R_5=H R_6=H	P.I.=0; dextrorotatory form inactive	Edery et al.,1971
(-)-Δ^8-trans-tetrahydrocannabinol-1-acetate	R_1=$COCH_3$ R_2=H R_3=C_5H_{11} R_4=H R_5=R_6=H	P.I.=0.1	Edery et al.,1971

Table V

SYNTHETIC CANNABINOIDS

Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
Δ^8-trans-tetrahydrocannabinol-2-acetate	R_1=OH R_2= $OCOCH_3$ R_3=C_5H_{11} R_4=R_5=R_6=H	P.I.=0	Edery et al.,1971
Δ^8-trans-tetrahydrocannabinol-2-carboxymethane	R_1=OH R_2=$COOCH_3$ R_3=C_5H_{11} R_4=R_5=R_6=H	P.I.=0	Edery et al.,1971
Δ^8-trans-tetrahydrocannabinol-4-carboxymethane	R_1=OH R_2=H R_3=C_5H_{11} R_4=$COOCH_3$ R_5=R_6=H	P.I.=0	Edery et al.,1971

Table V

SYNTHETIC CANNABINOIDS

Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
Δ^8-trans-tetrahydrocannabinol-1,4-diacetate ester	R_1=$OCOCH_3$ R_2=H R_3=C_5H_{11} R_4=$OCOCH_3$ R_5=R_6=H	P.I.=0	Edery et al.,1971
Δ^8-trans-tetrahydrocannabinol-3-α,β-dimethylheptane	R_1=OH R_2=H R_3=$CH(CH_3)CH(CH_3)C_5H_{11}$ R_4=R_5=R_6=H	P.I.=0.5-50	Edery et al.,1971 Loev et al.,1973
Δ^8-trans-tetrahydrocannabinol-3-α,β-dimethylpentane	R_1=OH R_2=H R_3=$CH(CH_3)CH(CH_3)C_3H_7$ R_4=R_5=R_6=H	P.I.=0.17	Edery et al.,1971

Table V

SYNTHETIC CANNABINOIDS

Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
Δ^8-trans-tetrahydrocannabinol-2-ethane	R_1=OH R_2=C_2H_5 R_3=C_5H_{11} R_4=R_5=R_6=H	P.I.=0.5	Edery et al.,1971
Δ^8-trans-tetrahydrocannabinol-4-ethane	R_1=OH R_2=H R_3=C_5H_{11} R_4=C_2H_5 R_5=R_6=H	P.I.=0	Edery et al.,1971
Δ^8-trans-tetrahydrocannabinol-3-hexane	R_1=OH R_2=H R_3=C_6H_{13} R_4=R_5=R_6=H	P.I.=1-20	Edery et al.,1971 Loev et al.,1973

Table V

SYNTHETIC CANNABINOIDS

Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
Δ^8-trans-tetrahydrocannabinol-10β-hydroxide	R_1=OH R_2=H R_3=C_5H_{11} R_4=H R_5=OH R_6=H	P.I.=0.1	Edery et al.,1971
Δ^8-trans-tetrahydrocannabinol-10α-hydroxide	R_1=OH R_2=H R_3=C_5H_{11} R_4=H R_5=H R_6=OH	P.I.=1.0	Edery et al.,1971
Δ^8-trans-tetrahydrocannabinol-2-methane	R_1=OH R_2=CH_3 R_3=C_5H_{11} R_4=R_5=R_6=H	P.I.=0.05	Edery et al.,1971

Table V

SYNTHETIC CANNABINOIDS

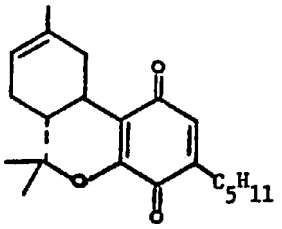
Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
Δ^8-trans-tetrahydrocannabinol-4-methane	R_1=OH R_2=H R_3=C_5H_{11} R_4=CH_3 R_5=R_6=H	P.I.=0	Edery et al.,1971
Δ^8-trans-tetrahydrocannabinol-1-methyl ether	R_1=OCH_3 R_2=H R_3=C_5H_{11} R_4=R_5=R_6=H	P.I.=0	Edery et al.,1971
Δ^8-tetrahydrocannabinols		numerous cis, trans and iso forms encountered during synthesis	Vree et al.,1972 Vree et al.,1973
Δ^8-trans-tetrahydrocannabinol-quinone		P.I.=0	Edery et al.,1971

Table V

SYNTHETIC CANNABINOIDS

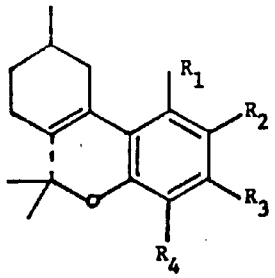
Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
			
$\Delta^{6a,10a}$-tetrahydrocannabinol-3-butane	R_1=OH R_2=H R_3=C_4H_9 R_4=H	P.I.=0.4	Adams et al.,1942a
$\Delta^{6a,10a}$-tetrahydrocannabinol-3-α-butylpentane	R_1=OH R_2=H R_3=$CH(C_4H_9)_2$ R_4=H	P.I.=0.3	Loev et al.,1973

Table V

SYNTHETIC CANNABINOIDS

Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
$\Delta^{6a,10a}$-tetrahydrocannabinol-1-dimethylaminocarbamate ester	R_1=OCON$(CH_3)_2$ R_2=H R_3=C_5H_{11} R_4=H	P.I.=0.1; sedative effect; probably converted to $\Delta^{6a,10a}$-THC-C_5 in vivo	Hardman et al.,1971
$\Delta^{6a,10a}$-tetrahydrocannabinol-3-dimethylaminoethane	R_1=OH R_2=H R_3=$CH_2CH_2N(CH_3)_2$ R_4=H		Pars and Razdan,1971
$\Delta^{6a,10a}$-tetrahydrocannabinol-3-α,β-dimethylheptane (DMH-THC)	R_1=OH R_2=R_4=H R_3=$CH(CH_3)CH(CH_3)C_5H_9$ R_4=H	P.I.=250-500; long duration and hypotensive action	Adams et al.,1949 Sidell et al.,1973 Loev et al.,1973
$\Delta^{6a,10a}$-tetrahydrocannabinol-3-α-dimethylheptane	R_1=OH R_2=R_4=H R_3=$C(CH_3)_2C_6H_{13}$	P.I.=500-1000; most potent derivative synthesized	Loev et al.,1973

Table V

SYNTHETIC CANNABINOIDS

Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
$\Delta^{6a,10a}$-tetrahydrocannabinol-3-α,β-dimethyl-2-heptene	R_1=OH R_2=H R_3=$C(CH_3)$=$C(CH_3)C_5H_{11}$ R_4=H	P.I.=250	Loev et al.,1973
$\Delta^{6a,10a}$-tetrahydrocannabinol-3-α,β-dimethyloctane	R_1=OH R_2=R_4=H R_3=$CH(CH_3)CH(CH_3)C_6H_{13}$	P.I.=50	Loev et al.,1973
$\Delta^{6a,10a}$-tetrahydrocannabinol-3-α,β-dimethylhexane	R_1=OH R_2=R_4=H R_3=$CH(CH_3)CH(CH_3)C_4H_9$	P.I.=10-50	Loev et al.,1973
$\Delta^{6a,10a}$-tetrahydrocannabinol-3-heptane	R_1=OH R_2=R_4=H R_3=C_7H_{15}	P.I.=1.0	Adams et al.,1942a

Table V

SYNTHETIC CANNABINOIDS

Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
$\Delta^{6a,10a}$-tetrahydrocannabinol-3-hexane (parahexyl; synhexyl (syn))	R_1=OH R_2=R_4=H R_3=C_6H_{13}	P.I.=0.5-1.5	Adams et al.,1942a
$\Delta^{6a,10a}$-tetrahydrocannabinol-3-methane	R_1=OH R_2=R_4=H R_3=CH_3	P.I.=0.1	Adams et al.,1942a
$\Delta^{6a,10a}$-tetrahydrocannabinol-3-β-methylheptane	R_1=OH R_2=R_4=H R_3=$CH_2CH(CH_3)C_5H_{11}$	P.I.=1.0	Loev et al.,1973
$\Delta^{6a,10a}$-tetrahydrocannabinol-3-α-methylheptane	R_1=OH R_2=R_4=H R_3=$CH(CH_3)C_6H_{13}$	P.I.=50	Loev et al.,1973

Table V

SYNTHETIC CANNABINOIDS

Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
$\Delta^{6a,10a}$-tetrahydrocannabinol-3-α-methylhexane	R_1=OH R_2=R_4=H R_3=$CH(CH_3)C_5H_{11}$	P.I.=10.0	Loev et al.,1973
$\Delta^{6a,10a}$-tetrahydrocannabinol-3-α-methylhexyl ether	R_1=OH R_2=R_4=H R_3=$OCH(CH_3)C_5H_{11}$	P.I.=5.0	Loev et al.,1973
$\Delta^{6a,10a}$-tetrahydrocannabinol-1-methyl,isopropyl ether phosphate ester	R_1=$OPO(CH_3)(OCH(CH_3)_2)$ R_2=R_3=R_4=H	P.I.=0	Hardman et al.,1971
$\Delta^{6a,10a}$-tetrahydrocannabinol-3-α-methyloctane	R_1=OH R_2=R_4=H R_3=$CH(CH_3)C_7H_{15}$	P.I.=25-300; hypotensive and sedative effects of long duration	Dagirmanjian and Boyd,1962 Hardman et al.,1971 Loev et al.,1973

Table V

SYNTHETIC CANNABINOIDS

Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
$\Delta^{6a,10a}$-tetrahydrocannabinol-3-nonane	R_1=OH R_2=R_4=H R_3=C_9H_{19}	P.I.=0.05-2.0	Loev et al.,1973
$\Delta^{6a,10a}$-tetrahydrocannabinol-3-octane	R_1=OH R_2=R_4=H R_3=C_8H_{17}	P.I.=0.6	Adams et al.,1942a
$\Delta^{6a,10a}$-tetrahydrocannabinol-3-pentane	R_1=OH R_2=R_4=H R_3=C_5H_{11}	P.I.=0.25-0.5; 8 possible isomers synthesized by Aaron	Aaron and Ferguson,1968 Adams and Baker,1940c Hardman et al.,1971 Ghosh et al.,1940a Loev et al.,1973 Edery et al.,1971
$\Delta^{6a,10a}$-tetrahydrocannabinol-3-propane	R_1=OH R_2=R_4=H R_3=C_3H_7	P.I.=0.4	Adams et al.,1942a

Table V

SYNTHETIC CANNABINOIDS

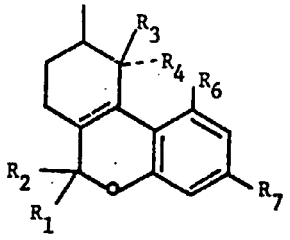
Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
			
$\Delta^{6a,10a}$-tetrahydrocannabinol-12, 13-diethane	$R_1=C_2H_5$ $R_2=C_2H_5$ $R_3=R_4=H$ $R_5=CH_3$ $R_6=OH$ $R_7=C_5H_{11}$;(dimethylheptane)	P.I.=0.12 (0.5)	Adams et al.,1941a Loev et al.,1973
$\Delta^{6a,10a}$-tetrahydrocannabinol-6-one-3-dimethylheptane	$R_1=R_2=(=O)$ $R_3=R_4=H$ $R_5=CH_3$ $R_6=OH$ $R_7=CH(CH_3)CH(CH_3)C_5H_{11}$	P.I.=0	Loev et al.,1973

Table V

SYNTHETIC CANNABINOIDS

Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
$\Delta^{6a,10a}$-tetrahydrocannabinol-12, 13-dipropane	$R_1=R_2=C_3H_7$ $R_3=R_4=H$ $R_5=CH_3$ $R_6=OH$ $R_7=C_5H_{11}$	P.I.=0.04	Adams et al.,1941a
$\Delta^{6a,10a}$-tetrahydrocannabinol-10-methane	$R_1=R_2=R_3=CH_3$ $R_4=R_5=H$ $R_6=OH$ $R_7=C_5H_{11}$	P.I.=0.25	Adams et al.,1941a

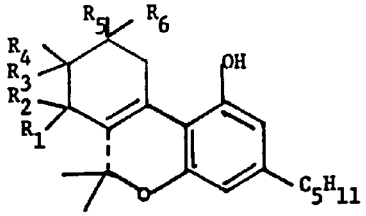


Table V

SYNTHETIC CANNABINOIDS

Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
$\Delta^{6a,10a}$-tetrahydrocannabinol-9-dimethane	$R_1=R_2=R_3=R_4=H$ $R_5=R_6=CH_3$	P.I.=0.1	Adams et al.,1942b
$\Delta^{6a,10a}$-tetrahydrocannabinol-9-ethane	$R_1=R_2=R_3=R_4=R_6=H$ $R_5=C_2H_5$	P.I.=0.22	Adams et al.,1942b
$\Delta^{6a,10a}$-tetrahydrocannabinol-8-methane	$R_1=R_2=R_3=R_6=H$ $R_4=R_5=CH_3$	P.I.=0.11	Adams et al.,1942b
$\Delta^{6a,10a}$-tetrahydrocannabinol-7-methane	$R_1=R_5=CH_3$ $R_2=R_3=R_4=R_6=H$	P.I.=0.75	Adams et al.,1942b
$\Delta^{6a,10a}$-tetrahydrocannabinol-pentamethylene	$R_1=R_2=R_3=R_4=R_5=R_6=H$	P.I.=0.21	Adams et al.,1942b

Table V

SYNTHETIC CANNABINOIDS

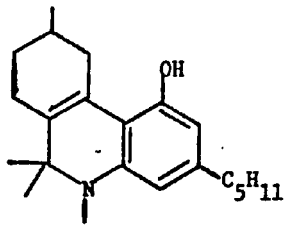
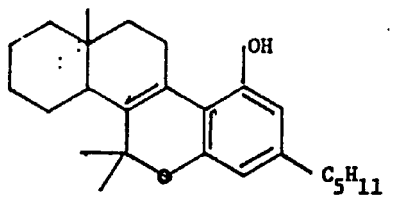
Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
$\Delta^{6a,10a}$-5-azatetrahydrocannabinol-5-methyl		P.I.=0	Adams et al.,1940c Hoops et al.,1968
$\Delta^{6a,10a}$-tetrahydrocannabinol (steroid analog)		P.I.=0	Razdan et al.,1968

Table V

SYNTHETIC CANNABINOIDS

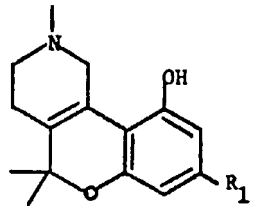
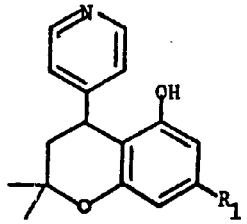
Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
$\Delta^{6a,10a}$-azatetrahydrocannabinol analog	 R_1=$CH(CH_3)CH(CH_3)C_5H_{11}$ or C_5H_{11}	P.I.=0.1-1.0	Dewey et al.,1970 Pars and Razdan,1971 Pars et al.,1966
dibenzopyran-pyridine azine analog	 R_1=$CH(CH_3)CH(CH_3)C_5H_{11}$	P.I.=0.1	Razdan and Pars ,1971

Table V

SYNTHETIC CANNABINOIDS

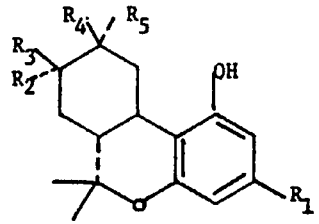
Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
			
hexahydrocannabinol	R_1=C_5H_{11} R_2=R_3=R_5=H R_4=CH_3	P.I.=0.2-0.5	Edery et al.,1971 Adams et al.,1942a
hexahydrocannabinol	R_1=C_5H_{11} R_2=R_3=R_4=H R_5=CH_3	P.I.=0.025; may be natural degradation product of one of the cannabinoids (Turner)	Edery et al.,1971 Turner et al.,1973
hexahydrocannabinol-3-butane	R_1=C_4H_9 R_2=R_3=R_5=H R_4=CH_3	P.I.=0.37	Adams et al.,1942a

Table V

SYNTHETIC CANNABINOIDS

Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
hexahydrocannabinol-3-heptane	$R_1=C_7H_{15}$ $R_2=R_3=R_5=H$ $R_4=CH_3$	P.I.=0.8	Adams et al.,1942a
hexahydrocannabinol-3-hexane	$R_1=C_6H_{13}$ $R_2=R_3=R_5=H$ $R_4=CH_3$	P.I.=0.2	Adams et al.,1942a
hexahydrocannabinol-8α-hydroxide	$R_1=C_5H_{11}$ $R_2=OH$ $R_3=R_5=H$ $R_4=CH_3$	P.I.=0	Edery et al.,1971
hexahydrocannabinol-8β-hydroxide	$R_1=C_5H_{11}$ $R_2=R_5=H$ $R_3=OH$ $R_4=CH_3$	P.I.=0.05	Edery et al.,1971

Table V

SYNTHETIC CANNABINOIDS

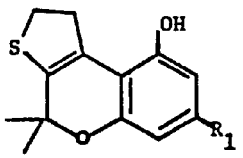
Trivial Name / substitution	Structure	Psychoactivity Index and Properties	Citation
hexahydrocannabinol-3-octane	$R_1=C_8H_{17}$ $R_2=R_3=R_5=H$ $R_4=CH_3$	P.I.=0.25	Adams et al.,1942a
hexahydrocannabinol-3-propane	$R_1=C_3H_7$ $R_2=R_3=R_5=H$ $R_4=CH_3$	P.I.=0.25	Adams et al.,1942a
dibenzopyran-cyclopentene-sulphur analog	 $R_1=CH(CH_3)CH(CH_3)C_5H_{11}$	P.I.=0.1-0.25; faster onset of action	Dewey et al.,1970 Pars and Razdan,1971

Table VI. Gives the approximate minimum effective dose for various cannabinoids and animals with reference to the route of administration and source of information.

Table VI

MINIMAL EFFECTIVE DOSE OF THE CANNABINOIDS

Cannabinoid	Animal	Minimum Effective Dose	Route	Citation
Δ^9-THC	mouse	0.1 mg/kg	i.p.	Dolby et al., 1974
Δ^9-THC	mouse	15 mg/kg	s.c.	Razdan et al., 1969
Δ^9-THC	rat	<0.5 mg/kg	i.p;i.g.	Elsmore et al., 1972
Δ^9-THC or Δ^8-THC	monkey	50-500 ug/kg	i.v.	Ho et al., 1972a
Δ^9-THC	monkey	200 ug/kg	i.g.	Ferraro et al., 1972
Δ^9-THC	man	5 ug/kg	smoked	Isbell et al., 1967 Perez-Reyes et al., 1971 Kiplinger et al., 1971
Δ^9-THC	man	300 ug/kg	i.g.	Hollister et al., 1968
Δ^9-THC	man (chronic user)	5-7 ug/kg	i.v.	Lemberger et al., 1972
Δ^9-THC	man	18±6 ug/kg	i.v.	Perez-Reyes et al., 1972; 1973
11-OH-Δ^9-THC	man	17±4 ug/kg	i.v.	Perez-Reyes et al., 1972; 1973
11-OH-Δ^9-THC	man	40 ug/kg	i.v.	Lemberger et al., 1972
8β-OH-Δ^9-THC	man	48±15 ug/kg	i.v.	Perez-Reyes et al., 1973
8α-OH-Δ^9-THC	man	>186 ug/kg	i.v.	Perez-Reyes et al., 1973

Table VII. This table gives the dose of various cannabinoids required to produce death in 50% of the tested animals (LD_{50}). All LD_{50} have the units of mg/kg. § means dose administered was non-lethal.

Table VII

LD_{50} For Various Cannabinoids

Cannabinoid	Animal	Route of Administration	LD_{50}	Citation
Δ^9-THC	rat(male)	i.v.	29 mg/kg	Phillips et al., 1971
Δ^9-THC	rat(male)	i.v.	100 mg/kg	Dewey et al., 1972
Δ^9-THC	rat(male)	i.p.	373 mg/kg	Phillips et al., 1971
Δ^9-THC	rat(male)	i.p.	430 mg/kg	Dewey et al., 1972
Δ^9-THC	rat(male)	i.v.	60 mg/kg	Harris, 1971
Δ^9-THC	rat(male)	i.p.	430 mg/kg	Harris, 1971
Δ^9-THC	rat(male)	i.g.	666 mg/kg	Phillips et al., 1971
Δ^9-THC	rat(male)	i.g.	>2000 mg/kg	Dewey et al., 1972
Δ^9-THC	rat(male)	i.g.	1015-1910 mg/kg	Thompson et al., 1973
Δ^9-THC	rat(female)	i.g.	800-1410 mg/kg	Thompson et al., 1973
Δ^9-THC	mice(male)	i.v.	42 mg/kg	Phillips et al., 1971
Δ^9-THC	mice(male)	i.v.	60 mg/kg	Dewey et al., 1972
Δ^9-THC	mice(male)	i.p.	455 mg/kg	Phillips et al., 1971
Δ^9-THC	mice(male)	i.p.	168 mg/kg	Dewey et al., 1972
Δ^9-THC	mice(male)	i.v.	100 mg/kg	Harris, 1971
Δ^9-THC	mice(male)	i.p.	500 mg/kg	Sofia, 1972
Δ^9-THC	mice(male)	i.g.	482 mg/kg	Phillips et al., 1971
Δ^9-THC	mice(male)	i.g.	1900 mg/kg	Dewey et al., 1972

Table VII

LD_{50} For Various Cannabinoids

Cannabinoid	Animal	Route of Administration	LD_{50}	Citation
Δ^9-THC	dog	i.v.	100 mg/kg	U.S. Commission, 1972
Δ^9-THC	monkey	i.v.	690 mg/kg §	U.S. Commission, 1972
Δ^8 or Δ^9-THC	dog	i.g.	3000 mg/kg §	Thompson et al., 1973
Δ^8 or Δ^9-THC	monkey	i.g.	9000 mg/kg §	Thompson et al., 1973
Δ^8-THC	rat(male)	i.v.	97 mg/kg	Dewey et al., 1972 Harris, 1971
Δ^8-THC	rat(male)	i.p.	560 mg/kg	Dewey et al., 1972 Harris, 1971
Δ^8-THC	rat(male)	i.g.	1980 mg/kg	Thompson et al., 1973 Dewey et al., 1972
Δ^8-THC	mice(male)	i.v.	31 mg/kg	Harris, 1971 Dewey et al., 1972
Δ^8-THC	mice(male)	i.p.	210 mg/kg	Dewey et al., 1972
Δ^8-THC	mice(male)	i.g.	>2000 mg/kg	Dewey et al., 1972

Table VIII. The table should be used in conjunction with Figure 8. This table gives the pathway number which codes the reaction in Figure 8 to the animals in which this process is observed, the reaction site when in vitro, the psychoactivity index, which represents the ratio of the approximate equalvalent dose of metabolite to that of Δ^9-THC required to produce behavioral or perceptual (in case of man) alterations.

Table VIII

METABOLISM OF Δ^9-THC

Pathway number	Animal	Reaction site	Psychoactivity Index	Method	Citation
1	man	--	1.0-1.2	in vivo	Lemberger et al., 1972; 1971 Perez-Reyes et al., 1972; 1973
1	rat	liver	2(i.v.) 14(i.c.)	in vivo	Wall, 1971 Ho et al., 1973
1	monkey	--	--	in vivo	Ho et al., 1972
1	mice	liver	2(i.v.) 15(i.c.)	in vitro	Christensen et al., 1971
1	rabbit	liver	--	in vitro	Nilsson et al., 1970 Wall, 1971
1	rat	liver	--	in vitro	Kupfer et al., 1973 Wall, 1970; 1971 Nakazawa and Costa, 1971 Burstein and Kupfer, 1971
2	man	--	0.25(i.v.)	in vivo	Lemberger et al., 1972 Lemberger, 1973
2	monkey	--	--	in vivo	Ben-Zvi et al., 1971 Ho et al., 1972
2	rat	liver	--	in vivo	Ho et al., 1973 Wall, 1971
2	rat	lung	--	in vivo	Cohn, 1971 Leighty, 1973
2	mice	liver	0.02(i.v.) 0.6(i.c.)	in vitro	Christensen et al., 1971
2	rat	liver	--	in vitro	Wall, 1971 Kupfer et al., 1972

Table VIII

METABOLISM OF Δ^9-THC

Pathway number	Animal	Reaction site	Psychoactivity Index	Method	Citation
2	rabbit	liver	--	in vitro	Nilsson et al., 1970 Wall, 1971
3	rabbit	liver	0.25	in vitro	Ryrfeldt et al., 1971 Wall, 1971
3	monkey	--	0.25(i.v.)	in vivo	Ben-Zvi et al., 1971
4	not reported; assumed intermediate				
5	rabbit	--	--	in vivo	Lemberger et al., 1972a Burstein et al., 1972
6					Mechoulam et al., 1972
7	proposed metabolites				Ryrfeldt et al., 1973
8	rat	--	--	in vivo	Mikes et al., 1971
9	rat	liver	--	in vitro	Wall et al., 1971

Table IX. This table gives the metabolites of Δ^8-THC recovered from the excreta and plasma of several animal species. It should be used in conjunction with Figure 9.

Table IX

METABOLISM OF Δ^6-THC

Pathway number	Animal	Reaction site	Psychoactivity Index	Method	Citation
1	mice	liver	2(i.v.) 14(i.c.)	in vitro	Christensen et al., 1971
1	rabbit	--	--	in vivo	Burstein et al., 1970
1	rat	liver	1-2(i.v.)	in vitro	Foltz et al., 1970 Wall, 1971
2	rat	liver	0.05(i.v.) 0.3(i.c.)	in vitro	Wall, 1971
2	mice	liver	0.05(i.v.) 0.5(i.c.)	in vitro	Christensen et al., 1971
3	mice	liver	0.05(i.v.) 0.5(i.c.)	in vitro	Christensen et al., 1971
3	rat	liver	0.05(i.v.) 0.5(i.c.)	in vitro	Wall, 1971
4	rat	--	--	in vivo	Estevez et al., 1973
5	rabbit	--	0.0	in vivo	Nilsson et al., 1973
6	dog	liver	--	in vitro	Maynard et al., 1971

Table X. This table gives the metabolites formed *in vitro* from the incubators of CBN and CBD with liver homogenates from several animal sources. It should be used in conjunction with Figure 10.

Table X

METABOLISM OF CBN

Pathway number	Animal	Reaction site	Psychoactivity Index	Method	Citation
1,2,3	rat	liver	--	in vitro	Wall, 1971
1	rabbit	liver	--	in vitro	Agurell et al., 1971a
1	rat	liver	0.0	in vitro	Widman et al., 1971a

METABOLISM OF CBD

Pathway number	Animal	Reaction site	Psychoactivity Index	Method	Citation
1,2,3	rat	liver	--	in vitro	Nilsson et al., 1971

Table XI. This table gives the percent distribution of the labeled cannabinoid metabolites in the feces or bile and urine of several species after the administration of the listed isotopically labeled cannabinod. The body half life is the time required for 50% of the administered labeled cannabinoid to be received from the urine and feces in the form of metabolites.

Table XI

METABOLITE DISTRIBUTION AND BODY HALF-LIVES OF THE CANNABINOIDS

Cannabinoid	Animal	Route	Metabolite Distribution feces or bile	urine	Body Half-life	Citation
Δ^9-THC	rat(female)	i.v.	80% (f)	20%	7 days	Agurell et al., 1969
Δ^9-THC	rat(male)	i.g.	42% (f) 22% (b)	<10%	96 hours	Turk et al., 1973
Δ^9-THC	rat(male)	i.v.	2.7% (f) 59.4% (b)	<10%	96 hours	Turk et al., 1973
Δ^9-THC	mice(female)	i.p.	80% (f)	9%	48 hours	Harbison et al., 1972
Δ^9-THC	rat(male)	i.p.	30% (f)	8%	—	Miras, 1965 Leighty, 1973
Δ^9-THC	rabbit	i.v.	10% (f)	35%	48 hours	Agurell et al., 1970
Δ^9-THC	human(male)	i.v.	40% (f)	22-30%	3 days	Lemberger et al., 1971b
Δ^9-THC	human(male)	i.v.	41-45% (f)	22-31%	3 days	Lemberger, 1973
Δ^8/Δ^9-THC	rat(male)	i.v.	50-60% (f)	10-15%	—	Klausner and Dingell, 1971
11OH-Δ^9THC	human(male)	i.v.	50% (f)	20-25%	—	Lemberger et al., 1972 Lemberger, 1973
11OH-Δ^8-THC	rabbit	i.v.	3-10% (f)	38-57%	60 hours	Nilsson et al., 1973

Table XII. Effects of decapitation and microwave irradiation on brain levels of cyclic AMP, adenyl cyclase and phosphodiesterase.
Mice were sacrificed either by decapitation or microwave irradiation, following which brain levels of cyclic AMP, adenyl cyclase and phosphodiesterase were determined as described in the text. Blank values for adenyl cyclase and phosphodiesterase were obtained by boiling samples for 3 minutes. Each value represents the mean value obtained by assaying one animal in duplicate.

Table XII

Treatment	Cyclic AMP (pmoles/mg brain)	Adenyl Cyclase (counts/min/mg protein)	Phosphodiesterase (counts/min/mg protein)
Decapitation	8.6; 11.6	2056; 2007	12,236; 11,361
Decapitation and immersion in liquid nitrogen	2.6; 4.2	--- ---	--- ---
Microwave irradiation	2.2; 2.8	6; 0	0;0

Table XIII. Statistical analysis of Δ^9-THC effects on cyclic AMP levels in mouse brain. Legend as in Table XII.
* N is the number of animals tested, each assayed in duplicate.
†P values generated from the statistical method for testing the difference between two populations with the population variance unknown but assumed equal, significance for one-sided upper tail hypothesis test.

Table XIII

Dose of Δ^9-THC	N*	Cyclic AMP p moles/mg brain protein ± 1 S.D.	P value
Control	8	4.9 ± .98	
0.1 mg/kg	8	12.6 ± 1.49	.0005
0.25 mg/kg	8	8.3 ± .84	.0005
0.5 mg/kg	8	7.4 ± .84	.0005
1.0 mg/kg	8	10.8 ± .88	.0005
2.0 mg/kg	3	3.5 ± .22	.025
10.0 mg/kg	8	2.0 ± .66	.0005
50.0 mg/kg	9	4.6 ± .54	.4

Table XIV. Dose effects of Δ^9-THC on cyclic AMP levels in various regions of mouse brain.
Legend as in Table XIII.

Table XIV

		Cortex		Cerebellum		Hypothalamus		Medulla	
		Cyclic AMP		Cyclic AMP		Cyclic AMP		Cyclic AMP	
Dose	N	(pmoles/mg protein)	P Value	(pmoles/mg protein)	P Value	(pmoles/mg protein)	P Value	(pmoles/mg protein)	P Value
Control	8	3.7 ± .89		7.0 ± 1.30		19.9 ± 3.78		9.4 ± 1.32	
0.1 mg/kg	8	13.4 ± 1.12	.0005	13.6 ± 1.88	.0005	15.9 ± 3.99	.025	14.1 ± 2.86	.0005
0.25 mg/kg	8	7.9 ± .28	.0005	8.4 ± 2.36	.08	14.7 ± 1.09	.0008	12.5 ± 1.32	.0005
0.5 mg/kg	8	4.0 ± .25	.2	8.3 ± .60	.01	20.3 ± 4.95	.05	13.6 ± 1.76	.0005
1.0 mg/kg	8	8.4 ± .49	.0005	18.4 ± 3.08	.0005	20.0 ± 1.17	.4	10.4 ± .3	.025
2.0 mg/kg	3	2.5 ± .16	.02	7.6 ± .50	.2	9.4 ± 1.45	.0005	6.8 ± .28	.007
10.0 mg/kg	8	1.0 ± .48	.0005	4.8 ± .58	.0005	25.8 ± 4.27	.004	7.2 ± 2.24	.1
50.0 mg/kg	9	3.5 ± .33	.15	5.6 ± .80	.004	14.6 ± 4.1	.005	11.7 ± 1.30	.0008

Table XV. The effect of 0.25 mg/kg Δ^9-THC injected intraperitoneally on the levels of cyclic AMP in the mouse liver, 30 minutes postinjection. Cyclic AMP assayed by the Gilman method described in the text.

Treatment	Animal	Pmol CAMP/mg wet tissue
control	1	0.80
	2	0.70
	3	1.00
0.25mg/kg-Δ^9-THC	1	0.70
	2	0.80
	3	0.80

Table XV

Table XVI. The effect of Δ^9-THC and CBN when administered *in vivo* (i.p.) to mice on the activity of adenyl cyclase, measured 30 minutes postinjection *in vitro*. The assay procedure is described in the text. Propranolol was used to block β-adrenergic responses. t-tests were performed on the pairs as indicated. N is the number of animals used; assays equal the number of replicates.

Table XVI

Code	N	Assays	Treatment	Cpm CAMP/ug-10 min. ± 1S.D.	Pair	P value
I	3	6	control	16.62 ± 0.44		
II	3	6	0.25 mg/kg Δ^9-THC	19.32 ± 0.55	I:II	<0.0005
III	3	6	10 mg/kg Δ^9-THC	16.13 ± 1.00	I:III	0.15
IV	3	2	0.25 mg/kg Δ^9-THC + 0.3mM propranolol	16.11 ± 0.44	I:IV II:IV	0.01 0.0005
I	3	6	control	14.45 ± 0.44		
II	3	6	0.25 mg/kg Δ^9-THC	17.63 ± 0.59	I:II	<0.0005
III	3	6	10 mg/kg Δ^9-THC	9.68 ± 0.26	I:III	<0.0005
IV	3	2	0.25 mg/kg Δ^9-THC + 0.3mM propranolol	18.75 ± 0.58	I:IV II:IV	<0.0005 0.03
I	3	6	control	12.46 ± 1.46		
II	3	6	0.25 mg/kg Δ^9-THC	14.17 ± 1.39	I:II	0.03
III	3	6	10 mg/kg Δ^9-THC	11.88 ± 1.16	I:III	0.25
IV	3	2	0.25 mg/kg Δ^9-THC + 0.3mM propranolol	13.70 ± 2.85	I:IV II:IV	0.25 0.35

Table XVI

Code	N	Assays	Treatment	Cpm CAMP/ug-10 min. ± 1S.D.	Pair	P value
I	3	6	control	10.83 ± 1.85		
II	3	6	0.25 mg/kg CBN	9.89 ± 1.80	I:II	0.2
III	3	6	10 mg/kg CBN	9.46 ± 0.27	I:III	0.06
IV	3	2	0.25 mg/kg CBN + 0.3mM propranolol	8.64 ± 0.25	I:IV II:IV	0.08 0.20
I	3	4	control	12.85 ± 2.06		
II	3	6	0.25 mg/kg CBN	13.16 ± 1.86	I:II	>0.40
III	3	6	10 mg/kg CBN	13.07 ± 0.86	I:III	>0.40
IV	3	2	0.25 mg/kg CBN + 0.3mM propranolol	10.77 ± 4.38	I:IV II:IV	0.25 0.15

Table XVII. The effect of the cannabinoids on the *in vitro* activity of adenyl cyclase from mouse brain homogenates. N - number of animals assayed separately, Assays - replecate of single animals, t - test used in conjunction with t tables to generate p values as in Appendix.

N	Assays	Treatment	Cpm CAMP/ug-10 min ±1S.D.	Signifigance
2	5	control	10.44 ±2.45	--
2	5	20nM-Δ^9-THC	12.14 ±5.30	(0.27)
3	9	control	10.22 ±2.70	--
3	8	2uM-Δ^9-THC	12.84 ±2.46	(0.03)
2	4	control	9.78 ±0.66	--
1	2	110uM-Δ^9-THC	9.45 ±0.07	(0.28)
4	11	control	7.28 ±1.32	--
4	11	212uM-Δ^9-THC	7.82 ±1.45	(0.15)
2	5	control	10.44 ±3.73	--
2	6	20nM-11-OH-Δ^9-THC	15.67 ±6.86	(0.08)
2	5	control	10.44 ±3.73	--
2	6	2uM-11-OH-Δ^9-THC	13.47 ±2.58	(0.08)
3	9	control	6.32 ±0.25	--
3	9	212uM-11OH-Δ^9-THC	6.17 ±0.90	(0.32)
2	5	control	10.44 ±3.73	--
2	5	20nM-CBN	15.68 ±2.75	(0.023)

Table XVII

N	Assays	Treatment	Cpm CAMP/ug-10 min ±1S.D.	Signifigance
1	3	control	9.72 ±1.86	--
1	2	2uM-CBN	8.12 ±4.14	(0.28)
3	6	control	5.63 ±0.16	--
2	4	200uM-CBN	5.91 ±0.70	(0.18)

Table XVII

Table XVIII. The interactions between the cannabinoids and biogenic amines (5-HT; NE) on the activity of adenyl cyclase in brain homogenates. N = number of animals; Assays = replicates; p value from t test pairs indicated.

Code	N	Assays	Treatment	Cpm CAMP/ug-10 min. ± 1S.D.	Pair	P value
I	2	4	control	5.05 ± 0.25		
II	2	4	control + 1 uM 5-HT	4.52 ± 1.05	I:II	0.18
III	2	4	212 uM Δ^9-THC + 1 uM 5-HT	4.98 ± 0.57	I:III	>0.40
					II:III	0.25
IV	2	4	212 uM 11-OH-Δ^9-THC + 1 uM 5-HT	4.65 ± 1.08	I:IV	0.16
					II:IV	>0.40
V	2	4	212 uM CBN + 1 uM 5-HT	5.31 ± 0.17	I:V	0.075
					II:V	0.10
VI	2	2	control + 10 mM KF	9.64 ± 0.73	I:VI	<0.0005
					II:VI	0.001

Table XVIII

Table XVIII

Code	N	Assays	Treatment	Cpm CAMP/ug-10 min. ± 1S.D.	Pair	P value
I	3	9	control	6.43 ± 0.45		
II	3	9	control + 1 uM NE	10.41 ± 0.91	I:II	<0.0005
III	3	9	20 nM Δ^9-THC + 1 uM NE	10.31 ± 2.25	I:III II:III	<0.0005 >0.40
IV	2	4	2 uM Δ^9-THC + 1 uM NE	13.70 ± 1.03	I:IV II:IV	<0.0005 <0.0005
V	3	9	212 uM Δ^9-THC + 1 uM NE	12.10 ± 2.50	I:V II:V	<0.0005 0.03
VI	2	4	20 nM 11-OH-Δ^9-THC + 1 uM NE	13.38 ± 2.03	I:VI II:VI	<0.0005 0.001
VII	2	4	2 uM 11-OH-Δ^9-THC + 1 uM NE	11.46 ± 3.26	I:VII II:VII	<0.0005 0.15
VIII	2	4	212 uM 11-OH-Δ^9-THC + 1 uM NE	7.98 ± 0.03	I:VIII II:VIII	<0.0005 <0.0005
IX	2	4	20 nM CBN + 1 uM NE	16.71 ± 1.25	I:IX II:IX	<0.0005 <0.0005
X	2	4	2 uM CBN + 1 uM NE	19.90 ± 1.06	I:X II:X	<0.0005 <0.0005
XI	1	2	212 uM CBN + 1 uM NE	8.60 ± 0.42	I:XI II:XI	<0.0005 0.015
XII	1	2	control + 10 mM KF	16.45 ± 3.41	I:XII II:XII	<0.0005 <0.0005
XIII	2	4	control + 50 uM β-estradiol	6.48 ± 0.60	I:XIII	>0.40
XIV	2	4	control + 50 uM β-estradiol + 1 uM NE	12.37 ± 2.61	I:XIV	0.035

Table XIX The effect of several cannabinoids at various concentrations on the conversion of ^{14}C-ATP (from ^{14}C-adenine) to ^{14}C-cyclic AMP in longitudinal mouse brain slices. Brain slices in which the cerebellum, cerebrum, hypothalamus and medulla (median plane of approximately ten 300 u slices) are all represented were incubated with Δ^9-THC and 11-OH-Δ^9-THC in the presence or absence of several biogenic amines (NE or 5HT). Each treatment was run in duplicate (N=2) with several median slices selected from the useable pool. The enzyme activity (adenyl cyclase) was corrected to a weight specific basis (protein). All assays were incubated for 20 minutes at 37°C as described in the text. The p value was generated for a student t test.

Table XIX

Code	N	Treatment	Cpm CAMP/mg-20 min. ± 1S.D.	Pair	P value
I	2	control	873.5 ± 256.7		
II	2	control + 166 nM NE	1075.0 ± 461.0	I:II	0.35
III	2	control + 166 nM 5-HT	1018.0 ± 244.7	I:III	0.35
IV	2	10.6 nM Δ^9-THC	673.0 ± 374.8	I:IV	0.35
V	2	10.6 nM 11-OH-Δ^9-THC	775.0 ± 162.6	I:V	0.40
VI	2	10.6 nM Δ^9-THC + 166 nM NE	973.0 ± 83.4	II:VI	0.40
VII	2	10.6 nM 11-OH-Δ^9-THC + 166 nM 5-HT	969.0 ± 173.9	II:VII	0.40
I	2	control	424.8 ± 10.1		
II	2	control + 166 uM NE	625.8 ± 67.1	I:II	0.025
III	2	control + 166 uM 5-HT	471.1 ± 2.3	I:III	0.015
IV	2	318 uM Δ^9-THC	400.1 ± 26.7	I:IV	0.15
V	2	318 uM Δ^9-THC + 166 uM NE	699.3 ± 135.5	II:V	0.25
VI	2	318 uM Δ^9-THC + 166 uM 5-HT	529.3 ± 15.6	III:VI	0.012

APPENDIX III

Procedure for normalizing data to a single grand mean from a series of repeated experiments.

(GM) Grand Control Mean = $\frac{\Sigma \bar{x}_i}{N}$

where x_i = mean of replicate assays of control groups; N = total number of control groups.

Weighted Grand Standard Deviation = $\frac{\Sigma n_i SD_i}{N}$

where n_i = number of replicate assays; $N = \Sigma n_i$ and SD_i = standard deviation of the replacate assays. Although the relative levels of cyclic AMP varied with animal batch, the control variation about the mean remained very consistent.

Adjusted Treatment Means = GM (F)

Adjusted Treatment Standard Deviation = SD_i (F)

where F = $\frac{\text{observed treatment mean for that experiment}}{\text{paired control mean for that experiment}}$

APPENDIX IV

Procedure for t test.

Hypothesis Test: $|\bar{x}_{control} - \bar{x}_{treatment}| > 0$

Null Hypothesis: $|\bar{x}_{control} - \bar{x}_{treatment}| = 0$

Test Statistic = $$\frac{|\bar{x}_{control} - \bar{x}_{treatment}|}{s_{\bar{x}1.\bar{x}2}}$$

where $$s_{\bar{x}1.\bar{x}2} = \sqrt{\frac{n_1+n_2}{n_1 \cdot n_2} \left\{ \frac{(n_1-1)\, s_{x1}^2 + (n_2-1)\, s_{x2}^2}{n_1+n_2-2} \right\}}$$

where $\bar{x}_{control}$ = control mean

$\bar{x}_{treatment}$ = drug treatment mean

$s_{\bar{x}1.\bar{x}2}$ = weighted pooled standard deviation

n_1 = replicates of assay (control)

n_2 = replicates of assay (treatment)

s_{x1}^2 = variance of control replicates

s_{x2}^2 = variance of treatment replicates

P value = the significance when the test statistic equals the table value.

Assumptions: Independent observations and population variances unknown but assumed equal.

Level of Significance: I consider the level of significance based on the total variation observed during these investigations to be p=0.025-0.03. Therefore, any p values less than this are considered statistical significant and any values equal to or greater than this are considered insignificant.

APPENDIX V

For additional bibliographical listings on the Cannabis literature, see United Nations Commission on Narcotic Drugs (1965), Bulletin on Narcotic (1951 to present), Gamage and Zerkin (1969), Moore (1969), Waller and Denny (1971), Canadian Commission (1972) and Mechoulam (1973).

REFERENCES

Aaron, H. S., and C.P. Ferguson. 1968. J. Org.Chem. 33:684.

Abdulla, Y. H., and K. Hamadah. 1970. Lancet Feb. 21:378.

Abel, E. L. 1970. J. Pharm. Pharmacol. 22:785.

Abel, E. L. 1971. Sci. 173:1038

Abel, E. L. 1971a. Nature (Lon.) 231:260

Abel, E. L. 1972. Experientia 28:1187

Abel, E. L., D. E. McMillian and L. S. Harris. 1972. Experientia 28:1188.

_____. 1973. Brit. J. Pharmac. 47:452

Abel, E. L. 1973. Experientia 29:1527

Adams, R., M. Hunt and J. H. Clark. 1940a. J. Amer. Chem. Soc. 62:196.

Adams, R., D. C. Pease and J. H. Clark. 1940b. J. Amer. Chem. Soc. 62:2194.

Adams, R. and B. R. Baker. 1940c. J. Amer. Chem. Soc. 62:2405.

Adams, R., C.K. Cain, W. C. McPhee and R. B. Wearn. 1941. J. Amer. Chem. Soc. 63:2209.

Adams, R., C. M. Smith and S. Loewe. 1941a. J. Amer. Chem. Soc. 63:1973.

Adams, R. 1942. Bull. N. Y. Acad. Med. 18:705.

Adams, R., S. Loewe, C. M. Smith and W. D. McPhee. 1942a. J. Amer. Chem. Soc. 64:694.

Adams, R., S. Loewe, C. W. Theobald and C. M. Smith. 1942b. J. Amer. Chem. Soc. 64:2653.

Adams, R., K. Chen and S. Loewe. 1945. J. Amer. Chem. Soc. 67:1534.

Adams, R., M. Harfenist and S. Loewe. 1949. J. Amer. Chem. Soc. 71: 1624.

Adams, H. R. and R. D. Sofia. 1973. Experientia 29:181.

Adams, T. C. Jr. and L. A. Jones. 1973. Agric. Food Chem. 21(6):1129.

Adgida, P. R., P. V. N. Murthy and J. M. McKenzie. 1971. Biochem. 10(4):711.

Agurell, S., I. M. Nilsson and F. Sandberg. 1968. Svensk. Farm. Tidskr. 72:662.

Agurell, S. 1970. In: The Botany and Chemistry of Cannabis. C. R. B. Joyce and S. H. Curry (eds.). Churchill, London, p. 175.

Agurell, S., I. M. Nilsson, A. Ohlsson and F. Sandberg. 1970. Biochem. Pharmacol. 19:1333.

Agurell, S. and K. Leander. 1971. Acta Pharmaceu. Suec. 8:391.

Agurell, S., I. M. Nilsson, J. L. G. Nilsson, A. Ohlsson, M. Widman and K. Leander. 1971a. Acta Pharmacaceu. Suec. 8(6):698.

Agurell, S., B. Gustafsson, T. Gosztonyi, K. Hedman and K. Leander. 1973. Acta Chem. Scand. 27(3):1090.

Allentuck, S. and K. M. Bowman. 1942. Amer. J. Psychiat. 99:248.

Alles, G. A., R. N. Icke and G. A. Feigen. 1942. J. Amer. Chem. Soc. 64:2031.

Ames, F. 1958. J. Mental Sci. 104:972.

Andrews, G., and S. Vinkenoog. 1967. The Book of Grass, An Anthology of Indian Hemp. Grove Press, N. Y., p. 1-76.

Appleman, M. M., L. Birnbaumer and N. Torres. 1966. Arch. Biochem. Biophys. 116:39.

Aramaki, H., N. Tomiyaso, H. Yoshimora and H. Tsukamoto. 1968. Chem. Pharm. Bull. (Tokyo). 16:822.
Arnoux, M. 1966. Ann. Amelior Plantes 16(2):123.
Aschoff, J. 1960. Cold Spr. Har. Symp. Quan. Biol. 25:11.
Atal, C. K., and J. K. Sethl. 1961. Curr. Sci. 30:177.
Atkinson, R. C., and R. M. Shiffrin. 1971. Sci. Amer. 224:82.
Bailey, K, and P. Toft. 1973. Biochem. Pharmacol. 22:2780.
Baker, J. R., and H. Rosenkrantz. 1972. J. Histochem. Cytochem. 20(10):827.
Ballard, C. W. 1915. J. Amer. Pharmaceu. Assoc. 4:1299.
Barondes, S. H. 1970. Int. Rev. Neurobiol. 12:177.
Barry, H. III, and R. K. Kubena. 1969. Proc. 77th Ann. Convention Amer. Psychol. Assoc. 4:865.
Bartholomew, G. A., and V. A. Tucker. 1964. Physiol. Zool. 37:341.
Beam, W. 1911. 4th Report, Wellcome Trop. Res. Lab. Report. No. 25 Sudan Gov.
Ben-Zvi, Z., R. Mechoulam and S. H. Burstein. 1970. J. Amer. Chem. Soc. 92:3468.
Ben-Zvi, Z., R. Mechoulam, H. Edery and G. Porath. 1971. Sci. 174: 951.
Benetowa, S. 1968. In: The Book of Grass. G. Andrews and S. Vinkenoog (eds.). Groove Press, N. Y. p. 31.
Bercht, C. A. L., F. J. E. M. Kuppers, R. J. J. Lousberg and C. A. Salemik. 1971. United Nations ST/SOA/SER .5/29:1.
Berl, S., S. Puszkin and W. J. Nicklas. 1973. Sci. 179:441.
Bessey, E. A. 1928. Amer. J. Botany 15:405.
Betts, T. J., and P. J. Holloway. 1967. J. Pharm. Pharmacol. 19: 97s.
Bibliography of Cannabis sativa. 1951. Bull. on Narc. 3(1):59; 3(2) :42 and each year thereafter.
Bieger, D., and C. H. Hockman. 1973. Neuropharmacology 12:269.
Bino, T., A. Chari-Bitron and A. Shahar. 1972. Biochim. Biophys. Acta 228:195.
Birmingham, M. K. 1973. Brit. J. Pharmacol. 48:169.
Black, C. A. 1945. Bot. Gazette 107:114.
Black, C. A., and A. J. Vessel. 1944. Soil Sci. Soc. Proc. 9:179.
Black, M. B., J. H. Woods and E. F. Domino. 1970. Pharmacologist 12:258.
Blackie, W. J. 1941. Industr. Eng. Chem. 13:96.
Blatt, A. H. 1938. J. Wash. Acad. Sci. 28:465.
Bloomquist, E. R. 1968. Marihuana. Glencoe Press, Calif.
Boggan, W. O., R. A. Steele and D. X. Freedman. 1973. Psychopharmacologia (Berl.). 29:101.
Booth, D. A. 1972. Nature New Biol. 237:222.
Borgeat, P. G., L. Chavancy, A. Dupont, F. Labrie, A. Arimura and A. V. Schally. 1972. Proc. Nat. Acad. Sci. (USA). 69(9):2677.
Borgen, L. A., W. M. Davis and H. B. Pace. 1971. Toxic. Appl. Pharmac. 20:480.
Borgen, L. A., and W. M. Davis. 1973. J. Pharmaceu. Sci. 62(3):479.
Borgen, L. A., G. C. Lott and W. M. Davis. 1973a. Res. Comm. Chem. Path. Pharm. 5(3):621.
Borgen, L. A., W. M. Davis and H. B. Pace. 1973b. Pharmacol. Biochem. Behav. 1:203.

Bose, B. C., A. Q. Saifi and A. W. Bhawat. 1964. Arch. Intl. Pharmacodyn. 147:285.
Bose, R. D. 1930. Agr. J. India 25:495.
Bosmann, H. B. 1971. Cytobios 4(14):121.
Boucher, R., M. Gauthier, P. Jolicoeur and F. Labrie. 1973. Can. J. Biochem. 51:913.
Bouquet, J. 1950. Bull. Narc. 2(4):14.
Bouquet, J. 1951. Bull. Narc. 3(1):22.
Bourne, H. R., G. M. Tomkins and S. Dion. 1973. Sci. 181:952.
Bowman, M., and R. O. Pihl. 1973. Psychopharmacologia (Berl). 29: 159.
Boyce, S. S. 1900. Hemp (Cannabis sativa). Orange Judd, N.Y.
Boyd, E. S., E. H. Boyd, J. S. Muchmore and L. E. Brown. 1971. J. Pharmacol. Exp. Therap. 176:480.
Breckenridge, B. 1970. Ann. Rev. Pharmac. 10:19.
Breimer, D. D., T. B. Vree, C. A. M. Van Ginneken, P. T. Henderson and J. M. Van Rossum. 1972. Proc. Intl. Sym. Gas Chrom. Mass Spec. p. 89.
Breslavec, L. 1937. Genetica 19:393.
Brill, N. O., E. Crumpton, I. M. Frank, J. S. Hochman, P. Lomax, W. H. McGlothlin and L. J. West. 1970. Ann. Intl. Med. 73(3): 449.
Bromberg, W. 1939. J. Amer. Med. Ass. 113:4.
Brooker, G., L. J. Thomas Jr., and M. M. Appleman. 1968. Biochem. 7:4177.
Brown, H. 1971. Psychopharmacologia (Berl.). 21:294.
Brown, J. H., and M. H. Makman. 1972. Proc. Nat. Acad. Sci. (USA). 69(3):539.
Bueno, O. F., and E. A. Carlini. 1972. Psychopharmacologia (Berl). 25:49.
Bullock, F. J., R. J. Bruni and E. Werner. 1970. Abstr. 160th Nat. ACS Meeting, Chicago, Ill., (Anal 077).
Bureau of Narcotics. 1933. In: Traffic in Opium and Other Dangerous Drugs. U. S. Government Printing Office, p. 35.
Burstein, S., and R. Mechoulam. 1968. J. Amer. Chem. Soc. 90:2420.
Burstein, S. H., F. Menezes, E. Williamson and R. Mechoulam. 1970. Nature 225:87.
Burstein, S., and D. Kupfer. 1971. Ann. N. Y. Acad. Sci. 191:61.
Burstein, S., J. Rosenfeld and T. Wittstruck. 1972. Sci. 176:422.
Burstein, S., and A. Raz. 1972a. Prostaglandins 2(5):369.
Burstein, S., E. Levin and C. Varanelli. 1973. Biochem. Pharmacol. 22:2905.
Buxbaum, D. M. 1972. Psychopharmacologia (Berl.). 25:275.
Byck, R., and J. M. Ritchie. 1973. Sci. 180:84.
Cahn, R. S. 1933. J. Chem. Soc. (Lon.). p.1400.
Caldwell, D. F., S. A. Meyers, E. F. Domino and P. E. Merriam. 1970. In:Psychotomimetic Drugs. Raven Press, N. Y., p. 299.
Camp, W. H. 1936. J. New York Bot. Gardens 37:110.
Campbell, A. M., M. Evans, J. L. G. Thompson and M. J. Williams. 1971. Lancet Dec. 4:1219.
Canadian Commission. 1972. Cannabis: A report of the Commission of Inquiry into the Non-medicinal Use of Drugs. Information Canada, Ottawa,

Cappell, H., C. D. Webster, B. S. Herring and R. Ginsberg. 1972. J. Pharmacol. Exper. Ther. 182(2):195.
Carlini, E. A., and C. Kramer. 1965. Psychopharmacologia (Berl.). 7:175.
Carlini, E. A., and J. Masur. 1969. Life Sci. 8(I):607.
Carlini, E. A., A. Hamaoui, D. Bieniek and F. Korte. 1970. Pharmacology 4:359.
Carlini, E. A., A. Hamaoui and M. W. Martz. 1972. Brit. J. Pharmac. 44(4):794.
Casswell, S., and D. F. Marks. 1973. Sci.181:803.
Cavero, I., J. P. Buckley and B. S. Jandhyala. 1972. Eur. J. Pharmac. 19:301.
Cavero, I., R. K. Kubena, J. Dziak, J. P Buckley and B. S. Jandhyala. 1972a. Res. Comm. Chem. Path. Pharmacol. 3(3):483.
Chari-Bitron, A., and T. Bino. 1971. Biochem. Pharmacol. 20:473.
Chasin, M., I. Rivkin, F. Mamrak, S. G. Samaniego and S. M. Hess. 1971. J. Biol Chem. 246:3037.
Chasin, M., F. Mamrak, S. G. Samaniego and S. M. Hess. 1973. J. Neurochem. 21:1415.
Cheuvart, C. 1954. Bull. Acad. Royal Med. Bel. Chem. Sci. 40:1152.
Chopra, G. S. 1969. Intl. J. Addictions 4(2):215.
Chopra. I. C., and R. N. Chopra. 1957. Bull. Narc. 9:4.
Christensen, H. D., R. I. Freudenthal, J. T. Gidley, R. Rosenfeld, G Boegli, L. Testino, D. R. Brine, C. G. Pitt and M. E. Wall. 1971. Sci. 172:165.
Clark, L. D., and E. N. Narashima. 1968. Amer. J. Psychiatry 125: 379.
Clark, L. D., R. Hughes and E. N. Nakashima. 1970. Arch. Gen. Psychiatry 23(3):193.
Claussen, U., and F. Korte. 1968. Justus Liebigs Ann. Chem. 713:162.
Cohen, G. M., D. W. Peterson and G. J Mannering. 1971. Life Sci. 10(I):1207.
Cohen, S. 1971. Acta Pharmaceu. Suec. 8(6):686.
Conney, A. H. 1967. Pharmacol. Rev. 19:317.
Consroe, P. F. 1973. Res. Comm. Chem. Path. Pharm. 5(3):705.
Constantinidis, J., and C. J. Miras. 1971. Psychopharmacologia (Berl.). 22:80.
Contreras, E., J. Castillo and L. Quijada. 1972. J. Pharm. Pharmac. 24:65.
Cools, A. R. 1972. Psychopharmacologia (Berl.). 25:229.
Cooper, J. R., F. E. Bloom and R. H. Roth. 1970. The Biochemical Basis of Neuropharmacology. Oxford University Press, N. Y.
Corcoran, M. E. 1973. Life Sci. 12(I):63.
Correspondant. 1974. Nature 247:128.
Couselinis, A. S., and C. J. Miras. 1970. U. N. Secretariat Doc. ST/SOA/SER .5123.
Cramer, H., D. G. Johnson, I. Hanbauer, S. D. Silberstein and I. J. Kopin. 1973. Brain Res. 53:97.
Dagirmanjian, R., and E. S. Boyd. 1962. J. Pharmacol. Exp. Ther. 135:25.
Darley, C. F., J. R. Tinklenberg, L. E. Hollister and R. C. Atkinson. 1973. Psychopharmacologia (Berl.). 29:231

D'Armiento, M., G. S. Johnson and I. Pastan. 1972. Proc. Nat. Acad. Sci. (USA). 69(2):459.
David, J. P., and H. H. Ramsey. 1949. Fed. Proc. 8:56.
Davis, T. W. M., C. G. Farmilo and M. Osadchuk. 1963. Anal. Chem. 35:751.
Davis, Jr., K. H., N. H. Martin, C. G. Pitt, J. W. Wildes and M. E. Wall. 1970. Lloydia 33(4):453.
Davis, W. M., J. E. Moreton, W. T. King and H. B. Pace. 1972. Res. Comm. Chem. Path. Pharm. 3(1):29.
deBelleroche, J. S., and H. F. Bradford. 1972. J. Neurochem. 19:585.
Deguchi, T. 1973. Mol. Pharmacol. 9(2):184.
De Robertis, E., G. Arnaiz, M. Alberici, R. W. Butcher and E. W. Sutherland. 1967. J. Biol. Chem. 242:3487.
De Ropp, R. S. 1960. J. Amer. Pharm. Ass. Sci. Ed. 49:756.
De Ropp, R. S., L. H. Kastl and L. Balbos. 1972. Proc. West Pharmacol. Soc. 15:21.
deZeeuw, R. A., T. M. Malingré, F. W. H. M. Merkus. 1972. J. Pharm. Pharmac. 24:1.
deZeeuw, R. A., J. Wijsbeek, D. D. Breimer, T. B. Vree, C. A. M. van Ginneken, J. M. van Rossum. 1972a. Science 175:778.
deZeeuw, R. A., J. Wijsbeek and T. M. Malingre. 1973. J. Pharm. Pharmac. 25:21.
Dewey, L. H. 1914. In: Yearbook of U. S. Department of Agriculture. Washington, D. C. Government Printing Office. p. 283.
Dewey, W. L., L. S. Harris, J. F. Howes, J. S. Kennedy, F. E. Granchelli, H. G. Pars, R. K. Razdan. 1970. Nature 226:1265.
Dewey, W. L., L. R. Yonce, L. S. Harris, W. M. Reavis, E. D. Griffin, Jr. and V. E. Newby. 1970a. Pharmacologist 12: 259.
Dewey, W. L., L. S. Harris and J. S. Kennedy. 1972. Arch. Internat'l. Pharmacodyn. Ther. 196(1):133.
Dewey, W. L., J. Jenkins, T. O'Rourke and L. S. Harris. 1972a. Arch. Int. Pharmacodyn. 198:118.
Dingell, J. V., H. G. Wilcox and H. A. Klausner. 1971. Pharmacologist 13:296.
Dingell, J. V., K. W. Miller, E. C. Heath and H. A. Klausner. 1973. Biochem. Pharmac. 22:949.
DiHrich, A., K. Battig and I. von Zeppelin. 1973. Psychopharmacologia (Berl.). 33:369.
Dokas, L. A., M. D. Botney and L. J. Kleinsmith. 1973. Arch. Biochem. Biophys. 159(2):712.
Dokas, L. A., and L. J. Kleinsmith. 1971. Sci. 172:1237.
Dolby, T. W. 1973. Unpublished Observations.
Dolby, T. W., and L. J. Kleinsmith. 1974. Biochem. Pharm. In Press.
Domino, E. F. 1971. Ann. N. Y. Acad. Sci. 191:166.
Domino, E. F., H. F. Hardman and M. H. Seevers. 1971. Pharm. Rev. 23(4):317.
Doorenbos, N. J., P. S. Fetterman, M. W. Quinby and C. E. Turner. 1971. Ann. N.Y. Acad. Sci. 191:3.

Dornbush, R. L., M. Fink and A. M. Freedman. 1971. Amer. J. Psychiatry 128(3):194.
Dorrance, D., O. Janiger and L. Teplitz. 1970. J. Amer. Med. Ass. 212(9):1488.
Dorywalski, J. 1936. Biol. Abstr. 10(7):15443.
Drake, Jr., W. D. 1971. The Connoisseur's Handbook of Marijuana. Straight Arrow Books, Calif.
Drew, W. G., L. L. Miller and A. Wikler. 1972. Psychopharmacologia (Berl.) 23:289.
Drew, W. G., and D. E. Slagel. 1973. Neuropharm. 12:909.
Drew, W. G., and L. L. Miller. 1973a. Pharmacology 9:41.
Drummond, G. I., and L. Duncan. 1970. J. Biol. Chem. 245:976.
Duquenois, P. 1950. Bull. Narc. 2:30.
Duquenois, P., and H. Negm. 1938. Med. Leg. 18:493.
Edery, H., Y. Grunfeld, Z. Ben-Zvi and R. Mechoulam. 1971. Ann. N.Y. Acad. Sci. 191:40.
El-Sourogy, M., A. Y. Maiek, H. H. Ibrahim, J. Farag and A. El-Shiky. 1966. J. Egypt Med. Ass. 49:626.
Elsmore, T. F., and G. V. Fletcher. 1972. Sci. 175:911.
Estevez, V. S., L. F. Englert and B. T. Ho. 1973. Res. Comm. Chem. Path. Pharmacol. 6(3):821.
Exley, J. 1968. Biochem. J. 107:285.
Fahrenholtz, K. E., M. Lurie and R. W. Kierstead. 1966. J. Amer. Chem. Soc. 88:2079.
_____. 1967. 89:5934.
Fairbairn, J. W. and J. A. Liebmann. 1973. J. Pharm. Pharmac. 25:150.
Farmilo, C. G., and T. W. M. Davis. 1961. J. Pharm. Pharmac. 13: 767.
Farmilo, C. G., T. W. M. Davis, F. A. Vandenheuvel and R. Lane. 1962. United Nations Document ST/SOA/SER.S/7.
Fehr, K. O., and H. Kalant. 1972. Can. J. Physiol. Pharmacol. 50:761.
Ferguson, J. 1970. Sci. 168:499.
Fernandes, M., W. Warning, W. Christ and P. Hill. 1973. Biochem. Pharm. 22:2981.
Fernstrom, J. D., and R. J. Wurtman. 1974. Sci. Am. 230:84.
Ferraro, D. P., D. M. Grilly and W. C. Lynch. 1971. Psychopharmacologia (Berl.). 22:333.
Ferraro, D. P. W. C. Lynch and D. M. Grilly. 1972. Pharmacology 7:273.
Ferraro, D. P., and M. G. Grisham. 1972a. Physiol. Behav. 9:49.
Ferraro, D. P., and D. K. Billings. 1972b. Psychopharmacologia (Berl.) 25:169.
Fetterman, P. S., E. S. Keith, C. W. Waller, O. Guerrero, N. J. Doorenbos and M. W. Quimby. 1971. J. Pharmaceutical Sci. 60(8):1240.
Fetterman, P. S., N. J. Doorenbos, E. S. Keith and M. W. Quimby. 1971a. Experientia 27:988.
Florendo, N. T., R. T. Barrnett and P. Greengard. 1971. Sci. 173:745.
Foltz, R. L., A. F. Fentiman, Jr., E. G. Leighty, J. L. Walter, H. R. Drewes, W. E. Schwartz, T. F. Page, Jr. and E. B. Truitt, Jr. 1970. Sci. 168:844.

Ford, R. D., and D. E. McMillian. 1971. Fed. Proc. 30:279.
Forn, J., A. Tagliamonte, P. Tagliamonte and G. L. Gessa. 1972. Nature New Biol. 237:245.
Forney, P. B., and G. F. Kiplinger. 1971. Ann. N.Y. Acad. Sci. 191:74.
Forrest, I. S., D. E. Green, S. D. Rose, G. C. Skinner and D. M. Torres. 1971. Res. Comm. Chem. Path. and Pharm. 2(6):787.
Forrest, I. S., L. S. Otis, M. T. Serra and G. C. Skinner. 1972. Proc. West Pharmacol. Soc. 15:83.
Freudenthal, R. I. S. Martin and M. E. Wall. 1972. Brit. J. Pharmacol. 44(2):244.
Fried, P. A., and G. W. Nieman. 1973. Pharmacol. Biochem. and Behav. 1:371.
Friedman, J. J., and E. E. Selkurt. 1966. In: Physiology. E. E. Selkurt (Ed.) p.369.
Fujimori, M., D. M. Trusty and H. E. Himwich. 1973. Life Sci. 12(I):553.
Fulton, C. C. 1942. Industr. Eng. Chem. Analyt. Ed. 14:407.
Gaballah, S., C. Popoff and S. Sooknandan. 1971. Brain Res. 31: 229.
Galanter, M. R. J. Wyatt, L. Lemberger, H. Weingartner, T. B. Vaughn, W. T. Roth. 1972. Sci. 176:934.
Gallager, D. W., E. Sandersbush and F. Sulser. 1972. Psychopharmacologia (Berl.) 26:337.
Gamage, J. R., and E. L. Zerkin. 1969. A Comprehenisve Guide to the English Language Literature on Cannabis. Stash Press, Bel it, Wisconsin.
Ganoi, Y. and R. Mechoulam. 1964. J. Amer. Chem. Soc. 86:1646.
Gaoni, Y., and R. Mechoulam. 1971. J. Amer. Chem. Soc. 93:217.
Garattini, S. 1965. In: Hashish: Chemistry and Pharmacology. G. E. W. Wolstenholme and J. Knight (Eds.) Boston p.70.
Garelis, E., and N. H. Neff. 1974. Sci. 183:532.
Garrett, E. R., and C. A. Hunt. 1973. J. Pharmaceu. Sci. 62(7): 1211.
Gascon, A. L., and M. T. Peres. 1973. Can. J. Physiol. Pharmacol. 51:12.
Gastinel, J. B. 1849. Repertoire de Pharmacie. 6:129.
Gayer, H. 1928. Arch. Exp. Pathol. Pharmakol. 129:312.
Geller, A., F. Robustelli and M. Jarvik. 1970. Psychopharmacologia. (Berl.) 16:281.
Gerber, W. F. 1969. Toxic Appl. Pharmacol. 14:276.
Gerber, W. F., and L. Schramm. 1969. Arch. Intl. Pharmacodyn. Ther. 177(1):244.
Gericke, D., P. Chadra. 1969. Hoppe-Seylers' Z. Physiol Chem. 350:1469.
Gessa, G. L., G. Krishna, J. Forn, A. Tagliamonte and B. B. Brodie. 1970. Adv. Biochem. Psychopharmacologia. 3:371.
Ghosh, R., D. C. S. Pascall and A. R. Todd. 1940. J. Amer. Chem. Soc. (Lon.) p. 1118.
Ghosh, R., A. R. Todd and S. Wilkinson. 1940a. J. Amer. Chem. Soc. (Lon.) p.1393.
Gianutsos, G., and E. R. Abbatiello. 1972. Psychopharmacologia. (Berl.) 27:117.

Gibbs, F. A. 1970. Clin. Electroenceph. 1:127.
Gill, E. W., W. D. M. Paton and R. G. Pertwee. 1970. Nature 228:134.
Gill, E. W., and G. Jones. 1971. J. Labelled Compounds. 8(2):237.
Gilman, A. G. 1970. Proc. Natl. Acad. Sci. (USA) 67:305.
Gilman, A. G., J. D. Minna. 1973. J. Biol. Chem. 248(19):6610.
Gilmour, D. G., A. D. Bloom, K. P. Leli, E. S. Robbins and C. Maximilian. 1971. Arch. Gen. Psychiatry. 24:268.
Glassman, E. 1969. Ann. Rev. Biochem. 38:605.
Glowinski, J., and L. Iversen. 1966. J. Neurochem. 13:655.
Goldberg, N. D., and A. G. O'Toole. 1969. J. Biol. Chem. 244: 3053.
Goldstein, M., B. Anagnoste and C. Shirron. 1973. J. Pharm. Pharmac. 25:348.
Grant, J. D., S. J. Gross, P. Lomax and R. Wong. 1972. Nature, New Biol. 236:216.
Greene, M. L., D. R. Saunders and K. Smith. 1972. Gastroenterology. 62:757.
Greengard, P., G. A. Robison and R. Paoletti. 1972. Advances in Cyclic Nucleotide Research. Vol. 1. Raven Press, N.Y.
Greengard, P., D. A. McAfee and J. W. Kebabian 1972a. Advances in Cyclic Nucleotide Research. Vol. 1. p. 373.
Greengard, P., and G. A. Robison (Eds.) 1973. Advances in Cyclic Nucleotide Research. Vol. 3. Raven Press, N.Y.
Grierson, G. A. 1892. Refererence to the Hemp Plant in Sanskrit and Hindi Literature. Howra, India.
Grilly, D. M., D. P. Ferraro and R. G. Marriott. 1973. Nature 242:119.
Grinspoon, L. 1971. Marihuana Reconsidered. Harvard University Press. Cambridge, Mass.
Grisham, M. G., and D. P. Ferraro. 1972. Psychopharmacologia. (Berl.) 27:163.
Grlić, L. 1962. Bull. on Narc. 14(3):37.
Grunfeld, Y., and H. Edery. 1969. Psychopharmacologia (Berl.) 14:200.
Guidotti, A., and E. Costa. 1973. Sci. 179:902.
Haagen-Smit, A. J., C. Z. Wawra, J. B. Koepfli, G. A. Alles, G. A. Feigen and A. N. Prater. 1940. Sci. (N.Y.) 91:602.
Haney, A., and Bazzaz, F. A. 1970. In: The Botany and Chemistry of Cannabis. Eds. Joyce, C. R. B., and Curry, S. H. Churchill, London.
Harbison, R. D., and B. Mantilla-Plata. 1972. J. Pharm. Exp. Ther. 180(2):446.
Hardman, H. F., E. F. Domino, M. H. Seevers. 1971. Pharm. Rev. 23(4):295.
Harris, L. S. 1971. Pharmacol. Rev. 23(4):285.
Harris, R. T., W. Waters and D. McLendon. 1972. Psychopharmacologia (Berl.) 26:297.
Hattori, T., A. Jakubovic and P. L. McGeer. 1973. Neuropharm. 12: 995.
Heald, P. J. 1961. Biochem. J. 78:340.
Hegi, G. 1958. Illustrierte Flora von Mittel - Europa. 2nd ed. 3:194.

Henriksson, B. G., and T. Jarbe. 1971. Psychopharmacologia. (Berl.) 22:23.
Henriksson, B. G., T. Jarbe. 1971a. J. Pharm. Pharmac. 23:458.
Herman, Z. S. 1973. Neuropharmacol. 12:705.
Herring, B. 1972. Psychopharmacologia (Berl.) 26:401.
Heslop-Harrison, J. 1957. Biol. Review 32:1.
Hill, S. Y., R. Schwin, B. Powell and D. W. Goodwin. 1973. Nature 243:241.
Hirata, K. 1927. J. Genetics. 19(1):65.
Hively, R. L., W. A. Mosher and F. W. Hoffman. 1966. J. Amer. Chem. Soc. 88:1832.
Ho, B. T., G. E. Fritchie, P. M. Kralik, L. F. Englert, W. M. McIsaac, J. Idanpaan-Heikkila. 1970. J. Pharm. Pharmac. 22:538.
Ho, R. J., and E. W. Sutherland. 1971. J. Biol. Chem. 246:6822.
Ho, B. T., G. E. Fritchie, L. F. Englert, W. M. McIsaac and J. E. Idanpaan-Heikkila. 1971. J. Pharm. Pharmacol. 23:309.
Ho, B. T., A. Rong, G. E. Fritchie, L. F. Englert, W. M. McIsaac, B. MacKay and D. H. W. Ho. 1971a. J. Pharmaceu. Sci. 60(11): 1761.
Ho, B. T., D. Taylor, L. F. Englert and W. M. McIsaac. 1971b. Brain Res. 31:233.
Ho, I. K., H. H. Loh and E. L. Way. 1972. Nature. 238:397.
Ho, B. T., V. Estevez, L. F. Englert, W. M. McIsaac. 1972. J. Pharm. Pharmac. 24:416.
Ho, B. T., D. Taylor, G. E. Fritchie, L. F. Englert and W. M. McIsaac. 1972a. Brain Res. 38:163.
Ho, B. T. V. Estevez and L. F. Englert. 1973. Res. Comm. Chem. Path. Pharmacol. 5:215.
Ho, B. T., D. Taylor, L. F. Englert. 1973a. Res. Comm. Chem. Path. Pharm. 5(3):851.
Ho, B. T., V. S. Estevex and L. F. Englert. 1973b. J. Pharm. Pharmac. 25:490.
Hockman, C. H. P. G. Perrin and H. Kalant. 1971. Sci. 172:968.
Hoffer, B. J., G. R. Siggins and F. E. Bloom. 1970. Psychopharmacal. 3:349.
Hoffer, B. J., G. R. Siggins, A. P. Oliver and F. E. Bloom. 1972. Advances in Cyclic Nucleotide Research. Vol. 1:411. Raven Press, N.Y.
Hollister, L. E. 1971. Ann. N.Y. Acad. Sci. 191:132.
Hollister, L. E., R. K. Richards and H. K. Gillespie. 1968. Clin. Pharmacol. Ther. 9:783.
Hollister, L. E., S. L. Sherwood and A. Cavasino. 1970. Pharm. Res. Comm. 2(4):305.
Hollister, L. E. 1971. Sci. 172:21.
Hollister, L. E., S. L. Kanter, F. Moore and D. E. Green. 1972. Clin. Pharm. Ther. 13(6):849.
Hollister, L. E. 1973. Experientia. 29:825.
Hollister, L. E. 1973. Clin. Pharm. Ther. 15(1):18.
Holtta, E., and A. Raina. 1973. Acta. Endocrinologica. 73:794.
Holtzman, D., R. A. Lovell, J. H. Jaffee and D. X. Freedman. 1969. Sci. 163:1464.
Hood, L. V. S. M. E. Rames and G. T. Barry. 1973. Nature. 242:402.

Hoops, J. F., H. Bader and J. H. Biel. 1968. J. Org. Chem. 33: 2995.
Howes, J. 1970. Pharmacologist. 12:258.
Hsie, A. W., T. T. Puck. 1971. Proc. Nat. Acad. Sci. (USA) 68: 358.
Huard, P., and M. Wong. 1968. Chinese Medicine. McGraw-Hill, N.Y.
Idanpaan-Heikkila, J., G. E. Fritchie, L. F. Englert, B. T. Ho and W. M. McIsaac. 1969. N. Engl. J. Med. 281:330.
Irwin, S. 1968. Psychopharmacologia. 13:222.
Isbell, H., D. R. Jasinski, C. W. Gorodetsky, F. Kurte, U. Claussen, M. Haage, H. Sieper and F. Von Spulak. 1967. Bull. Prob. Drug Depend. Nat. Acad. Sci. p. 4832.
Isbell, H. C. W. Gorodetzsky, D. Janiski, U. Claussen, F. N. Spulak and F. Kurte. 1967a. Psychopharmacologia. 11:184.
Izquierdo, I., O. A. Orsingher and A. C. Berardi. 1973. Psychopharmacologia. (Berl.) 28:95.
Izquierdo, I., and A. G. Nasello. 1973a. Psychopharmacologia. (Berl.) 31:167.
Jaffe, P. G., and M. Baum. 1971. Psychopharmacologia. (Berl.) 20:97.
Jarbe, T. U. C., and B. G. Henriksson. 1973. Psychopharmacologia (Berl.) 30:315.
Jard, S., J. Premont and P. Benda. 1972. Febs letters. 26(1):344.
Jen, T. Y., G. A. Hughes and H. Smith. 1967. J. Amer. Chem. Soc. 89:4551.
Johnson, S., and E. F. Domino. 1971. Clin. Pharmacol. Ther. 12(5): 762.
Johnson, E. M., H. Maeno and P. Greengard. 1971a. J. Biol. Chem. 246(24):7731.
Jolicoeur, F., and F. Labrie. 1971. Febs. Letters 17(I):141.
Jones, G., and R. G. Pertwee. 1972. Brit. J. Pharmac. Chem. Ther. 45:375.
Jones, R., and G. Stone. 1970. Psychopharmacologia (Berl.) 18(1): 108.
Jones, R. T. 1971. Ann. N.Y. Acad. Sci. 191:155.
Jost, J., A. Hsie, S. D. Hughes and L. Ryan. 1970. J. Biol. Chem. 245(2):351.
Kabelik, J., Z. Krejci and F. Santavy. 1960. Bull. on Narc. 12(3)13.
Kabelikovi, J. 1952. Lerarske Listy. 15(7):500.
Kakiuchi, S., and T. W. Rall. 1968. Mol. Pharmacol. 4:367.
Kakiuchi, S., and T. W. Rall. 1968a. Mol. Pharmacol. 4:397.
Kakiuchi, S., T. W. Rall and H. McIlwain. 1969. J. Neurochem. 16:485.
Kakiuchi, S. , R. Yamazaki, Y. Teshima and K. Venishi. 1973. Proc. Nat. Acad. Sci. (USA) 70(K)I:3526.
Karniol, I. G., and E. A. Carlini. 1972. J. Pharm. Pharmac. 24:833.
Katz, B., and R. Miledi. 1969. J. Physiol. (Lon.) 203:689.
Kebabian, J. W., and P. Greengard. 1971. Sci. 174:1346.
Kebabian, J. W., G. L. Petzoid and P. Greengard. 1972. Proc. Nat. Acad. Sci. (USA) 69:2145.
Kechatov, E. A. 1959. Bull. on Narc. 1(2):5.
Keller, R. 1972. Life Sci. 11(II):485.

Kelly, L. A., and R. W. Butcher. 1973. Biochem. Biophys. Acta 320:540.
Kennedy, J. S., and W. J. Waddell. 1972. Toxic. Applied Pharmacol. 22:252.
Khandwala, A. S., and C. B. Kasper. 1973. Biochem. Biophys. Res. Comm. 54(4):1241.
Kilbey, M., G. E. Fritchie, D. M. McLendon and K. M. Johnson. 1972. Nature 238:463.
Kiplinger, G. F., and J. E. Manno. 1971. Pharm. Rev. 23(4):339.
Kiplinger, G. F., J. E. Manno, B. E. Rodda, R. B. Forney, S. E. Haine, R. East, A. B. Richards. 1971. Clin. Pharm. Ther. 12(4):650.
Kish, V. M. 1973. Doctoral Thesis. University of Michigan
Klainer, L. M., Y. M. Chi, S. L. Freidberg, T. W. Rall and E. W. Sutherland. 1962. J. Biol. Chem. 237:1239.
Klausner, H. A., H. G. Wilcox and J. V. Dingell. 1971. Acta Pharm. Suec. 8:671.
Klausner, H. A., and J. V. Dingell. 1971a. Life Sci. 10(I):49.
Klein, C. C., G. R. Berg and J. Weller. 1970. Sci. 168:979.
Klein, F. K., H. Rapoport and H. W. Elliott. 1971. Nature 232:258.
Koch, A. L., and S. L. Putnam. 1971. Anal. Biochem. 44:239.
Koehler, B. 1946. Phytopath. 33:937.
Kolansky, H., and W. T. Moore. 1972. Amer. J. Med. Ass. 222(1):35.
Korinek, J., T. C. Spelsberg and W. M. Mitchell. 1973. Nature 246: 435.
Korte, F. 1970. In: Botany and Chemistry of Cannabis. p. 119.
Korte, F., and H. Sieper. 1960. Justus Liebigs Ann. Chem. 630:71.
Korte, F., and H. Sieper. 1964. J. Chromatog. 13:90.
Kosersky, D. S., W. L. Dewey and L. S. Harris. 1973. Eur. J. Pharmacol. 24:1.
Krantz, J. C., H. J. Bergen and B. L. Welch. 1971. Amer. J. Pharmacy 143(5):149.
Krejci, Z. 1970. In: The Botany and Chemistry of Cannabis. eds. Joyce, C. R. B., and S. H. Curry. Churchill, London
Krishna, G., J. Forn, K. Voight, M. Paul and G. L. Gessa. 1970. Adv. Biochem. Psychopharmacologia 3:155.
Krnjevic, K., and S. Schwartz. 1966. Exp. Brain Res. 3:320.
Kubena, R. K., J. L. Perhach Jr., and H. Barry III. 1971. Eur. J. Pharmacal. 14:89.
Kubena, R. K., J. Cavero, B. S. Jandhyala and J. P. Buckley. 1971a. Pharmacologist 13:247.
Kubena, R. K., and H. Barry III. 1972. Nature 235:397.
Kupfer, D., I. Jansson and S. Orrenios. 1972. Chem-Biol. Interactions 5:201.
Kupfer, D., E. Levin and S. H. Burstein. 1973. Chem.-Biol. Interactions 6:59.
Kvetnansky, R., G. P. Gewirtz, V. K. Weise and J. Kopin. 1971 Endocrinology 89:50.
Lamprecht, F., R. Kvetnansky, L. K. Y. Ng, R. B. Williams and I. J. Kopin. 1973. Eur. J. Pharmacol. 21:249.
Langan, T. 1973. In: Advances in Cyclic Nucleotide Research. Vol. III:99. P. Greengard and G. A. Robison (eds.). Raven Press, N. Y.

Laskowska, R. 1961. Nature 192:147.
Layman, J. M., and A. S. Milton. 1971. Brit. J. Pharmacol. 42(2): 308.
Layman, J. M., and A. S. Milton. 1971a. Brit. J. Pharmacol. 41(2): 379.
Lemberger, L., S. D. Silberstein, J. Axelrod and I. J. Kopin. 1970. Sci. 170:1320.
Lemberger, L., N. R. Tamarkin, J. Axelrod and I. J. Kopin. 1971. Sci. 173:72.
Lemberger, L., J. Axelrod and I. J. Kopin. 1971a. Ann. N. Y. Acad. Sci. 191:142.
Lemberger, L., J. Axelrod and I. J. Kopin. 1971b. Pharmacol. Rev. 23(4):371.
Lemberger, L., R. E. Crabtree and H. M. Rowe. 1972. Sci. 177:62.
Lemberger, L., J. L. Weiss, A. M. Watanake, I. M. Galanter, R. J. Wyatt and P. V. Cardon. 1972a. New Eng. J. Med. March.
Lemberger, L. 1973. Amer. Soc. Pharmacol. Exper. Therap. 1(1):461.
Lemberger, L., R. E. McMahon, R. A. Archer, K. Matsumoto and H. Rowe. 1973. J. Pharmacol. Exper. Therap. 187:169.
Lemberger, L. 1973a. Drug Metab. Dispos. 1(1):461.
Lemberger, L., R. Martz, B. Rodda, R. Forney and H. Rowe. 1973b. J. Clin. Invest. 52(10):2411.
Leonard, B. E. 1971. Pharm. Rse. Comm. 3(2):139.
Lerner, M., and J. T. Zeffert. 1968. Bull. Narc. 20(2):53.
Leung, B. S., A. R. Means and B. W. O'Malley. 1971. Endocrin. 89(1):70.
Levey, S. G. 1971. J. Biol. Chem. 246:7405.
Lewis, M. F. (ed.). 1972. Current Research in Marijuana. Academic Press, N. Y. p. inclusive.
Libet, B. 1970. Fed. Proc. 29:1945.
Libet, B, and T. Tosaka. 1970. Proc. Natl. Acad. Sci. (USA). 67: 667.
Ling, G. M., R. Mechoulam and D. R. Usher. 1971. Proc. 11th Ann. Meeting Assoc. Psychophysiol.
Ling, G. M., J. A. Thomas, D. R. Usher and R. L. Singhal. 1973. Int. J. Clin. Pharmacol. 7(1):1.
Lipparini, F., A. S. Decarolis and V. G. Longo. 1969. Physiol. Behav. 4:527.
Loev, B., P. E. Bender, F. Dowalo, E. Macko and P. J. Fowler. 1973. J. Med. Chem. 16(11):1200.
Loewe, S. 1945. J. Pharmacol. Exp. Therap. 84:78.
Loewe, S. 1946. J. Pharmacol. Exper. Therap. 88:154.
Loewe, S. 1947. Sci. 106:89.
Loewe, S. 1950. Arch. Exp. Pathol. Pharmacol. 211:175.
Lomax, P. 1971. Proc. West. Pharmacol. Soc. 14:10.
Lowry, O. H., N. J. Rosebrough, A. L Farr and R. J. Randall. 1951. J. Biol. Chem. 193:265.
Maeno, H., E. M. Johnson and P. Greengard. 1971. J. Biol Chem. 246:134.
Mahoney, J. M., and R. A. Harris. 1972. Biochem. Pharmacol. 21:1217.
Maitre, L. 1971. Acta Pharmaceu. Suec. 8:693.
Malinovski, S. M. 1930. Biol. Abstr. 4:23899.
Malkin, M., and F. Lipmann. 1969. Proc. Nat. Acad. Sci. 64:973.

Manning, F. T., J. H. McDonough Jr., T. F. Elsmore, C. Saller and F. J. Sodetz. 1971. Sci. 174:424.
Manno, J. E., G. F. Kiplinger, J. F. Bennett, S. Haine and R. B. Forney. 1970. Clin. Pharmacol. Therap. 11:808.
Marcotte, J., and H. P. Witschi. 1972. Res. Comm. Chem. Path. Pharmacol. 4(3):561.
Martin, P. A. 1969. Lancet. I:370.
Martinez, J. L., S. W. Stadnicki and V. H. Schaeppi. 1972. Life Sci. 11(I):643.
Martz, R., D. J. Brown, R. B. Forney, T. B. Bright, G. F. Kiplinger and B. E. Rodda. 1972. Life Sci. 11(I):999.
Marx, J. L. 1972. Sci. 178:1188.
Masui, H and L. D. Garren. 1970. Proc. Nat. Acad. Sci. (USA). 60: 3206.
Masur, J. 1973. Pharmacol. 9:35.
Masur, J., and N. Khazan. 1970. Life Sci. ((I):1275.
Masur, J., R. M. Martz and E. A. Carlini. 1971. Psychopharmacologia (Berl.). 19:388.
Masur, J. M., R. M. W. Martz, D. Bieniek and F. Korte. 1971a. Psychopharmacologia (Berl.). 7:175.
Masur, J., I. G. Karniol and J. P. Neto. 1972. J. Pharm. Pharmacol. 24:262.
Matchett, J. R., J. Levine, L. Benjamin, B. B. Robinson and O. A. Pope. 1940. J. Amer. Pharmaceu. Ass. (Balt.). 29:399.
McAfee, D. A., M. Schorderet and P. Greeengard. 1971. Sci. 171:1156.
McCallum, N. K. 1973. J. Chromatog. Sci. 11:509.
McGeer, P. L. 1971. Amer. Sci. 59:221.
McIsaac, W. M., G. E. Fritchie, J. E. Idanpaan-Heikkila, B. T. Ho and L. F. Englert. 1971. Nature 230:593.
McMeens, R. R. 1860. Report of the Committee on Cannabis indica, Transactions of the 15th Annual Meeting of the Ohio State Medical Soc. Follett, Foster Co. Colombus, Ohio.
McMillan, D. E., L. S. Harris, J. M. Frankenheim and J. S. Kennedy. 1970. Sci. 169:501.
McMillan, D. E., R. D. Ford, J. M. Frankenheim, R. A. Harris and L. S. Harris. 1972. Arch. Intl. Pharmacodyn. Therap. 198(1):132.
McMillan, D. E., W. L. Dewey, R. F. Turk, L. S. Harris and J. H. McNeil Jr. 1973. Biochem. Pharmacol. 22:383.
McPhee, H. C. 1924. Bot. Gaz. (Chic.). 78:335.
Mechoulam, R., and Y. Gaoni. 1967. Fortschr. Chem. Org. Naturst. 25:175.
Mechoulam, R., B. Yagnitinsky and Y. Gaoni. 1968. J. Amer. Chem. Soc. 90:2418.
Mechoulam, R. 1970. Sci. 168:1159.
Mechoulam, R., A. Shani, H. Edery and Y. Grunfeld. 1970. Sci. 164: 611.
Mechoulam, R., H. Varconi, Z. Ben-Zvi, H. Edery and Y. Grunfeld. 1972. j. Amer. Chem. Soc. 94:7930.
Mechoulam, R. (ed.). 1973. Marijuana. Academic Press, N. Y.
Meleges, F. T., J. R. Tinklenberg, L. E. Hollister and H. K. Gillespie. 1970. Sci. 168:1118.
Melikian, A. P., and I. S. Forrest. 1973. J. Pharmaceu. Sci. 62(6): 1025.

Merari, A., A. Barak and M. Plaves. 1973. Psychopharmacologia (Berl.). 28:243.
Merkus, F. W. H. M. 1971. Nature 232:579.
Merlin, M. D. 1972. Man and Marihuana. Fairleigh Dickinson Univ. Press. New Jersey
Messiha, F. S., and R. R. Soskin. 1973. Res. Comm. Chem. Path. Pharmacol. 6(1):325.
Meyer, P. E., R. C. Pillard, S. M. Mirin and L. S. Shapiro. 1971. Amer. J. Pshychiatry 128(3):198.
Mikes, F., and P. G. Waser. 1971. Sci. 172:1158.
Mikes, F., A. Hofmann and P. G. Waser. 1971. Biochem. Pharm. 20:2469.
Mikuriya, T. H. 1968. International Journal of Addiction. 3(2):253.
Mikuriya, T. H. 1969. Calif. Med. 110.
Mikuriya, T. H. 1970 Medical Times 98(4).
Mills, L., and P. Brawley. 1972. Agents and Actions 2(5):201.
Miras, C. J., S. Simos and J. Kiburis. 1964. Bull. on Narc. 16(1): 13.
Miras, C. J. 1965. In: Hashish: Its Chemistry and Pharmacology. Wolstenholme, G. E. W., and Knight, J. (eds.). Little, Brown. Boston. p.37.
Miras, C. J., and A. Coulselinis. 1970. United Nations Secretariat Publication. 25: ST/SOA/SER S/25.
Miyamoto, E., J. K. Kuo and P. Greengard. 1969. J. Biol. Chem. 224:6395.
Mole, M. L., and C. E. Turner. 1974. J. Pharmaceu. Sci. 63(1):154.
Monn, E., and R. O. Christiansen. 1971. Sci. 173:540.
Montemartini, L. 1928. Biol. Abstracts 2:9577.
Moore, L. A. 1969. Marijuana Bibliography. Bruin Humanist Forum, Calif.
Morrison, A. L., H. Rinderknecht, F. Bergel, A. R. Todd, A. D. MacDonald and G. Woolfe. 1943. J. Chem. Soc. p. 286.
Morrison, P. R. 1948. J. Cell. Comp. Phys. 31:281.
Mukhopadhyay, B. K., K. S. Subramanian and H. B. Donnicliff. 1943. Analyst 68:70.
Murphy, H. B. M. 1963. Bull. on Narc. 15(1):20.
Nahas, G. G., D. Zagury, I. W. Schwartz and M. Nagel. 1973. Nature 243:407.
Nahas, G. G., I. W. Schwartz, J. Adamec and W. M. Manger. 1973a. Proc. Soc. Exp. Biol. Med. 142(1):58.
Nahas, G. G., N. Suciu-Foca, J. Armand, A. Morishima. 1974. Sci. 183:419.
Nakazawa, K., and E. Costa. 1971. Nature 234:48.
National Institute of Mental Health. 1971. Report on Marihuana and Health. U. S. Government Printing Office, Washington, D. C.
Neish, A. C. 1964. In: Biochemistry of Phenolic Compounds. Harborne, J. B. (ed.). Academic Press. p. 295.
Neto, J. P., and E. A. Carlini. 1972. Eur. J. Pharmacol. 17:215.
Neu, R. L., H. O. Powers, S. King and L. I. Gardner. 1969. Lancet I-675.
Neumeyer, J. L., and R. A. Shagoury. 1971. J. Pharmaceu. Sci. 60(10):1433.

Newman, L. M., M. P. Lutz, M. H. Dould and E. F. Domino. 1972. Sci. 171:1022.
Ng, L. K. Y., F. Lamprecht, R. B. Williams and I. J. Kopin. 1973. Sci. 180:1368.
Nicholson, M. T., H. B. Pace and W. M. Davis. 1973. Res. Comm. Chem. Path. Pharm. 6(2):427.
Nigam, M. C., K. L. Handa, I C. Nigam and L. Leui. 1965 Can. J. Chem. 43:3372.
Nilsson, I. M., S. Agurell, J. L. G. Nilsson, A. Ohlsson, F. Sandberg and M. Walqvist. 1970. Sci. 168:1228.
Nilsson, I. M., S. Agurell, K. Leander, J. L. G. Nilsson and W. Widman. 1971. Acta Pharm. Suec. 8(6):701.
Nilsson, I. M., S. Agurell, J. L. G. Nilsson, A. Ohilsson, J. Lindgren and R. Mechoulam. 1973. Acta. Pharm. Suec. 10:97.
Nilsson, J. L. G., I. M. Nilsson and S. Agurell. 1969. Acta Chem. Scand. 23:2207.
Nir. I., D. Ayalon, A. Tsafriri, T. Cordova and H. R. Lindner. 1973. Nature 243:470.
Nordai, A. 1970. In: The Botany and Chemistry of Cannabis. Joyce, C. R. B., and Curry, S. H. (eds.). Churchill, London. p.63.
Novotny, M., and M. L. Lee. 1973. Experientia (Basel). 29:1038.
Obata, Y., and Y. Ishikawa. 1960. Bull. Agr. Chem. Soc. (Japan). 24:667.
Ohlsson, A., C. I. Abov-Chaar, S. Agurell, I. M. Nilsson, K. Olofsson and F. Sandberg. 1971. Bull. on Narc. 23(1):29.
Okamoto, T., P. Chan and B. T. So. 1972. Life Sci. 11(II):733.
Orsingher, O. A., and S. Fulginiti. 1970. Pharmacology 3:337.
Oskoui, M. 1972. Fed. Proc. Fed. Amer. Soc. Exp. Biol. 31:505.
Ott, T., B. Lobner and H. Matthies. 1972. Psychopharmacologia (Berl). 23:261.
Oh, T., H. Matthies. 1973. Psychopharmacologia (Berl.). 28:195.
Otten, J., G. S. Johnson and I. Pastan. 1971. Biochem. Biophys. Res. Comm. 44:1192.
Pace, H. B., M. W. Davis and L. A. Borgen. 1971. Ann. N.Y. Acad. Sci. 191:123.
Palmer, G. C. 1973. Life Sci. 12(II):345.
Palmer, G. C., F. Sulser and G. A. Robison. 1973. Neuropharm. 12: 327.
Park, Y. Y., and R. E. Tilton. 1970. Proc. West Pharm. Soc. 13:151.
Parker, J. N., and T. C. Dubas. 1973. Int. J. Clin. Pharmacol. Ther. Toxicol. 7(1):75.
Pars, H., G., F. E. Granchelli, J. K. Keller and R. K. Razdan. 1966. J. Amer. Chem. Soc. 88:3664.
Pars, H. G., and R. K. Razdan. 1971. Ann. N.Y. Acad. Sci. 191:15.
Pastan, I., and R. L. Perlman. 1971. Nature New Biol. 229:5.
Paton, W. D. M., and R. G. Pertwee. 1971. Acta Pharmaceu. Suec. 8:691.
Paul, M. I., B. R. Ditzion and D. S. Janowsky. 1970. Lancet Jan. 10:88.
Paul, M. I., H. Cramer and W. E. Bunney Jr. 1971. Sci. 171:300.
Pearl, J. H., E. F. Domino and P. Rennick. 1973. Psychpharmacologia. (Berl.). 31:13.
Pearson, O. P. 1948. Sci. 108:44.

Perez-Reyes, M., and M. A. Lipton. 1971. Paper for National Institute of Mental Health. Contract #HSM 42-69-62.
Perez-Reyes, M., M. C. Timmons, M. A. Lipton, K. H. Davis and M. E. Wall. 1972. Sci. 177:633.
Perez-Reyes, M., M. C. Timmons, M. A. Lipton, A. D. Christensen, K. H. Davis and M. E. Wall. 1973. Experientia. 29:1009.
Perkins, J. P. 1970. Trans. Am. Soc. Neurochem. 1:60.
Perkins, J. P. 1973. Advances in Cyclic Nucleotide Research. Vol. 3. Raven Press, New York.
Persaud, I., and A. Ellington. 1968. Lancet II(7564):406 and II:1306.
Petcoff, D. G., S. M. Strain, W. R. Brown and E. Ribi. 1971. Sci. 173:824.
Peterson, D. W., G. M. Cohen and S. B. Sparber. 1971. Life Sci. 10(1):1381.
Petrzilka, T., and C. Sikemeier. 1967. Helv. Chim. Acta 50:1416 and 50:2111.
Petrzilka, T., W. Haefliger and C. Sikemeier. 1969. Helv. Chim. Acta 52:1102.
Phillips, R. N., R. F. Turk, J. Manno, N. Jain and R. Forney. 1970. J. Forensic Sci. 15(2):191.
Phillips, R. N., R. F. Turk and R. B. Forney. 1971. Proc. Exp. Biol. and Med. 136(1):260.
Phillips, R. N., M. A. Neel, D. J. Brown and R. B. Forney. 1971a. Pharmacologist 13:297.
Pirch, J. H., R. A. Cohn, P. R. Barnes and E. S. Barratt. 1972. Neuropharm. 11:231.
Pitt, C. G., and M. E. Wall. 1969. Tetrahedran Letters. 321:1.
Pohle, W., and H. Matthies. 1971. Brain Res. 29:123.
Porter, C. D., T. N. Burbridge and K. G. Scott. 1970. Proc. West Pharm. Soc. 13:144.
Potvin, R. J., and P. A. Fried. 1972. Psychopharmacologia.(Berl.). 26:369.
Pradhan, S. W., P. T. Bailey and B. Ghosh. 1972. Res. Comm. Chem. Path. Pharm. 3(2):197.
Prasad, K. N., and B. Mandal. 1972. Exp. Cell. Res. 74:532.
Pratt, R., and H. W. Youngken Jr. 1951. Pharmacognosy. J. P. Lippincott Co., Phila., Penn.
Pritchard, I. J. 1916. J. Heredity (Wash.). 7:325.
Rall, T. W. 1971. Ann. N. Y. Acad. Sci. 185:520.
Rall, T. W., and A. G. Gilman. 1970. The Role of Cyclic AMP in the Nervous System. Neurosciences Research Program Bull. 8(3).
Rasmussen, H. 1970. Sci. 170:404.
Rasmussen, K. E., S. Rasmussen and B. Svendsen. 1972. Acta Pharm. Suec. 9:457.
Rathenasinkam, E. 1948. Analyst (Lon.). 73:509.
Rating, D., I. Broemann, H. Honecker, S. Klowe and H. Coper. 1972. Pshychopharmacologia (Berl.). 27:349.
Raz, A., A. Schurr and A. Livne. 1972. Biochim. Biophys. Acta 274:269.
Raz, A., A. Schurr, A. Livne and R. Goldman. 1973. Biochem. Pharmacol. 22:3129.

Razdan, R. K., V. V. Kane, H. G. Pars, J. L. Kucera, D. H. Reid, L. S. Harris, W. L. Dewey and J. F. Howes. 1969. Minutes 31st Meeting, Committee on Problems of Drug Dependence, NAS-NRC, 6135.

Razdan, R. K., and G. R. Handrick. 1970. J. Amer. Chem. Soc. 92: 6061.

Razdan, R. K., A. J. Puttick, B. A. Zitko and G. R. Handrick. 1972. Experientia 28:121.

Reis, D. J., A. Corvelli and J. Conners. 1968. J. Pharmacol. Exp. Ther. 167:166.

Rethy, A., V. Tomasi and A. Trevisani. 1971. Arch. Biochem. Biophys. 147:36.

Ribi, E., R. F. Smith, S. M. Strain, D. G. Petcoff, R. Parker, G. Goode and I. Sorval Inc. 1973. Prep. Biochem. 3(3):209.

Richelson, E. 1973. Nature New Biol. 242:176.

Riolo, R. L. 1973. Personal Communication. University of Michigan.

Robichaud, R. C., M. A. Hefner, J. E. Anderson and M. E. Goldberg. 1973. Pharmacology 10:1.

Robison, B. B. 1943. J. Amer. Soc. Agron. 35:911.

Robison, B. B., and J. R. Matchett. 1940. J. Amer. Pharmaceu. Ass. 29:448.

Robison, G. A., M. J. Schmidt and E. W. Sutherland. 1970. Adv. Biochem. Psychopharmacol. 3:11.

Robison, G. A., M. J. Schmidt and E. W. Sutherland. 1970. In: Role of Cyclic AMP and Cell Function. Greengard, P., and Costa, E. (eds.). Raven Press, N. Y. p. 11.

Robison, G. A., G. G. Nahas and L. Triner (eds.). 1971. Ann. N.Y. Acad. Sci. 185.

Robison, G. A., R. W. Butcher and E. W. Sutherland (eds.). 1971a. Cyclic AMP. Academic Press, N.Y.

Robison, G. A., R. W. Butcher and E. W. Sutherland. 1971b. Cyclic AMP. Academic Press, N.Y. p. 91.

Rodin, E. A., E. F. Domino and J. P. Porzak. 1970. J. Amer. Med. Ass. 213:1300.

Rosenkrantz, H., G. R. Thompson and M. C. Braude. 1972. J. Pharmaceu. Sci. 61(7):1106.

Rosevear, J. 1967. Pot: A Handbook of Marihuana. University Books, N.Y. p. 16-26.

Rothe, C. F. 1966. In: Pysiology. Selkurt, E. E. (ed.). 2nd ed. p. 353.

Rubin, A., T. R. Tephly and G. J. Mannering. 1964. Biochem. Pharmacol. 13:1007.

Rubin, R. P. 1970. Pharmacol. Rev. 22:389.

Ryan, L. W., and L. H. Heidrick. 1968. Sci. 162:1484.

Ryrfeldt, A., C. H. Ramsay, S. Agurell, T. M. Nilsson and M. Widman. 1971. Acta Pharm. Suec. 8:704.

Ryrfeldt, A., C. H. Ramsay, I. M. Nilsson, M. Widman and S. Agurell/ 1973. Acta Pharmaceu. Suec. 10:13.

Sabalitschka, T. 1925. Heil-und Gewurz-Pflanzen. 8:73.

Sabelli, A. C., A. J. Vazquex, A. D. Musnaim and L. Madrid-Pedemonte. 1974. Nature 248:144.

Sattin, A., and T. W. Rall. 1970. Mol. Pharmacol. 6:13.

Scaringelli, F. 1961. Ass. Off. Agr. Chem. 44(2):296.

Schaffner, J. H. 1921. Bot. Gaz. (Chicago). 71:197.
Schaffner, J. H. 1923. Ecology 4:323.
Schaffner, J. H. 1928. Amer. J. Botany 15:77.
Schaffner, J. H. 1931. Amer. J. Botany 18:424.
Scheckel, C. L., E. Boff, P. Dahlen and T. Smart. 1968. Sci. 160:1467.
Scheving, L. E., W. H. Harrison, P. Gordon and J. E. Pauly. 1968. Amer. J. Physiol. 214:166.
Schildkraut, J. J., and D. H. Efron. 1971 Psychopharmacologia. (Berl.). 20:191.
Schmidt, M. J., D. E. Schmidt and G. A. Robison. 1971. Sci. 173:1142.
Schmidt, M. J., J. T. M. Hopkins, D. E. Schmidt and G. A. Robison. 1972. Brain Res. 42:465.
Schmidt, M. J., and L. Sokoloff. 1973. J. Neurochem. 21:1193.
Schultes, R. E. 1970. In: The Botany and Chemistry of Cannabis. Joyce, C. R. B., and Curry, S. H. (eds.). Churchill, London p. 11.
Seeman, P., M. Chau-Wong and S. Moyyen. 1972. Can. J. Physiol. Pharmacol. 50:1193.
Segelman, A. B., P. A. Babrock and B. L. Braun. 1973. J. Pharmaceu. Sci. 62(3):515.
Shannon, M. E., and P. A. Fried. 1972. Psychopharmacologia (Berl.). 27:141.
Shashoua, V. E. 1968. Nature 217:238.
Shashoua, V. E. 1971. Proc. Nat. Acad. Sci. (USA). 68(11):2835.
Sheppard, J. R. 1971. Proc. Nat. Acad. Sci. (USA). 68:1316.
Shiffrin, R. M. 1971. Sci. 173:1038.
Shimizu, H., C. R. Creveling and J. W. Daly. 1970. Adv. Biochem. Psychopharmacol. 3:135.
Shimizu, H., C. R. Creveling and J. Daly. 1970. Proc. Nat. Acad. Sci. (USA). 65:1033.
Shoyama, Y., A. Yamaguchi, T. Sato, T. Yamaochi and I. Nishioka. 1969. Yakagaku Zasshi. 89:842.
Shoyama, Y. T. Yamauchi and I. Nishioka. 1970. Chem. Pharm. Bull. (Tokyo). 18:1327.
Shoyama, T., R. Oku, T. Yamauchi and I. Nishioka. 1972. Chem. Pharm. Bull. 20(9):1927.
Sidell, F. R., J. E. Pless, H. Neitlich, P. Sussman, H. W. Copelan and V. M. Sim. 1973. Proc. Soc. Exp. Biol. and Med. 143 (3):867.
Silva, M. T., E. A. Carlini, V. Claussen and F. Korte. 1968. Psychopharmacologia (Berl.). 13:332.
Sinonsen, J., and A. R. Todd. 1942. J. Chem. Soc. p. 188.
Sjoden, P., T. V. C. Jarbe and B. G. Henriksson. 1973. Pharm. and Biochem. Behav. 1:395.
Slatkin, D. J., N. J. Doorenbos, L. S. Harris, A. N. Masoud, M. W. Quimby and P. L. Schiff Jr. 1971. J. Pharmaceu. Sci. 60 (12):1891.
Small, E., and H. D. Beckstead. 1973. Nature 245:147.
Smith, D. E. 1968. J. Psychedelic Drugs 2:37.
Smith, T., and H. Smith 1857. J. Chem. Soc. 21:47.
Smythies, J. R. 1972. Eur. J. Pharmacol. 19:18.

Snyder, S. H. 1971. Uses of Marijuana. Oxford Univ. Press, N.Y.
Sofia, R. D. 1972. Eur. J. Pharmacol. 20:139.
Sofia, R. D., and W. Barry III. 1970. Eur. J. Pharmacol. 13:134.
Sofia, R. D., R. K. Kubena and H. Barry III. 1971 J. Pharm. Pharmacol. 23:889.
Sofia, R. D., B. N. Dixit and H. Barry III. 1971a. Life Sci. 10(1):425.
Sofia, R. D., R. J. Ertel, B. N. Dixit and H. Barry III. 1971b. Eur. J. Pharmacol. 16:257.
Sofia, R. D. 1972 Res. Comm. Chem. Path. Pharmacol. 4:281.
Sofia, R. D., S. D. Nalepa, J. J. Harakal and H. B. Vassar. 1973. J. Pharmacol. Exp. Ther. 186(3):646.
Soloman, D. (ed.). 1966. The Marihuana Papers. New American Library, Bergenfield, N. J.
Sprague, R. A., H. Rosenkrantz and M. C. Braude. 1973. Life Sci. 12(II):409.
Stearn, W. T. 1970. In: The Botany and Chemistry of Cannabis. (eds.) Joyce, C. R. B., and Curry, S. H. Churchill, London p. 1.
Stellwagen, R. H. 1972. Biochem. Biophys. Res. Comm. (47(5):1144.
Stephan, J. 1932. Biol. Abstracts 6(3):6636.
Stockings, G. T. 1947. Brit. Med. Journal 1.
Straight, R., A. W. Wayne, E. G. Lewis and E. C. Beck. 1973. Biochem. Med. 8:341.
Tagliamonte, A., and P. Tagliamonte. 1969 Sci. 166:1433.
Tagliamonte, A., P. Tagliamonte, J. Forn, J. Perez-Cruet, G. Krishna and G. L. Gessa. 1971. J. Neurochem. 18:1191.
Talley, P. J. 1934. Plant Physiol. (Lancaster). 9(4):731.
Taskin, D. P., B. J. Shapiro and I. M. Frank. 1973. New Eng. J. Med. 289:336.
Tayleur-Stockings, G. 1947. Brit. Med. J. 1:919.
Taylor, E. C., E. J. Strojny. 1960. J. Amer. Chem. Soc. 82:5198.
Taylor, E. C., K. Lenard and Y. Shvo. 1966. J. Amer. Chem. Soc. 88:367.
Taylor, N. 1966. In: The Marihuana Papers. (ed.) Solomon, D. New American Library, Bergenfield, N.J. p. 31.
Taylor, N. 1966a. Narcotics: Natures Dangerous Gifts. Dell Books N. Y.
ten Ham, M., and J. van Noordwijk. 1973. Psychopharmacologia (Berl.) 29:171.
Thompson, L. J., and R. C. Proctor. 1953. North Carolina Med. J. 14.
Thompson, W. J., and M. M. Appleman. 1971. Biochem. 10:31.
Thompson, G. R., H. Rosenkrantz, U. H. Schaeppi and M. C. Braude. 1973. Toxicol. and Appl. Pharmacol. 25:363.
Timmons, M. L., S. C. G. Pitt and M. E. Wall. 1969. Tetrahedron Letters. p. 3129.
Tinklenberg, J. R., F. T. Melges, L. E. Hollister and H. K. Gillespie. 1970. Nature 226:1171.
Todd, A. R. 1946. Experientia 2:55.
Triner, L., G. G. Nahas, Y. Vulliemoz, N. I. A. Overweg, M. Verosky D. V. Habif and S. H. Ngai. 1971 Ann N.Y. Acad. Sci. 185:458.
Truitt, Jr., E. B. 1971. Pharm. Rev. 23:273.

Truitt, Jr., E. B., and S. M. Anderson. 1971. Ann. N.Y. Acad. Sci. 191:68.
Turk, R. F., J. E. Manno, N. C. Jain and R. B. Forney. 1971. J. Pharm. Pharmac. 23:190.
Turk, R. F., W. L. Dewey and L. S. Harris. 1973. J. Pharmaceu. Sci. 62(5):737.
Turner, C. E., K. W. Hadley, P. S. Fetterman, N. J. Doorenbos, M. W. Quimby and C. Waller. 1973. J. Pharmaceu. Sci. 62(10):1601.
Turner, C. E., and K. Hadley. 1973a. J. Pharmaceu Sci. 62(2):251.
United Nations Commission on Narcotic Drugs. 1965. Document E-CN 7-479. U. N. Econ. Soc. Council, N.Y.
United States Commission. 1972. First Report of the National Commission on Marihuana and Drug Abuse. Appendix Vol. I and II. U. S. Government Printing Office.
Uzunov, P., H. M. Shein and B. Weiss. 1973. Sci. 180:304.
VanderKloot, G., and H. Kita. 1974. Bioscience 24(1):13.
Van Wijk, R., K. Clay and W. D. Wicks. 1972. Cancer Res. 32:1905.
Van Wijk, R., W. D. Wicks, M. M. Bevers and J. Van Rijn. 1973. Cancer Res. 33:1331.
Veliky, I. A., and K. Genest. 1970. Lloydia 33(4)493.
Verwey, A. M. A., and A. H. Witte. 1972. Pharm. Weekblad. 107:153.
Volavka, J., R. Dornbush, S. Feldstein, G. Clare, A. Zaks, M. Fink and A. M. Freedman. 1971. Ann. N.Y. Acad. Sci. 191:206.
von Hungen, K., and S. Roberts. 1973. Eur. J. Biochem. 36:391.
von Hungen, K., and S. Roberts. 1973. Nature New Biol. 242:58.
Vree, T. B., D. D. Breimer, C. A. M. van Ginneken and J. M. van Rossum. 1971. LKB-Produkten N.V. Zeerant 35, the Hague, the Netherlands.
Vree, T. B., D. D. Breimer, C. A. M. van Ginneken, J. M. van Rossum, R. A. deZeeuw and A. H. Witte. 1971. Clinica Chim. Acta 314:365.
Vree, T. B., D. D. Breimer, C. A. M. van Ginneken and J. M. van Rossum. 1972. J. Chromatogr. 74:209.
Vree, T. V., D. D. Breimer, C. A. M. van Ginneken, J. M. van Rossum and N. M. M. Nibbering. 1973. J. Chromatogr. 79:81.
Vulliemoz, Y., L. Triner and G. G. Nahas. 1970. Fed. Proc. 29(2): 615.
Wahlqvist, M., I. M. Nilsson, F. Sandberg, S. Agurell and B. Granstrand. 1970. Biochem. Pharm. 19:2579.
Wall, M. E. 1971. Ann. N.Y. Acad. Sci. 191:23.
Wall, M. E., O. R. Brine, G. A. Brine, C. G. Pitt, R. I. Freudenthal and H. D. Christensen. 1970. J. Amer. Chem. Soc. 92:3466.
Wall, M. E., D. Brine, M. Perez-Reyes and M. Lipton. 1971. Acta Pharm Suec. 8(6):702.
Waller, C. W. 1971. Pharm. Rev. 23(4):265.
Waller, C. W., and J. J. Denney. 1971. Annotated Bibliography of Marihuana (Cannabis sativa L.) 1964-1970. The Research Institute of Pharmaceutical Science. University of Mississippi, University, Miss.
Walton, R. P. 1938. Marijuana: America's New Drug Problem. J. B. Lippincott Company, Phila., Penn.
Waskow, I. E., J. E. Olsson, C. Salzman and M. M. Katz. 1970. Arch. Gen. Psychiatry 22(2):97.

Waters, D. H., and S. D. Glick. 1973. Res. Comm. Chem. Path. Pharmacol. 6(1):57.
Waymire, J. C., N. Weiner and K. N. Prasad. 1972. Proc. Nat. Acad. Sci. (USA). 69(8):2241.
Weber, K., and M. Osborn. 1969. J. Biol Chem. 244:4406.
Webster, C. D., A. E. LeBlanc, J. A. Marshmann and J. M. Beaton. 1973. Psychopharmacologia (Berl.). 30:217.
Well, A. T., N. E. Zinberg and J. M. Nelsen. 1968. Sci. 162:1234.
Well, A. T., and N. E. Zinberg. 1969. Nature 222:434.
Weiner, M., G. G. Boterbaugh and D. A. Biake. 1972. Res. Comm. Chem. Path. Pharmacol. 4(1):37.
Weiner, M., G. G. Boterbaugh and D. A. Blake. 1972a. Res. Comm. Chem. Path. Pharmacol. 3(2):249.
Weiner, M., and J. W. Olson. 1973. Life Sci. 12(I):345.
Weiss, B., and E. Costa. 1968. J. Pharmacol. Exp. Ther. 161:310.
Weiss, B., and S. W. Crayton. 1970. Adv. Biochem. Psychopharmacol. 3:217.
Weiss, J. L., A. M. Watanabe, L. Lemberger, N. R. Tamarkin and P. V. Cardon. 1972. Clin. Pharm. and Ther. 13(5)I:671.
Welch, B. L. 1968. Biochem. Pharm. 17:2013.
Welch, B. L., A. S. Welch, F. S. Messiha and H. J. Berger. 1971. Res. Comm. Chem. Path. Pharm. 2(3):382.
Weller, M., and R. Rodnight. 1971. Biochem. J. 124:393.
Wellmann, W., and V. Schwabe. 1973. Brain Res. 59:371.
Wenkert, E., D. W. Cochran, F. M. Schell, R. A. Archer and K. Matsumoto. 1972. Experientia 28:250.
Wester, P. J. 1914. J. Heredity 5:207.
Whineray, E. 1967. In: The Book of Grass, An Anthology of Indian Hemp. (eds.). Andrews, G., and S. Vinkenoog. Grove Press, N.Y. p. 69.
White, E. R., and C. H. Hine. 1972. Abstracts 5th International Congress on Pharmacology. July. San Francisco, Calif.
Widman, M., I. M. Niisson, J. L. G. Nilsson, F. Sandberg, S. Agurell and B. Granstrand. 1971. Acta Pharm. Suec. 8(6):705.
Widman, M., J. M. Nilsson, J. L. G. Nilsson, S. Agurell and K. Leander. 1971a. Life Sci. (II) 10:157.
Williams, E., C. K. Himmelsbach, A. Wikler and D. C. Ruble. 1946. Public Health Report 61:1059.
Wilsie, C. P., C. A. Black and A. R. Aandahl. 1944. Iowa Agr. Exp. Sta. Bull. p. 63.
Witschi, H., B. Saint-Francois. 1972. Toxicol. App. Pharmacol. 23:165.
Wood, T. B., W. T. N. Spivey and T. H. Easterfield. 1897. J. Amer. Chem. Soc. 75:20.
Wooten, G. F., N. B. Thoa, I J. Kopin and J. Axelrod. 1973. Mol. Pharmacol. 9(2):178.
Yang, T. J., S. J. Nas. 1971. Experientia. 27:442.
Zimmberberg, B., S. D. Glick and M. E. Jaruik. 1971. Nature 233:343.
Zitko, B. A., J. F. Howes, R. K. Razdan, B. C. Dalzell, H. C. Dalzell J. C. Sheehan and H. G. Pars. 1972. Sci. 177:442.